The Analyst

The Analytical Journal
of The Chemical Society

A monthly international publication dealing with all branches of analytical chemistry

Volume 102 No 1210 Pages 1–72 January 1977

THE ANALYST

THE ANALYTICAL JOURNAL OF THE CHEMICAL SOCIETY

Published by The Chemical Society

Editorial: The Director of Publications, The Chemical Society, Burlington House, London, W1V 0BN. Telephone 01-734 9864. Telex No. 268001.

Advertisements: J. Arthur Cook, 9 Lloyd Square, London, WC1X 9BA. Telephone 01-837 6315.

Subscriptions (non-members): The Chemical Society Publications Sales Office, Blackhorse Road, Letchworth, Herts., SG6 1HN.

Volume 102 No 1210 January 1977

Summaries of Papers in this Issue

Potentiometric System for the Continuous Determination of Low Levels of Chloride in High-purity Power Station Waters

An analytical system for the continuous determination of low levels of chloride (0–150 $\mu g\ l^{-1}$) in high-purity power station waters has been developed that is based on the potential of a silver - silver chloride electrode *versus* a mercury - mercury(I) sulphate reference electrode, the electrodes being immersed in a buffered sample stream. The temperature of the electrode flow cell is controlled at 10 (± 0.1) °C, at which level the sensitivity of 3.54 mV per 100 $\mu g\ l^{-1}$ is approximately 50% greater than that at 25 (± 0.1) °C, and a linear response to changes in chloride concentration is observed over the range 0–150 $\mu g\ l^{-1}$. The standard deviations at 10 °C for nominal chloride concentrations of 50, 100 and 150 $\mu g\ l^{-1}$ are ± 1.7, ± 2.8 and ± 2.8 $\mu g\ l^{-1}$, respectively. Substances normally present in these waters do not interfere appreciably.

Keywords: *Potentiometry*; *chloride determination*; *high-purity waters*; *silver - silver chloride electrode*

K. TOMLINSON

Central Electricity Generating Board, Scientific Services Department, Wythenshawe, Manchester, M23 9LL.

and K. TORRANCE

Central Electricity Research Laboratories, Kelvin Avenue, Leatherhead, Surrey, KT22 7SE.

Analyst, 1977, **102**, 1–8.

Determination of Arsenic in Soil and Plant Materials by Atomic-absorption Spectrophotometry with Electrothermal Atomisation

A method is described for the determination of arsenic in soil and plant materials by hydride generation and atomic-absorption spectrophotometry using electrothermal atomisation. Soils are decomposed by leaching with a mixture of nitric and sulphuric acids or fusion with pyrosulphate, and plants by wet oxidation with nitric and sulphuric acids. The resultant acidic sample solution is made to react with sodium borohydride and the liberated arsenic hydride is swept into an electrically heated tube mounted on the optical axis of a simple, laboratory constructed atomic-absorption apparatus.

The advantages of high sensitivity, rapid analysis and simplicity of equipment are discussed, and the results for both types of sample material are compared with values obtained by use of the molybdenum-blue method.

Keywords: *Arsenic determination*; *soils*; *plants*; *atomic-absorption spectrophotometry*

A. J. THOMPSON and PANDORA A. THORESBY

Applied Geochemistry Research Group, Department of Geology, Imperial College of Science and Technology, London, SW7 2BP.

Analyst, 1977, **102**, 9–16.

Determination of Noble Metals in Geological Materials by Radiochemical Neutron-activation Analysis

A method for the determination of platinum, palladium, gold and iridium in geological materials following activation with thermal neutrons is described. Radionuclides formed from the elements are separated by a scheme based largely on liquid - liquid extractions. The procedure has been applied to the analysis of US Geological Survey standard rocks and to studies of the distribution of the noble metals in lateritic nickel ores

Keywords: *Noble metal determination*; *standard rocks*; *nickel ores*; *neutron-activation analysis*

I. AHMAD, S. AHMAD and D. F. C. MORRIS

Nuclear Science Centre, School of Chemistry, Brunel University, Uxbridge, Middlesex, UB8 3PH.

Analyst, 1977, **102**, 17–24.

A Selective Reagent for the Spectrophotometric Determination of Palladium

A spectrophotometric method for the determination of trace amounts of palladium(II) with ethyl 3-phenyl-5-isoxazolone-4-carboxylate was developed. With this reagent, palladium forms a yellow complex, stable in the pH range 1–6.5, which is extractable into 4-methylpentan-2-ol from 0.05–4 M perchloric acid medium. The coloured complex adheres to Beer's law at 370 nm in aqueous and in 4-methylpentan-2-ol solutions, with molar absorptivities of 3.5×10^3 and 3.9×10^3 l mol^{-1} cm^{-1}, respectively. Other platinum-group metals do not interfere. In the presence of highly coloured ions, extraction with 4-methylpentan-2-ol eliminates all interferences. Cyanide ions interfere in the method.

Keywords: *Palladium determination*; *spectrophotometry*; *ethyl 3-phenyl-5-isoxazolone-4-carboxylate reagent*

F. CORIGLIANO, S. DI PASQUALE and A. RANIERI

Istituto di Chimica Analitica, Università di Messina, 98100 Messina, Italy.

Analyst, 1977, **102**, 25–28.

Determination of Pyrimethamine, Ethopabate and Sulphaquinoxaline in Poultry Feeding Stuffs by High-performance Liquid Chromatography Using a Weak Cation-exchange Column Packing

A method based on high-performance liquid chromatography, using a weak cation-exchange column packing, is described for the simultaneous determination of pyrimethamine, ethopabate and sulphaquinoxaline in poultry feeding stuffs. The method is applicable to mash and pelleted feeds containing at least 1 mg kg^{-1} of sulphaquinoxaline. A comparison is made between this and other methods of analysis of these prophylactics and, with the exception of sulphaquinoxaline in pelleted feeds, good agreement is obtained. An alternative procedure is described for the determination of sulphaquinoxaline in pelleted feeds.

Keywords: *Prophylactics determination*; *poultry feeding stuffs*; *high-performance liquid chromatography*

G. B. COX and K. SUGDEN

Department of Industry, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, SE1 9NQ.

Analyst, 1977, **102**, 29–34.

JANUARY 1977 Vol. 102 No. 1210

The Analyst

Potentiometric System for the Continuous Determination of Low Levels of Chloride in High-purity Power Station Waters

K. Tomlinson

Central Electricity Generating Board, Scientific Services Department, Wythenshawe, Manchester, M23 9LL

and K. Torrance

Central Electricity Research Laboratories, Kelvin Avenue, Leatherhead, Surrey, KT22 7SE

An analytical system for the continuous determination of low levels of chloride (0–150 $\mu g\ l^{-1}$) in high-purity power station waters has been developed that is based on the potential of a silver - silver chloride electrode *versus* a mercury - mercury(I) sulphate reference electrode, the electrodes being immersed in a buffered sample stream. The temperature of the electrode flow cell is controlled at 10 (± 0.1) °C, at which level the sensitivity of 3.54 mV per 100 $\mu g\ l^{-1}$ is approximately 50% greater than that at 25 (± 0.1) °C, and a linear response to changes in chloride concentration is observed over the range 0–150 $\mu g\ l^{-1}$. The standard deviations at 10 °C for nominal chloride concentrations of 50, 100 and 150 $\mu g\ l^{-1}$ are ± 1.7, ± 2.8 and ± 2.8 $\mu g\ l^{-1}$, respectively. Substances normally present in these waters do not interfere appreciably.

Keywords: Potentiometry; chloride determination; high-purity waters; silver - silver chloride electrode

It is generally accepted that the presence of chloride in the steam - water circuit of power stations can be associated with corrosive conditions within the boiler; consequently chloride levels have to be controlled. The concentration of chloride tolerated in boiler waters varies with the design of the boiler and the chemical treatment accorded to the boiler water, but in every instance it is essential that any ingress of chloride is detected so that remedial action can be taken. In order to identify the source of the chloride, it is necessary to monitor plant streams, *e.g.*, feed water or the effluent from a water-treatment plant, where chloride levels may be as low as a few micrograms per litre. It was for this purpose that the apparatus described in this paper was developed.

Potentiometric techniques using various forms of silver chloride electrode[1,2] have been shown to be capable of determining chloride at concentrations less than 100 $\mu g\ l^{-1}$ but no method has been reported for the continuous determination of chloride at these levels in plant streams. A continuous on-stream analytical method was developed that was based on a manual method for the determination of chloride in boiler waters,[3] in which the potential of a silver - silver chloride electrode *versus* a mercury(I) sulphate reference electrode was measured in a sample the pH and ionic strength of which were controlled by the addition of an acetate buffer. Thus, changes in the potential of a silver - silver chloride electrode immersed directly in a buffered sample stream were used to detect small changes (2–5 $\mu g\ l^{-1}$) in the chloride concentration of some power station waters. In order to achieve this sensitivity considerable attention was given to the choice and control of temperature, the preparation of high-purity water and the constancy of flow of sample and standard solutions.

Theoretical Basis of the Electrode Response at Low Chloride Levels

At chloride concentrations in the sample below $\sqrt{K_s}$ (where K_s is the solubility product of silver chloride) the contribution to the total chloride present at equilibrium made by

dissolution of the electrode surface is significant. The electrode responds to the total chloride present and an expression[3] for its potential under these conditions is

$$E = E^{\circ}_{Ag/Ag^+} + \frac{RT}{F} \ln K_s - \frac{RT}{F} \ln \left[\frac{\gamma_{\pm} m}{2} + \left(\frac{\gamma_{\pm}^2 m^2 + 4K_s}{4} \right)^{\frac{1}{2}} \right] \quad .. \quad (1)$$

where m is the original concentration of chloride in the sample and $\gamma_{\pm}$ is the mean univalent activity coefficient. On rearrangement:

$$E = E^{\circ}_{Ag/Ag^+} + \frac{RT}{F} \ln K_s - \frac{RT}{F} \ln \left[\frac{\gamma_{\pm} m}{2\sqrt{K_s}} + \left(\frac{\gamma_{\pm}^2 m^2}{4K_s} + 1 \right)^{\frac{1}{2}} \right] \quad .. \quad (2)$$

If m is considerably less than $\sqrt{K_s}$, then the following approximation is valid:

$$E \approx \text{Constant} - \frac{RT}{F} \ln \left(\frac{\gamma_{\pm} m}{2\sqrt{K_s}} + 1 \right) \quad .. \quad .. \quad (3)$$

As the binomial expansion of the series $\ln (x + 1)$ is $\left(\frac{x}{1} - \frac{x^2}{2} + \frac{x^3}{3} \ldots \right)$, it can be seen that at chloride concentrations of less than 100 μg l^{-1} the potential of a silver - silver chloride electrode can be considered to be directly proportional to $\gamma_{\pm} m$, *i.e.*, the first term of the binomial expansion of equation (3).

Experimental

Apparatus

Electrode flow cell

This cell was constructed from a Perspex block in which four holes were bored vertically to form a thermometer pocket, an air vent and two electrode pockets. These holes were connected by horizontal or sloping passages to form the flow cell, details of which are given in Fig. 1.

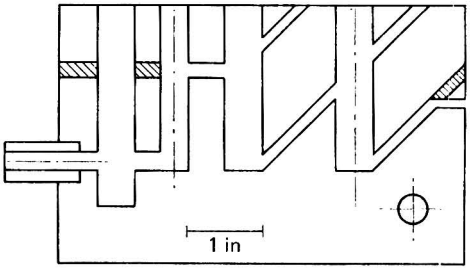


Fig. 1. Electrode compartment.

Silver - silver chloride electrode

These electrodes were constructed from silver rod, 3.18 mm in diameter (Johnson Matthey Ltd., London), and mounted in a PTFE holder. A hole of the same diameter as the silver rod was drilled in the PTFE holder and the contraction of the plastic after drilling was such that a water-tight seal was formed round the silver rod when it was pushed into the hole.

The length of the rod that was to be chloridised (16 mm) was first de-greased with acetone, then washed with distilled water and lightly etched with dilute nitric acid (1 + 10). It was then chloridised in 0.01 N hydrochloric acid, *versus* a platinum cathode, for 24 h at a current of 0.1–0.2 mA. After electrolysis, the chloridised rod was removed from the acid solution, washed with distilled water and stored in the dark in water for about 1 week before use.

Mercury(I) sulphate reference electrode

A mercury - mercury(I) sulphate reference electrode, Type 33/1380/230 (Electronic Instruments Ltd., Chertsey, Surrey), with a ground-glass sleeve junction was used. The glass

barrel of the electrode was fitted with a side-arm, which was connected to a reservoir containing 0.5 M sodium sulphate salt bridge solution.

Millivoltmeter

The e.m.f. of the cell was measured on a Vibron 33B2 electrometer (H. W. Sullivan Ltd., Orpington, Kent), which was fitted with a voltage back-off circuit such that readings were always made on the 0–10 mV scale. The cell potentials were recorded on a potentiometric chart recorder.

Analysis stream

Four sample streams were brought through nylon or stainless-steel sampling lines to constant-head devices, from which streams were abstracted by a Technicon Mark II proportioning pump (Technicon Instruments Ltd., Basingstoke, Hants.) at flow-rates of 7.8 ml min^{-1}, whenever they were selected by a six-channel valve system (Hook and Tucker, Croydon, Surrey). The two remaining channels were reserved for blank water and a standard chloride solution. For most plant investigations, the timer was set for a 2-h cycle with a 20-min period selected for each channel.

The samples passed from the pump to a glass T-piece de-bubbler, 40 cm above the pump, where a 3.9-ml min^{-1} stream was abstracted and pumped to waste from the vertical leg of the T-piece. This device was found to be necessary for the removal of any stray air bubbles and it also provided an additional constant head for the analysis stream before it was returned to the pump at a flow-rate of 3.9 ml min^{-1}, after which it was combined with a buffer stream flowing at 0.4 ml min^{-1}. The buffered stream was first mixed in a glass coil and then passed through a second de-bubbler T-piece as a further precaution against stray bubbles reaching the flow cell. A flow of 0.8 ml min^{-1} was abstracted from the vertical outlet of the T-piece and pumped to waste while the main flow continued through two heat-exchange coils before reaching the flow cell (see Fig. 2). The primary heat exchanger consisted of 3.5 m of 3.18 mm o.d. stainless-steel tubing coiled inside a 1.5-l capacity plastic vessel, through which a stream of water from the thermocirculator flowed. The secondary heat exchanger consisted of two

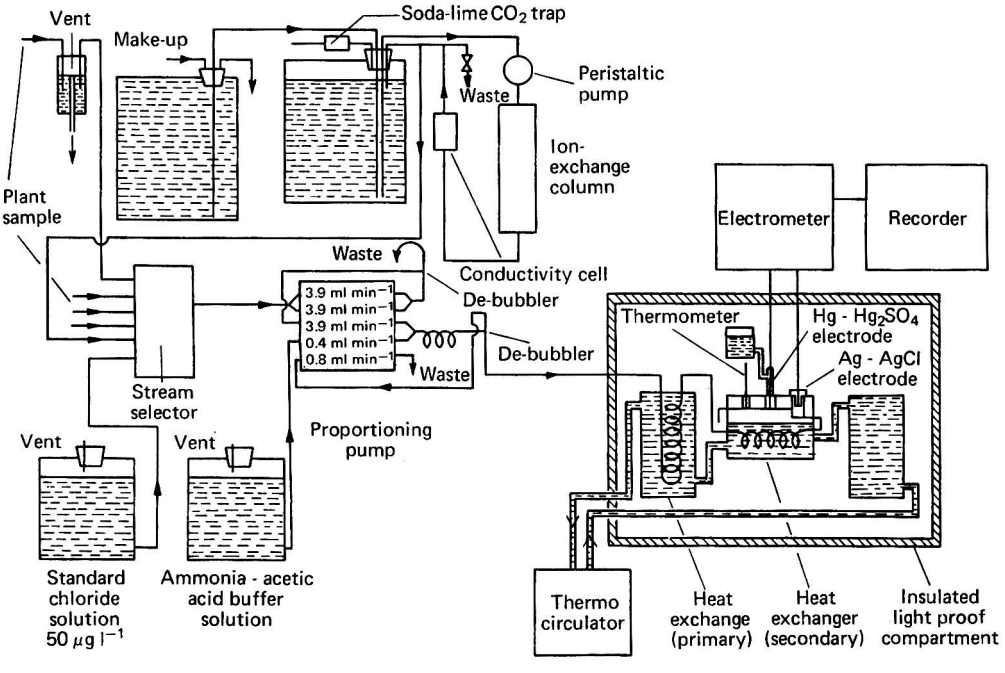


Fig. 2. Flow diagram of on-stream analyser for the determination of chloride in feed and boiler water.

2-m lengths of the same tubing wound in 25 mm diameter coils. These coils were mounted in two Perspex compartments (through which the water from the thermocirculator also flowed), cemented one on either side of the flow cell. The primary and secondary heat exchangers, together with the flow cell, were housed in a light-proof and heat-insulated metal box. This arrangement was necessary in order to obtain stability of temperature and freedom from photoelectric interference at the silver - silver chloride electrode.

Controlled-temperature circulating water

A Churchill Chiller Thermocirculator (Churchill Instruments Co. Ltd., Perivale, Middlesex) was used to control the temperature of the water circulating through the heat exchangers. A 5-l insulated polythene bottle was inserted into the circulating system immediately after the outlet from the thermocirculator in order to reduce any temperature fluctuations (not shown in Fig. 2). The water passed from this last bottle through the following series of vessels contained in the light-proof, heat-insulated box: firstly, through a 2.5-l capacity polythene bottle, acting as a second thermal buffer, then through the secondary and primary heat-exchange vessels (described above) before returning to the circulator (see Fig. 2).

The temperature was controlled at a thermoregulator setting on the Thermocirculator such that the desired temperature (usually 10 °C) was indicated on a thermometer placed in the flow cell. The variation in temperature, when measured by a resistance thermometer in the same location, was less than ± 0.1 °C. The control of the sample temperature within these limits is of great importance.

Reagents

Low chloride content blank water. The blank water system was designed for the production of the relatively large volume of high-purity water that was needed for the preparation of standard chloride and buffer solutions. Under normal circumstances the water was circulated continuously when the apparatus was in use.

Two 40-l polythene aspirators were connected by a nylon siphon tube; the water in one aspirator was used as a reservoir for the system, while the contents of the other were circulated round a purification loop. De-ionised water was used to charge the reservoir. The water was pumped from one polythene aspirator to the top of a mixed-bed ion-exchange column at a rate of 100 ml min^{-1} by a peristaltic pump (Watson and Marlow Ltd., Penryn, Cornwall). The ion-exchange column consisted of an intimate mixture of 500 ml of Zerolit 325 in the H^+ form (Zerolit Ltd., Isleworth, Middlesex) and 500 ml of Zerolit FF (ip) in the OH^- form (Zerolit Ltd.). An in-line conductivity cell (cell constant 0.1; Electronic Instruments Ltd.) was used to monitor the effluent from the column, which was returned to the 40-l aspirator. Water was abstracted from this system immediately after the ion-exchange column, at which point its conductivity had fallen to within the range 0.06–0.07 $\mu S\ cm^{-1}$ at 25 °C.

Ammonium acetate buffer solution. A 114-ml (± 1 ml) volume of analytical-reagent grade acetic acid was mixed with approximately 200 ml of water, after which 55 ml (± 1 ml) of ammonia solution (sp. gr. 0.88) were added. The solution was made up to 1 l with water.

Standard chloride solutions. A solution containing 1 000 $\mu g\ ml^{-1}$ of chloride was prepared from dried analytical-reagent grade sodium chloride. This solution was subsequently diluted ten-fold to give a 100 $\mu g\ ml^{-1}$ standard solution, from which chloride solutions in the concentration range 10–150 $\mu g\ l^{-1}$ were prepared by appropriate dilution with blank water.

Procedure

When the conductivity of water in the blank-water system had fallen to approximately 0.06–0.07 $\mu S\ cm^{-1}$ it was used to prepare the buffer and standard chloride solutions. After the buffer solution had been prepared, a sample stream was manually selected to run through the analysis circuit. In most instances the blank water was used and a period of 4–6 h was required before a stable response was recorded. This period probably reflects the time required for the temperature of the system to stabilise. When the response was stable the channel selector was switched to automatic and, under normal operating conditions, four samples and two chloride standard lines were connected to the inlet of the selector. Each of the six channels was sampled in turn for 20 min and the cycle was then repeated. The sample temperatures were in the range 15–30 °C when they reached the constant-head device.

Results

Calibration Graph at Low Chloride Levels

The linearity of a graph of e.m.f. *versus* chloride concentration, predicted by equation (3) for solutions having chloride concentrations less than $\sqrt{K_s}$, was investigated at 25 °C by using the same cell and buffer as are described under Experimental but with a simplified heat-exchange system[4] because the operating temperature was close to ambient laboratory temperature. The response to five standard chloride solutions, nominally 20, 40, 60, 80 and 100 $\mu g\ l^{-1}$, was measured and a linear regression analysis of the e.m.f. *versus* concentration data gave a correlation coefficient greater than 0.99 and a slope of 2.26 mV per 100 $\mu g\ l^{-1}$.

Having verified the predicted linearity of the calibration graph using this cell and buffer system, simple ways of improving the sensitivity were considered and the most obvious was to lower the operating temperature of the system as equation (1) had indicated that the slope of the calibration graph would increase with decrease in temperature. In order to confirm this prediction the apparatus described under Experimental was used and its response to three standard chloride solutions, of nominal concentration 50, 100 and 150 $\mu g\ l^{-1}$, measured at 5, 10 and 15 °C; the results are shown in Table I.

TABLE I

OBSERVED EFFECT OF TEMPERATURE ON SENSITIVITY

	Response* (standard minus blank)/mV		
Temperature/°C	50 $\mu g\ l^{-1}$	100 $\mu g\ l^{-1}$	150 $\mu g\ l^{-1}$
5	2.24 (0.03)†	4.23 (0.06)	6.22 (0.07)
10	1.86 (0.03)	3.44 (0.02)	5.13 (0.02)
15	1.40 (0.02)	2.65 (0.04)	3.90 (0.05)

* Each result is the mean of 12 determinations.
† The figures in parentheses are the standard deviations for single determinations with 11 degrees of freedom.

The results confirmed the theoretically predicted increase in sensitivity with decrease in temperature and in subsequent work a temperature of 10 °C was chosen. This temperature gave the best compromise between an increase in sensitivity and an apparent loss of precision at the lowest temperature of 5 °C. A more extensive investigation of the response to standard chloride solutions was made at 10 °C. Six standard solutions with concentrations 25, 50, 75, 100, 125 and 150 $\mu g\ l^{-1}$ were prepared, and the e.m.f. of each was recorded for a period of 20 min. This procedure was repeated 12 times and the results are given in Table II.

TABLE II

RESPONSE TO STANDARD CHLORIDE SOLUTIONS AT 10 °C

Concentration of chloride added/$\mu g\ l^{-1}$	Mean e.m.f.,* normalised with respect to 150 $\mu g\ l^{-1}$ solution/mV	Standard deviations†	
		mV	$\mu g\ l^{-1}$
25	4.45	0.02	0.6
50	3.52	0.06	1.7
75	2.63	0.04	1.1
100	1.79	0.05	1.4
125	0.89	0.06	1.7
150	0	0.05	1.4

*Each result is the mean of 12 determinations.
† The standard deviations of a single result with 11 degrees of freedom.

From these results a correlation coefficient of greater than 0.99 and a gradient equivalent to 3.54 mV per 100 $\mu g\ l^{-1}$ were calculated.

Recovery of Chloride from Spiked Samples of Power Station Waters

A number of samples of power station waters were collected and their chloride concentrations determined as described under Procedure (at 10 °C) by connecting the sample bottles to the

selector. Ten-litre aliquots of these samples were then spiked with standard chloride solution and the chloride level in the spiked samples re-determined. The results are shown in Table III.

TABLE III

RECOVERY OF CHLORIDE FROM SAMPLES OF POWER STATION WATERS

Sample	Initial chloride concentration/$\mu g\ l^{-1}$	Chloride spike added/$\mu g\ l^{-1}$	Final chloride concentration/$\mu g\ l^{-1}$	Recovery, %
Condensed steam (A) ..	<1	5.0	5.3* (0.23)†	106.0
Condensed steam (B) ..	<1	19.7	19.5 (0.40)	98.9
Boiler water	108.0	47.2	156.9 (0.40)	103.6

* Each result is the mean of six determinations.

† The figures in parentheses are the standard deviations for single determinations with five degrees of freedom.

Effect of Other Substances

The range of potentially interfering substances investigated was limited to those expected to be present in the steam - water circuit of most power stations and, in each instance, the concentrations of these species tested were greatly in excess of those expected under normal operating conditions.

For each interfering substance, the responses to four solutions, blank water, blank water plus interfering substance, 50 $\mu g\ l^{-1}$ chloride standard and 50 $\mu g\ l^{-1}$ chloride standard plus interfering substance, were sequentially recorded for periods of 20 min each. This cycle was recorded twice for each substance. The apparent chloride concentration in each solution containing an interfering substance was calculated from a linear calibration graph based on the blank water and 50 $\mu g\ l^{-1}$ standard solution. The results are shown in Table IV.

TABLE IV

EFFECT OF OTHER SUBSTANCES AT 10 °C

Substance	Concentration of substance/mg l^{-1}	Interference effects ($\mu g\ l^{-1}$) of chloride at chloride concentrations of— 0 $\mu g\ l^{-1}$*	50 $\mu g\ l^{-1}$*
Na_2SO_4	75	1.7	3.1
Na_2HPO_4	50	1.4	2.7
NaOH	4	<1	<1
NaOH	40	4.1	3.8
Na_3PO_4	50	2.7	<1
Na_2CO_3	50	2.7	<1
NH_3	10	<1	<1
Morpholine	10	<1	<1
Cyclohexylamine	10	<1	<1
Hydrazine	1	<1	<1
H_2SO_4	10	<1	<1
Na_2SO_3	5	4.4	4.5
Fe^{2+}, Cu^{2+}, Zn^{2+}	1 + 1 + 1	−2.9	<1
Ca^{2+}, Mg^{2+}, K^+	2 + 2 + 20	1.5	<1
O_2	~4	<1	—

* If the other substances had no effect the results would have been expected to fall within the limits of 0 ± 1.2 and 50 ± 3.4 $\mu g\ l^{-1}$ at the 95% confidence level.

In most instances there was no significant interference, and in every instance except one, where an interference was detected, the effect was positive. The small effects noted for sodium hydroxide, trisodium orthophosphate and sodium carbonate could be explained by a change in the pH of the system.[3] The unexpected interference from sodium sulphate and sodium sulphite could arise from trace amounts of chloride in the reagent chemicals. However, the effects were so small, even at the excess levels of these substances added, that for most practical purposes they could be ignored.

As a precaution against interference from particulate matter, a Millipore filter (0.45 μm) was placed in the sample line. Under power station conditions the electrode flow cell was cleaned out once a month to prevent the accumulation of any particulate (colloidal) matter that might be adhering to the cell.

Application to Power Station Systems

The apparatus has been used successfully in a number of investigations requiring detailed knowledge of the chloride content of various parts of the power station's steam - water system that are not normally included in routine analyses. In one such investigation the chloride apparatus, together with a commercial sodium ion monitor, was used to monitor the inlet and outlet streams of a condensate polishing plant. An example of a recorded trace is shown in Fig. 3, in which the apparatus was set up to sample four streams (inlets A and B, outlets A and B) and two standard solutions (blank and 50 μg l^{-1}) in a 2-h cycle. The chloride levels at the outlets were approximately 2.5–3.0 μg l^{-1} greater than those of the blank water and when these values were compared with sodium-ion measurements in the same streams a significant excess of chloride was observed. In this instance the use of the chloride monitor identified the fault as an anion-exchange resin charge not performing up to design specification, a conclusion that could not have been reached from a reading of the electrical conductivity meter, which is normally used to monitor water quality at this point in the plant.

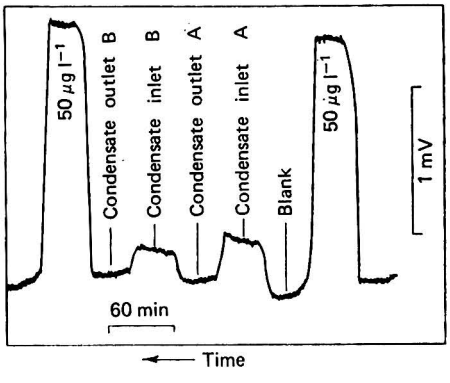


Fig. 3. Recording from condensate polishing plant.

Discussion

The slope of the experimentally determined calibration graph at 25 °C, 2.26 mV per 100 μg l^{-1}, is identical with the value found by Florence[2] at the same temperature using a solid-state Orion chloride electrode in a null-point potentiometric technique. No experimental data were available for comparison at 10 °C. A value for the sensitivity, at 10 °C, of 3.55 mV per 100 μg l^{-1} was calculated from equation (2), using $\gamma_{\pm} = 0.8$ (estimated from the Davies equation[5]) and $K_s = 5.3 \times 10^{-11}$ from Linke,[6] and this result is in excellent agreement with the experimentally determined value of 3.54 mV per 100 μg l^{-1}. The upper limits of the linear responses at 25 and 10 °C were not accurately determined but were considered to be of the order of 200 and 150 μg l^{-1}, respectively.

The response time of the apparatus was such that it took 3 min from a sample change at the selector valve to change the response of the electrode. A further 7 min were required before a plateau response of this sample was reached and normally, using a 2-h cycle for six channels, the 100% response for any constant chloride level was recorded for 13 min. If a longer period than 13 min was required, the automatic cycle could be by-passed and the selector valve operated manually for the required period.

It was observed that the response of a silver - silver chloride electrode was less stable when there was insufficient chloride (as observed by visual examination) remaining on the electrode surface to cover the silver metal substrate completely. The time taken for an

electrode to reach this condition depended on the mass of the halide precipitated on the silver during the chloridising stage of the preparation of the electrode and also on the volumes and concentrations of the solutions which had passed through the flow cell. Calculations based on the coulombs of electricity passed during the chloridising process (assuming 100% current efficiency) and the volume and chloride content of the water which had passed through the flow cell indicated that the lifetime of the electrode was considerably greater than that expected from the silver chloride solubility equilibrium. The most probable explanation is that at the rates of flow used only a small volume of solution surrounding the silver - silver chloride electrode reaches the theoretical equilibrium concentration of chloride, as expressed by equation (2). The electrodes, prepared as described previously, have a lifetime in excess of 4 months but in regular plant use they were replaced every 3 months. In preliminary experiments the performance of an Orion solid-state silver chloride - silver sulphide electrode was compared with that from a silver - silver chloride electrode but the advantages that were to be expected from the operational lifetime of the former were outweighed by its slow response at concentrations below $\sqrt{K_s}$ at 10 °C.

The apparatus has been shown to be capable of detecting changes in chloride concentration of less than 1 μg l^{-1}, but the absolute levels of chloride detected in the plant samples are partially dependent on the chloride content of the blank water that was used to prepare the calibration standards. The range of conductivity of the blank water at 25 °C (0.06–0.07 μS cm^{-1}) indicates that the chloride level is very low, and if the anionic contribution to the conductivity (other than that from the dissociation of water) was considered to be entirely from chloride ions, then the chloride content of the blank water would be in the range 0.7–3.0 μg l^{-1}. Levels of this order have been found by other workers[2] in distilled de-ionised water. This level of chloride must be kept in mind when quoting the limit of detection of the apparatus as the values determined by use of this technique are detectable changes above an unknown blank level.

This work was carried out at the Central Electricity Research Laboratories and at the Central Electricity Generating Board's North West Regional Scientific Services Laboratories, and is published by permission of the Central Electricity Generating Board.

References

1. Bardin, V. V., *Ind. Lab.*, 1962, **28**, 967.
2. Florence, T. M., *J. Electroanalyt. Chem.*, 1971, **31**, 77.
3. Torrance, K., *Analyst*, 1974, **99**, 203.
4. Torrance, K., and Parker, A. J., *Lab. Pract.*, 1969, **18**, 948.
5. Robinson, R. A., and Stokes, R. H., "Electrolyte Solutions," Second Edition, Butterworths, London, 1965, p. 232.
6. Linke, W. F., "Solubilities: Inorganic and Metal - Organic Compounds," Volume 1, Van Nostrand, Princeton, New York, Toronto and London, 1958.

Received *July 9th*, 1976
Accepted *September 6th*, 1976

Analyst, January, 1977, Vol. 102, pp. 9–16

Determination of Arsenic in Soil and Plant Materials by Atomic-absorption Spectrophotometry with Electrothermal Atomisation

A. J. Thompson and Pandora A. Thoresby

Applied Geochemistry Research Group, Department of Geology, Imperial College of Science and Technology London, SW7 2BP

A method is described for the determination of arsenic in soil and plant materials by hydride generation and atomic-absorption spectrophotometry using electrothermal atomisation. Soils are decomposed by leaching with a mixture of nitric and sulphuric acids or fusion with pyrosulphate, and plants by wet oxidation with nitric and sulphuric acids. The resultant acidic sample solution is made to react with sodium borohydride and the liberated arsenic hydride is swept into an electrically heated tube mounted on the optical axis of a simple, laboratory constructed atomic-absorption apparatus.

The advantages of high sensitivity, rapid analysis and simplicity of equipment are discussed, and the results for both types of sample material are compared with values obtained by use of the molybdenum-blue method.

Keywords: *Arsenic determination*; *soils*; *plants*; *atomic-absorption spectrophotometry*

As part of a study of the uptake of heavy metals by plants growing in contaminated soils, rapid methods for the determination of arsenic in these materials were investigated. The limitations of the Gutzeit method are well known, and it was found that the colorimetric molybdenum-blue or silver diethyldithiocarbamate methods also tend to suffer from poor precision and accuracy when applied to large numbers of samples. Reports of collaborative studies of the colorimetric methods[1,2] show clearly that considerable analytical expertise is required in order to achieve acceptable results.

The determination of arsenic by atomic-absorption spectrophotometry with thermal atomisation and with hydride generation using sodium borohydride has been described by Thompson and Thomerson,[3] and it was evident that the method could be modified for the analysis of soil and plant materials. This paper describes the chemical pre-treatment for both types of sample, details the construction of suitable apparatus and compares the results obtained with those attained by use of the molybdenum-blue method.

Arsenic hydride is generated by the reaction of sodium borohydride with an acidified sample solution and is swept into a heated furnace tube by a current of nitrogen carrier gas. The hydride is thermally decomposed on contact with the hot walls of the tube, yielding a substantial population of ground-state atoms for atomic-absorption measurement. Since this paper was prepared, Wauchope[4] has described a somewhat similar method using an adapted commercial instrument, but recorded a variable arsenic recovery from spiked soil samples.

The principal advantages of the atomic-absorption spectrophotometric technique with electrothermal atomisation are high sensitivity and good precision and accuracy, coupled with speed of analysis. The apparatus required is simple, cheap and readily constructed from components available in most well equipped laboratories. The reagent blank values are negligible when compared with those found in colorimetric methods, being equivalent to 2 ng ml^{-1} of arsenic; consequently the detection limit is at least two orders of magnitude lower. A minor disadvantage of the method is that the small dynamic range spanned by atomic absorption makes large dilutions inevitable, especially with contaminated soils, and care must be taken to minimise errors from this source.

By using the rapid atomic-absorption spectrophotometric method with electrothermal atomisation it is possible to complete 50–100 determinations per analyst-day, inclusive of sample decomposition, with a precision comparable with that of the much slower colorimetric methods and the advantage of a detection limit at the nanogram level.

Experimental

Apparatus

A schematic diagram of the apparatus is given in Fig. 1. The hydride-generator cell, with a volume of approximately 60 ml, was constructed from a sealed Quickfit B24/29 socket fitted with an angled side-arm near to its base. A Drechsel head with matching joint was used to admit nitrogen carrier gas and vent the arsenic hydride to the furnace tube. The sample was introduced by means of an Eppendorf pipette, which formed a gas-tight seal with a short length of plastic tubing attached to the side-arm.

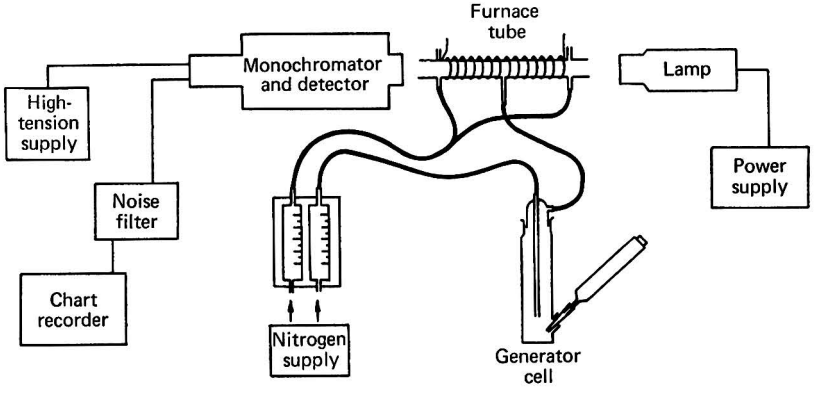


Fig. 1. Schematic diagram of apparatus.

The silica furnace tube (constructed to dimensions described by Thompson and Thomerson[3]) was wound with a heating element of flat cross-section of the type used in domestic irons. Approximately half of the length of a 750-W element, operated at 120 V from a variable-output transformer, was found to be adequate to give an optimum working temperature of 850–900 °C. Asbestos string was used in order to secure and insulate the wire as ceramic cement tended to shrink and distort the tube.

The arsenic hollow-cathode lamp, used as the source of the 193.7-nm resonance radiation, was of the rectangular, slotted-cathode type, manufactured by Hilger and Watts Ltd. The lamp current at 8 mA was supplied by an inexpensive constant-current unit obtained from Decade Electronics, Surbiton, Surrey. A Hilger D330 grating monochromator was used to select the analytical line but a cheaper prism and detector assembly would also serve the purpose. Alternatively, the furnace tube could be mounted in most commercial atomic-absorption instruments. The output signal from the detector was passed to a Servoscribe recorder via a simple noise filter and impedance matching circuit.

The hollow-cathode lamp, furnace tube and monochromator - detector were mounted on a framework of laboratory scaffolding. The components were aligned on a common optical axis until the maximum lamp emission signal was obtained. It was noticed that apparent visual alignment did not necessarily give the maximum signal; therefore, it was essential to observe the recorder response while adjusting the relative positions of the components. However, this adjustment could be carried out in a few minutes and performance was found to be adequate without the need for quartz focusing optics.

The nitrogen carrier gas and auxiliary gas flows were controlled by flow meters fitted with needle valves at rates of 1.0 and 0.5 l min^{-1}, respectively. It was noted that the carrier gas flow-rate was not critical if maintained constant, but that a reduced auxiliary gas flow-rate resulted in the combustion of hydrogen at the ends of the furnace tube, giving non-specific molecular absorption.

The most reproducible results were obtained by using a wide monochromator slit width of 0.5 mm, thus observing a large area of the furnace tube and minimising errors caused by inhomogeneity of the absorbing atom population. However, this slit width degraded the resolution (simulating the performance of a cheap monochromator) and dictated the

use of an arsenic lamp with a high line to background ratio. Fig. 2 shows the lamp spectrum at this slit width and the proximity of an unidentified line, assumed to be that of neon, to the 193.7-nm arsenic resonance line. The recommended lamp was found to have the most favourable characteristics of the four different makes tested, reducing the adverse effect of the partial background resolution on the sensitivity and calibration curvature.

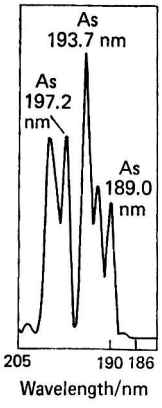


Fig. 2. Arsenic hollow-cathode lamp spectrum.

Hydride Generation

Thompson and Thomerson[3] and other workers[4,5] have reported that the absorption signal for arsenic is dependent on the oxidation state of the element prior to the hydride-generation stage. We confirmed these previous reports and also found that the ratio of signals for the two oxidation states varies with the major anion present. This behaviour could be indicative of anionic complexation of the arsenic, or simply a result of differences in the reaction kinetics for the reduction mechanism. Hydrochloric acid has been recommended as the sample matrix for the hydride-generation method,[3,4] but solutions in sulphuric acid were found to give more consistent results with only a slight reduction in sensitivity. In this work, the recommended chemical procedure resulted in a solution of arsenic(V) in 3 N sulphuric acid, and the absorption signal was 54% of that for arsenic(III) in the same solution.

The effect of other elements on generation of the hydride was tested by making standard additions of arsenic(V) to solutions containing 0.5 μg ml^{-1} of cadmium, 5 μg ml^{-1} of copper, lead and zinc, 20 μg ml^{-1} of magnesium and 50 μg ml^{-1} of calcium, iron and aluminium, these concentrations exceeding those normally encountered in diluted soil sample solutions such as are used for arsenic determination. No interference was detected and the average arsenic recovery over the calibration range was 99.85%.

The cell dimensions, carrier gas flow-rate and borohydride concentration are inter-related in that they dictate the rate at which the arsenic hydride is released to the furnace tube and thus the rise time of the absorption signal, which must lie within the response characteristics of the recorder. By use of the cell and gas flow-rates described above, the optimum conditions for quantitative generation of the hydride, together with a suitable rise time, were achieved by allowing 1 ml of 2% *m*/*V* sodium borohydride solution to react with 1 ml of a sample solution that was 3 N with respect to an acid. Because of the large excess of acid present, the precise normality of the sample solutions is not critical but should be maintained roughly constant by avoiding excessive loss of sulphur trioxide during the decomposition stage.

When generating the arsenic hydride, samples were introduced into the cell, previously charged with sodium borohydride, by means of an Eppendorf pipette. It was found to be essential to adopt a consistent technique for handling the pipette, as unduly slow or interrupted operation of the piston tended to yield poorly defined or double peaks.

Chemical Pre-treatment of Soils

According to Stanton,[6] arsenic can be determined in soils that are not not rich in sulphide

or organic matter after a rapid decomposition by fusion with potassium pyrosulphate. However, as is shown in Table I, we obtained significantly lower results when using the method of attack compared with acid leaching. Arsenic could be lost by volatilisation as the oxide, chloride or metal, especially under reducing conditions when large amounts of organic matter were present. Nevertheless, this method is included because it is very rapid and therefore worthy of consideration for routine geochemical reconnaissance work.

TABLE I

COMPARISON OF PYROSULPHATE FUSION AND NITRIC ACID - SULPHURIC ACID LEACHING FOR SOILS

Sample number	Arsenic found/μg g^{-1}		Fusion result as percentage of acid leaching result
	Fusion	Acid leaching	
146	106	145	73
154	211	304	69
159	488	687	71
160	492	646	76
176	844	876	96
186	327	399	82
191	1213	1375	88
225	65	59	110
231	374	406	92
432	982	1123	87
		Mean ..	84

Acid leaching of the soil samples by heating them to fumes of nitric and sulphuric acids, although somewhat slower than fusion, gave more reliable results, and no difficulties arose when dealing with sulphide- or organic-rich material. The results of recovery experiments using this method are shown in Table II.

Details of the two methods of decomposition are given below, the use of analytical-grade reagents and de-ionised water being assumed throughout this paper.

TABLE II

RESULTS OF RECOVERY EXPERIMENTS

The arsenic was added to 0.1 g of ultra-high purity silica and leached with nitric and sulphuric acids.

Arsenic added/μg	Arsenic found/μg	Mean arsenic found/μg	Recovery, %
0.0	0.0	0.0	—
1.0	0.99, 1.04	1.02	102
2.0	1.98, 1.62	1.80	90
5.0	4.95, 5.05	5.00	100
10.0	9.45, 9.58	9.52	95
20.0	18.25, 19.85	19.05	95
		Mean ..	96

Fusion procedure

Weigh 0.100 g of a dried soil sample, sieved to pass a 177-μm aperture, into a borosilicate glass test-tube, to it add 0.5 g of powdered potassium pyrosulphate, then mix and fuse the solids until a quiescent melt is obtained. Leach the cooled melt by warming it with 10 ml of 15 N sulphuric acid and transfer the solution into a 50-ml calibrated flask, washing the siliceous residue thoroughly with water. Oxidise the arsenic to the pentavalent state by addition of a few drops of 0.1 M potassium permanganate (just enough to colour the solution pink), cool the solution and dilute it to volume with water. Dilute an aliquot of this sample stock solution appropriately with 3 N sulphuric acid to obtain an arsenic concentration of not greater than 0.1 μg ml^{-1}.

Acid-leach procedure

Weigh 0.100 g of dried soil, sieved to pass a 177-μm aperture, into a wide-necked 250-ml conical flask and to it add 20 ml of concentrated nitric acid and 10 ml of 15 N sulphuric acid. Evaporate the solution on a hot-plate to low volume, with further dropwise additions of concentrated nitric acid if necessary in order to prevent charring of organic matter. When

the oxidation of organic matter is complete, increase the temperature until fumes of sulphur trioxide are evolved for a few minutes, but avoid an excessive loss of the acid. Cautiously dilute the cooled solution to about 25 ml with water and transfer it into a 50-ml calibrated flask, washing the siliceous residue thoroughly with water; when the solution is at room temperature, dilute it to volume with water. Dilute an aliquot of this sample solution with 3 N sulphuric acid to obtain an arsenic concentration of not greater than 0.1 μg ml^{-1}.

Chemical Pre-treatment of Plant Materials

Herbage samples were decomposed by wet oxidation with nitric and sulphuric acids. A comparison of this method with nitric acid - perchloric acid digestion was made and Table III shows that no significant differences in the results for arsenic were found. The nitric acid - sulphuric attack was therefore used because it was far more rapid, resulted in smaller insoluble residues and eliminated the need for an evaporation to dryness.

TABLE III

COMPARISON OF NITRIC ACID - SULPHURIC ACID AND NITRIC ACID - PERCHLORIC ACID MIXTURES FOR THE WET OXIDATION OF PLANT MATERIAL

Sample number	Arsenic found/μg g^{-1}		Nitric acid - perchloric acid result as percentage of nitric acid - sulphuric acid result
	Nitric acid - sulphuric acid	Nitric acid - perchloric acid	
155	5.9	4.9	83
167	4.6	3.2	70
179	6.4	6.8	106
194	2.4	2.2	92
202	4.3	4.3	100
221	1.3	1.2	92
228	1.4	1.7	121
230	6.6	6.2	94
232	1.6	1.8	113
244	2.3	2.4	104
		Mean ..	98

Residues from the wet oxidation with nitric and sulphuric acids typically contained more than 98% of silica and represented less than 2% of the dry mass of the sample. None of the residues examined was found to contain any detectable arsenic.

Wet oxidation procedure

Weigh 2.00 g of dried, finely milled sample into a wide-necked conical flask and add 20 ml of concentrated nitric acid and 10 ml of 15 N sulphuric acid. Rapidly evaporate the suspension on a hot-plate to the point of incipient fumes of sulphur trioxide, then begin the cautious dropwise addition of concentrated nitric acid in order to complete the oxidation and control any charring of organic matter. When the solution no longer darkens on ceasing the addition of nitric acid, allow it to fume for a further few minutes but avoid an excessive loss of sulphur trioxide. Cautiously dilute the cooled solution to about 25 ml with water, transfer it into a 50-ml calibrated flask, washing the residue thoroughly with water, and, when cool, dilute it to volume with water. Dilute an aliquot of this sample stock solution appropriately with 3 N sulphuric acid to obtain an arsenic concentration of less than 0.1 μg ml^{-1}.

Arsenic Determination

Before beginning the analysis of a batch of samples the apparatus was switched on and allowed to stabilise for 10–20 min. During this time the alignments of the hollow-cathode lamp, furnace tube and the monochromator were checked, and the gas flow-rates adjusted as has been previously described. The reference emission signal from the lamp (*i.e.*, the 193.7-nm resonance line) was then selected at the monochromator and displayed on the chart recorder.

The cell was charged with 1 ml of freshly prepared sodium borohydride reagent and the Drechsel head replaced. An Eppendorf pipette containing 1 ml of sample solution, or calibration standard, was then inserted into the side-arm seal. After allowing a few seconds for the ingressed air to be swept from the system, (as indicated by the recorder pen returning to the maximum emission signal datum), the sample was injected into the cell. The absorption

signal was instantaneously observed as a pulse at the recorder. The cell was then carefully rinsed with water and recharged with borohydride ready for the next sample.

Calibration was carried out by injecting solutions in 3 N sulphuric acid containing known amounts of arsenic within the range 0.005–0.10 μg ml^{-1}. It was normal practice to precede and follow each batch of samples with a complete series of calibration standards and reagent blanks. Further calibration solution injections were interspersed with the samples and the averaged measurements of each standard used to prepare a calibration graph. Fig. 3 shows typical calibration standard traces and an averaged calibration graph corrected for a reagent blank.

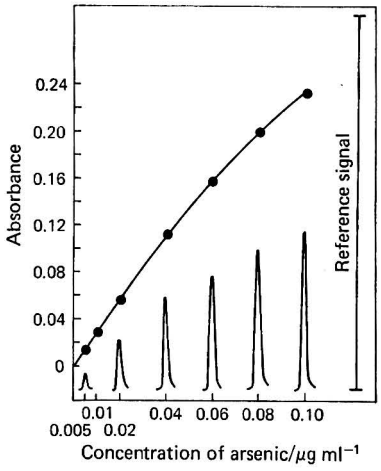


Fig. 3. Typical calibration standard traces and averaged graph.

The absorption signals were measured as peak heights from the recorder traces and calculated as percentages of the reference signal. The percentage absorptions, after blank correction, were converted into absorbances and the calibration graph drawn. Blank corrections were also made to the sample determinations.

Discussion of Results

The sensitivity (calculated as 1% absorption) of the rapid atomic-absorption spectrophotometric method with electrothermal atomisation was found to be 0.002 μg ml^{-1} of arsenic, with a detection limit (2σ) of 0.001 μg ml^{-1}. These figures are for the laboratory-constructed apparatus and could, no doubt, be improved upon.

The results of precision studies are given in Table IV. Those for calibration standards

TABLE IV

RESULTS OF PRECISION STUDIES

Method	Sample	Arsenic concentration: μg ml^{-1}	Arsenic concentration: μg g^{-1}	Number of measurements	Relative standard deviation, %
Atomic-absorption spectrophotometry with electrothermal atomisation ..	Calibration standards	0.01		9	10.7
		0.06		9	4.8
		0.10		9	7.3
	Plant material No. 478		0.79	10	12.7
	Soil No. 3003		11.0	10	12.7
Molybdenum blue ..	Soil No. 3003		10.8	6	11.4
	Soil No. 162		155.0	6	12.2

demonstrate that, as would be expected for an atomic-absorption method, the relative standard deviation is lowest near the mid-point of the calibration range. These results were calculated from absorbances measured over a period of 3 h and thus represent a realistic in-batch instrumental drift. The precisions obtained for the soil and herbage samples were identical and not significantly higher than those found for the two soils analysed by the molybdenum-blue method. These results are from one-off measurements of the replicates, as would be the case when exploiting the extreme speed of the method. However, the actual determination stage is so rapid compared with the sample attack and calculation of results that several injections of each sample can be made and the peak heights averaged for calculation without drastically reducing productivity. By averaging three injections, relative standard deviations of less than 5% have been obtained.

The lack of reliable data for arsenic in the available reference materials dictated that the accuracy of the method was best assessed on the basis of recovery experiments and by comparison of the atomic-absorption spectrophotometry with electrothermal atomisation results with those obtained by use of the molybdenum-blue method. In Table II the results of the recovery experiments are listed; the average arsenic recovery of 96% indicates a small negative bias. The comparison of results shown in Table V reveals that the values obtained by atomic-absorption spectrophotometry with electrothermal atomisation tend to be higher than those determined by the molybdenum-blue method. This phenonemon is believed to reflect the greater inherent accuracy of the atomic-absorption method rather than a systematic bias; the somewhat erratic recoveries of arsenic observed in the collaborative reports on the colorimetric method, referred to in the introduction, confirm this view.

TABLE V

COMPARISON OF RESULTS FOR SOILS AND PLANT MATERIALS BY ATOMIC-ABSORPTION SPECTROPHOTOMETRY WITH ELECTROTHERMAL ATOMISATION AND MOLYBDENUM-BLUE METHODS

		Arsenic found/μg g^{-1}	
Sample	Sample number	Atomic absorption	Molybdenum blue
Soil	152	299	238
	156	375	305
	157	293	259
	162	189	155
	164	103	71
	178	467	449
	186	207	172
Plant material ..	155	5.9	5.0
	167	4.6	4.4
	179	6.4	5.5
	194	2.4	2.3
	202	4.3	3.9
	221	1.3	1.1
	228	1.4	1.4
	230	6.6	5.1
	232	1.6	1.7
	244	2.3	3.6

Conclusions

The atomic-absorption spectrophotometric method with electrothermal atomisation was found to have considerable advantages of speed, sensitivity and accuracy over the colorimetric methods that are commonly used to determine arsenic in soils and plant materials. This atomic-absorption technique is eminently suited to the routine analysis of agricultural and geochemical samples and does not require the high degree of analytical expertise demanded by the colorimetric methods.

The cheaply constructed apparatus makes the method a viable proposition even for small-scale surveys, and the same basic equipment has potential use for the determination of other elements for which the analytical use of volatile hydrides has been described,[3] such as antimony, bismuth, germanium, lead, selenium, tellurium and tin. Its use for more than one

element would justify the inclusion of a logarithmic converter unit with the chart recorder, thus avoiding the need to measure percentage absorptions, which would simplify the calculation of results.

This work forms part of a continuing research programme currently supported by the Agricultural Research Council, under the general direction of Professor J. S. Webb and Dr. I. Thornton.

References

1. Analytical Methods Committee, *Analyst*, 1960, **85**, 629.
2. Analytical Methods Committee, *Analyst*, 1975, **100**, 54.
3. Thompson, K. C., and Thomerson, D. R., *Analyst*, 1974, **99**, 595.
4. Wauchope, R. D., *Atom. Absorption Newsl.*, 1976, **15**, 64.
5. Siemer, D. D., Koteel, P., and Jariwala, V., *Analyt. Chem.*, 1976, **48**, 836.
6. Stanton, R. E., "Rapid Methods of Trace Analysis," First Edition, Edward Arnold, London, 1966, p. 39.

Received *June 22nd*, 1976
Accepted *September 20th*, 1976

Analyst, January, 1977, Vol. 102, pp. 17–24

Determination of Noble Metals in Geological Materials by Radiochemical Neutron-activation Analysis

I. Ahmad, S. Ahmad and D. F. C. Morris

Nuclear Science Centre, School of Chemistry, Brunel University, Uxbridge, Middlesex, UB8 3PH

A method for the determination of platinum, palladium, gold and iridium in geological materials following activation with thermal neutrons is described. Radionuclides formed from the elements are separated by a scheme based largely on liquid - liquid extractions. The procedure has been applied to the analysis of US Geological Survey standard rocks and to studies of the distribution of the noble metals in lateritic nickel ores.

Keywords: *Noble metal determination*; *standard rocks*; *nickel ores*; *neutron-activation analysis*

The abundance of platinum, palladium, gold and iridium in geological materials is usually very low.[1,2] Methods for the determination of these elements have been reviewed by Beamish and Van Loon.[3–5] Neutron-activation analysis appears to be the most generally propitious method for the determination of sub-microgram amounts despite the fact that for the determination of the elements in most geological materials a procedure involving radiochemical separations is required.

A relatively rapid radioactivation procedure for the determination of platinum, palladium, gold and iridium is described; it is based on irradiation in a nuclear reactor, followed by carrier chemistry and liquid - liquid extraction, and it can be used for platinum and palladium with either beta- or gamma-ray counting.

The nuclear characteristics of platinum, palladium, gold and iridium that are relevant to activation with neutrons in a thermal nuclear reactor are summarised in Table I. Nuclear reactions appropriate for our analyses were: for platinum, $^{196}Pt(n,\gamma)^{197}Pt$; for palladium, $^{108}Pd(n,\gamma)^{109}Pd$; for gold, $^{197}Au(n,\gamma)^{198}Au$; and for iridium, $^{191}Ir(n,\gamma)^{192}Ir$ and $^{193}Ir(n,\gamma)^{194}Ir$.

The use of shorter-lived radioisotopes was ruled out because radiochemical and counting operations were performed at a considerable distance from the nuclear reactor. Possible conflicting nuclear processes that can limit the analyses are discussed later.

Experimental

Irradiation

Geological samples analysed were in the form of fine powders. Samples of material (approximately 0.4 g) were accurately weighed into silica irradiation tubes of 4 mm i.d. fitted with pure aluminium caps. A control was prepared by weighing 0.1-cm^3 aliquots of freshly diluted standard solutions of the individual noble metals into a silica ampoule of 4 mm i.d. The ampoule was sealed by fusion, the lower part being kept cool. Samples and controls were packed together with silica-wool into standard screw-topped aluminium isotope cans; four samples of powdered rock and two composite standards were the usual contents of each can.

In our work irradiations were carried out in the nuclear reactor HERALD at the Atomic Weapons Research Establishment, Aldermaston, usually with a thermal neutron flux of 2.5×10^{12} neutrons cm^{-2} s^{-1}. After irradiation for 10 h, the samples were allowed to decay overnight before delivery to our laboratory for radiochemical processing. (The radiation level 1 m from the surface of the can was usually 2.5–3.5 mR h^{-1}.)

Radiochemical Separation

A radiochemical separation scheme using isotope carriers and hold-back carriers was employed. Extraction and purification of gold(III) chloride was achieved by a sequence

TABLE I

NUCLEAR DATA FOR THERMAL NEUTRON ACTIVATION OF PLATINUM, PALLADIUM, GOLD AND IRIDIUM[6]

Target nuclide	Natural abundance, %	Isotopic activation cross-section/barn	Product of thermal neutron activation	Half-life of product	Major radiations emitted and energy*/MeV
^{190}Pt	0.012 7	~150	^{191}Pt	3.0 d	EC; γ 0.042–0.62
^{192}Pt	0.78	2	^{193}Pt^{m}	4.4 d	IT e$^-$ (γ) 0.136; e$^-$ (γ) 0.013
		<14	^{193}Pt	0.3–500 yr	EC
^{194}Pt	32.9	0.09	^{195}Pt^{m}	4.1 d	IT e$^-$ (γ) 0.130; e$^-$ γ 0.031, 0.099
^{196}Pt	25.2	0.05	^{196}Pt^{m}	1.3 h	IT 0.35 (β^-; γ 0.28)
		0.9	^{197}Pt	20 h	β^- 0.67 max. γ Au X-rays 0.077 (20%), 0.19
^{198}Pt	7.19	0.03	^{199}Pt^{m}	14.1 s	IT γ (e$^-$) 0.39; e$^-$ (γ) 0.032
		4	^{199}Pt	30 min	β^- 1.69 max. γ 0.074–0.96 Parent 3.15-d ^{199}Au
^{102}Pd	0.96	4.8	^{103}Pd	17 d	EC; γ 0.053 (others)
^{106}Pd	27.3	0.29	^{107}Pd^{m}	21 s	IT γ e$^-$ 0.21
^{108}Pd	26.7	0.2	^{109}Pd^{m}	4.7 min	IT γ e$^-$ 0.188
		12	^{109}Pd	13.5 h	β^- 1.028 max. Parent 40-s ^{109}Ag^{m}
^{110}Pd	11.8	0.04	^{111}Pd^{m}	5.5 h	IT e$^-$ γ 0.17; β^-; (γ)
		2	^{111}Pd	22 min	β^- 2.2 max.; (γ) Parent 74-s ^{111}Ag^{m} and 7.5-d ^{111}Ag
^{197}Au	100	98.8	^{198}Au	2.70 d	β^- 0.96 (others) γ 0.412 (others)
^{191}Ir	37.3	0.3	^{192}Ir^{m2}	>5 yr	IT 0.16 (to 74-d ^{192}Ir)
		250	^{192}Ir^{m1}	1.45 min	IT e$^-$ (γ) 0.058; (β^-)
		750	^{192}Ir	74.2 d	β^- 0.67 max.; γ 0.317, 0.468, 0.296 (others) EC
^{193}Ir	62.7	130	^{194}Ir	17.4 h	β^- 2.24 max. γ 0.328 (others)

* EC = orbital electron capture; IT = isomeric transition (decay from an excited metastable state to a lower state).

of liquid - liquid distribution steps using diethyl ether as extractant.[7,8] Gold was finally precipitated as the metal by addition of oxalic acid and was counted in that form. The average chemical yield was approximately 95%. Palladium(II), platinum(IV) and iridium(IV) were extracted from aqueous chloride solutions by use of di-n-heptyl sulphoxide in 1,1,2-trichloroethane.[9] The platinum(IV) and iridium(IV) were returned into an aqueous medium by back-washing with water and, following the removal of cations by extraction with dinonylnaphthalenesulphonic acid in heptane,[10] they were reduced successively by use of mercury(I) chloride.[11] Samples were counted as metals; the chemical yield for platinum was generally about 90% and that for iridium about 80%. Palladium(II), which was not removed from the diheptyl sulphoxide in trichloroethane extract by washing with water, was back-extracted with 10% aqueous dimethylamine.[9] The radiochemically purified palladium(II) was precipitated and mounted for counting as the butane-2,3-dione dioximate (dimethylglyoximate). The chemical yield was approximately 85%.

Separations of platinum and palladium from the irradiated materials were made first, because of the relatively short half-lives of platinum-197 and palladium-109. The next separation was that of gold and finally the iridium was isolated. With a single operator, radiochemical separation and counting of platinum and palladium were always achieved within 6 h of the delivery of the irradiated samples from Aldermaston.

Measurement of Radioactivity

The following sets of apparatus were used for counting the final precipitates: a thin end-window (1.7 mg cm^{-3}) Geiger - Müller counter, Model EHM2S, for measurement of β-activities; a sodium iodide (thallium) scintillation detector, diameter 3 in and 0.25 in thick, with a beryllium window 0.2 mm thick, for counting soft gamma-rays and X-rays; and a standard

sodium iodide (thallium) crystal, diameter 3 in and 3 in thick, for general gamma-ray counting. Both of the scintillation counters were used in conjunction with a Packard Spectrazoom 800-channel pulse-height analyser.

A coaxial germanium (lithium) detector, diameter 42.5 mm, P-core diameter 11 mm, together with an Intertechnique Didac 4096-channel analyser, was used for measurement of the more active gamma-emitting samples and also for confirmations of radiochemical purity. The system resolution was better than 2.5 keV (FWHM) and the peak to Compton ratio was better than 22:1, both for the 1.332-meV peak of cobalt-60.

Reagents

Carrier solutions

Gold, 10 mg cm^{-3} *as Au(III) in concentrated hydrochloric acid.*
Platinum, 10 mg cm^{-3} *as Pt(IV) in concentrated hydrochloric acid.*
Palladium, 10 mg cm^{-3} *as Pd(II) in concentrated hydrochloric acid.*
Iridium, 10 mg cm^{-3} *as Ir(IV) in concentrated hydrochloric acid.*
Silver, 20 mg cm^{-3} *as Ag(I) in* 0.1 M *nitric acid.*

The concentrations of metals in these solutions were confirmed by gravimetric methods.

Standard solutions for controls

These solutions were usually prepared from the carrier solutions by dilution of 1-cm^3 aliquots to 1 dm^3 with water in a calibrated flask, care being taken to ensure homogeneous mixing. Portions of such diluted solutions were weighed into the silica irradiation vessels immediately after preparation. The specific gravity of each solution was determined experimentally.

General reagents

Hydrochloric acid, 6, 4, 2.9, 1.4 *and* 0.5 M.
Nitric acid, concentrated.
Oxalic acid solution, 5% *m/V.*
Dinonylnaphthalenesulphonic acid solution, 0.5 *g-equiv* dm^{-3} *in heptane.*
Diethyl ether. Pre-equilibrate by shaking with 6 M hydrochloric acid.
Dimethylamine solution, 10% *m/V.*
Dimethylglyoxime solution, 1% *m/V in ethanol.*
Sodium peroxide, powder.
Mercury(I) nitrate.
Sodium bromide.
Potassium iodide.
Bromine solution in concentrated hydrochloric acid, saturated.
Diheptyl sulphoxide solution, 1 M *in trichloroethane.*[9]

Detailed Radiochemical Procedure

Sample dissolution and addition of carriers

Remove the aluminium cap from the irradiation tube and transfer the activated geological sample into a nickel crucible. Add 2.5 g of sodium peroxide and mix thoroughly. Cover the mixture with a thin layer of sodium peroxide powder. Place the lid on the crucible and heat at 480–500 °C in a muffle furnace for approximately 12 min. Remove the crucible from the furnace and cool it by immersing the outside walls in a beaker of water. Detach the cooled sintered cake from the crucible and transfer it into a 250-cm^3 beaker containing 1 cm^3 of palladium, 2 cm^3 each of gold, platinum and iridium and 1 cm^3 of silver carrier solutions; cover the beaker immediately with a watch-glass. Add 5 cm^3 of concentrated hydrochloric acid to the crucible and loosen any possibly adhering material by scraping with a glass rod. Heat the crucible and contents for a few minutes and transfer the acidic solution to the beaker containing the sintered cake and carrier solutions. Rinse the crucible with a small amount of water and add the washings to the contents of the beaker. Evaporate this solution to a low bulk, add 15 cm^3 of aqua regia and evaporate almost to dryness on a hot-plate. Add two 10-cm^3 portions of concentrated hydrochloric acid and again evaporate to a small bulk. Take up the residue in 10 cm^3 of 6 M hydrochloric acid and filter off the precipitate of silica. Reject the silica to active waste storage.

Radiochemical separations

1. Extract gold(III) from the final solution obtained above with three successive 10-cm³ portions of diethyl ether. Combine and retain the organic layers for the purification of gold step.
2. Extract platinum(IV), iridium(IV) and palladium(II) from the aqueous phase with three successive 10-cm³ portions of the diheptyl sulphoxide reagent (agitation of the phases together should not be continued for more than 1 min).
3. Combine the organic extracts and return platinum(IV) and iridium(IV) to an aqueous solution by shaking with three 10-cm³ portions of water. Palladium(II) remains in the organic phase.
4. To remove base metals from the aqueous phase, extract with two successive 10-cm³ portions of the dinonylnaphthalenesulphonic acid reagent (do not shake too vigorously). Retain the aqueous phase for the platinum and iridium purification steps.

Purification of palladium. Strip the palladium from the trichloroethane phase of step (3) by agitating with three successive 10-cm³ aliquots of 10% dimethylamine solution. Discard the organic layer. Acidify the aqueous solution with hydrochloric acid and add 1 cm³ of silver carrier solution, filter and discard the precipitate of silver chloride. To the filtrate add dimethylglyoxime reagent dropwise, centrifuge and discard the supernatant liquid. Dissolve the palladium dimethylglyoximate by the addition of 1 cm³ of aqua regia and evaporate to dryness. Add 2 cm³ of concentrated hydrochloric acid and reduce the solution to a low bulk. Repeat the addition of hydrochloric acid and evaporation. Add 1 cm³ of gold carrier solution and warm to reduce to low bulk. Dissolve in 10 cm³ of 6 M hydrochloric acid and extract the gold(III) with two successive 10-cm³ portions of diethyl ether reagent. Evaporate the aqueous phase to low bulk and dissolve in 50 cm³ of 0.5 M hydrochloric acid. Add ethanolic dimethylglyoxime solution dropwise to re-precipitate the palladium, centrifuge and discard the supernatant liquid. Wash the precipitate twice with small portions of water and then twice with 95% ethanol and transfer the precipitate as a slurry with 95% ethanol on to a weighed aluminium counting tray by using a transfer pipette. Make sure that the distribution of the precipitate on the tray is uniform. Dry under an infrared lamp at 110 °C, cool and weigh in order to determine the chemical yield.

Purification of platinum. Evaporate the solution from step (4) to a low bulk and add 2.9 M hydrochloric acid to make the volume up to about 50 cm³. Add 1 cm³ of silver carrier solution, filter and discard the precipitate of silver chloride. Boil the solution and add a freshly prepared slurry of mercury(I) chloride.[11] Boil the solution for a further 10 min and collect the precipitate on a filter-paper. Retain the filtrate for the purification of iridium step, and dissolve the precipitate by washing with hot brominated hydrochloric acid into a clean beaker. Evaporate the solution to about 2 cm³ and dilute to 50 cm³ with 2.9 M hydrochloric acid. Re-precipitate the platinum with more freshly prepared mercury(I) chloride slurry, filter the precipitate on an ashless Whatman No. 541 filter-paper and wash with hot 2% hydrochloric acid. Heat the paper and precipitate in a porcelain crucible in a fume cupboard in order to burn off the filter-paper and volatilise the mercury, then ignite the crucible and contents at 900 °C in an electric muffle furnace. Transfer the metallic platinum to a weighed aluminium counting tray and determine the chemical yield gravimetrically.

Purification of gold. Wash the diethyl ether extract of gold(III) from step (1) with three successive 10-cm³ portions of 1.4 M hydrochloric acid and discard the aqueous phases. To the organic phase add 20 cm³ of 5% oxalic acid solution and heat on a water-bath at 70–80 °C until the whole of the organic layer has evaporated. Filter the precipitate of gold on an ashless filter-paper and wash with 4 M hydrochloric acid, water and ethanol. Ignite in a weighed silica crucible at 800 °C, cool and transfer the metallic gold to a tared aluminium tray for activity measurements; determine the yield by weighing.

Purification of iridium. After the precipitation of platinum with mercury(I) chloride, iridium is left in solution. Evaporate the solution to low bulk and then make it up to 100 cm³ with 2.9 M hydrochloric acid. Add 10 g of sodium bromide and 5 g of potassium iodide. Boil the solution and add an excess of freshly prepared mercury(I) chloride slurry. Boil until the colour changes from brown to black. Allow the precipitate to settle and filter it on a Whatman No. 541 filter-paper, ignite the paper and precipitate in a silica crucible at 800 °C and then reduce the residue in an atmosphere of hydrogen at that temperature.

Transfer the iridium metal to a weighed aluminium counting tray for measurement of the activity and chemical yield.

Procedure for irradiated controls

Cut open the ampoule containing the irradiated standards and transfer the contents to a 250-cm³ beaker containing platinum, palladium, gold and iridium carrier solutions. Rinse out the ampoule several times with concentrated hydrochloric acid and transfer the washings into the same beaker. Evaporate the solution to a low bulk and take up in 10 cm³ of 6 M hydrochloric acid. Extract the gold(III) with diethyl ether and evaporate the extract with 5% oxalic acid solution to give a precipitate of gold.

The procedure for the extraction and purification of platinum, palladium and iridium is similar to that used for the rock samples except that the dinonylnaphthalenesulphonic acid treatment in step (4) is not needed.

Counting

In the analysis of materials containing very low concentrations of noble metals the final samples from palladium and platinum purifications were radiometrically assayed by Geiger - Müller counting. Radiochemical purity was confirmed by decay measurements. With richer specimens, palladium was assayed also by scintillation counting of the gamma- and X-rays from 40-s silver-109m, the daughter of palladium-109. Similarly, with the more active samples of platinum the 0.077-MeV photopeak from platinum-197 was measured. Where comparisons could be made, satisfactory agreement was achieved between results obtained from beta counting and from counting of electromagnetic radiations.

TABLE II

DETERMINATION OF NOBLE METALS IN US GEOLOGICAL SURVEY STANDARD ROCKS

Concentrations in parts per billion ($\mu g\ kg^{-1}$).

	Rock								
	Diabase W-1			Peridotite PCC-1			Dunite DTS-1		
Element	Found	Literature value	Reference	Found	Literature value	Reference	Found	Literature value	Reference
Au ..	5.4, 4.1,	3.6 ± 0.4	12	3.3, 3.3,	0.63, 0.73	15	1.1, 0.9$_4$,	0.45–0.98	15
	4.3, 5.6,	3.7	13	3.1, 3.0,	0.73, 1.3	22	1.1, 1.1,	0.6	24
	4.2, 5.5,	4.2 ± 0.8	14	3.1, 2.9,	0.8	23	1.1, 1.1,	0.8	23
	4.1, 4.3,	4.6 ± 0.7	15	2.7, 2.6	0.8 ± 0.5	24	1.1, 1.2	0.8	13
	4.7, 4.6,	5.8 ± 0.2	16	(Av. 3.0 ± 0.3)	1.42, 3.14	25	(Av. 1.1 ± 0.1)	0.8 ± 0.2	14
	5.0, 4.8	4.6 ± 0.7	17		1.6	13		0.85, 0.97	25
	(Av. 4.7 ± 0.5)	4.8 ± 1.1	18		3.2 ± 1.2	14		1.4 ± 0.5	17
					3.4 ± 1.4	17		0.72 ± 0.10	18
					0.67 ± 0.10	18			
Pt ..	17, 17,	12	13	4.9, 4.9,	3.5 ± 1.7	22	3.7, 4.8,	3	13
	17, 17,	16, 22	19	4.4, 4.7,	8	13	4.7, 4.9,	10–11	19
	17, 17	17 ± 3	17	4.8, 5.1	5.1 ± 1.5	17	5.1, 5.2	5	17
	(Av. 17.0 ± 0.3)	10	18	(Av. 4.8 ± 0.3)	13.5 ± 2.9	18	(Av. 4.7 ± 0.6)	10 ± 5	18
Pd ..	18, 18,	11.5 ± 0.3	16	6.4, 6.1,	5.9 ± 0.6	22	2.5, 2.6,	1	13
	18, 17	14	19	6.0, 5.7,	6.0 ± 0.3	22	2.5, 2.7,	6.1 ± 1.3	17
	(Av. 17.7 ± 0.4)	18.5 ± 1.4	20	6.8, 6.7,	7	19	2.8, 2.6	2	18
		25	13	6.3, 6.3	13	13	(Av. 2.6 ± 0.1)		
		16 ± 2	17	(Av. 6.3 ± 0.4)	3.0 ± 0.1	17			
		19.3 ± 3.0	18		7.1 ± 0.4	18			
Ir ..	0.33, 0.30,	0.26 ± 0.02	16	6.0, 5.9,	2.6 — 3.6	26	1.01, 0.86,	0.34—4.5	26
	0.32, 0.28,	0.28	13	6.2, 6.1,	5 ± 2	27	0.99, 1.17,	0.5	23
	0.29, 0.30	0.34 ± 0.09	21	5.7, 5.9,	5.1 ± 2.2	21	0.99, 1.06,	0.5 ± 0.1	27
	(Av. 0.30 ± 0.02)	0.26 ± 0.19	17	6.1, 6.3	5.2	13	0.92, 1.03	0.51, 1.51	29
		2.3 ± 0.4	18	(Av. 6.0 ± 0.2)	5.6	28	(Av. 1.0 ± 0.1)	0.56	28
					5.7	29		0.58 ± 0.21	21
					6.6	23		0.65	30
					6.65	29		1.0	13
					6.7, 7.1	30		0.60 ± 0.09	17
					3.5 ± 0.4	17		0.43 ± 0.06	18
					2.1 ± 0.6	18			

The 0.412-MeV gamma-rays of gold-198 were always used in the determination of gold, and for the determinations of iridium the 0.317- and 0.468-MeV gamma-rays of iridium-192 and the 0.328-MeV gamma-ray of iridium-194 were counted.

Results

Standard Rocks

Results for the analysis of samples of three US Geological Survey standard rocks are given in Table II. Magnitudes obtained by other workers using a variety of methods are listed for comparison. The agreement of our results with values published previously is mostly satisfactory, particularly when one takes into account doubts as to the homogeneity of distribution of the noble elements at the ultra-microconcentrations at which they are present in the standard rocks.[31]

Lateritic Nickel Ores

A major aim of our programme was to discover whether there was enrichment of gold, platinum, palladium and iridium in lateritic nickel ores.[32] The distribution of the elements has been determined as a function of depth through certain deposits. Results based on quadruplicate analyses of the samples are listed in Table III and the standard deviation for a single determination is included. It will be observed that there is a notable concentration, particularly of platinum and palladium, in parts of the profiles. The non-destructive neutron-activation analysis of many other elements in these deposits and the over-all geochemistry will be discussed elsewhere.

TABLE III

DISTRIBUTION OF NOBLE METALS AS A FUNCTION OF DEPTH THROUGH LATERITIC DEPOSITS

Deposit	Sample number	Depth/m	Description	Metal content/μg kg⁻¹ Au	Pt	Pd	Ir	Ni, %
La Gloria,	L1	1.5–2	Surficial laterite	11.8 ± 0.8	140 ± 4	84 ± 3	8.2 ± 0.1	1.7
Lake Izabal,	L2	2.5–3	Plastic laterite	13.8 ± 0.7	145 ± 1	79 ± 4	13.9 ± 0.4	1.9
Guatemala	L3	3.5–4	Saprolite	8.9 ± 0.1	173 ± 9	57 ± 1	15.2 ± 0.5	2.1
	L4	5.25–5.75	Boulders in saprolite zone	5.9 ± 0.4	135 ± 1	93 ± 3	13.2 ± 2.2	1.9
	L6	8–8.5	Fines of weathered rock	5.6 ± 0.4	101 ± 1	50 ± 1	7.4 ± 0.1	1.6
	L5	8–8.5	Weathered rock	4.8 ± 0.1	97 ± 1	46 ± 2	6.7 ± 0.4	1.2
	L7	10.25–10.75	Rock	3.2 ± 0.2	68 ± 2	37 ± 1	5.0 ± 0.6	0.65
New Caledonia:	BNC1	0–2	Ferricrete	30.4 ± 3.7	108 ± 9	80.0 ± 2.1	8.0 ± 0.7	0.73
Lat. 22° 17′ S	BNC2	2–7	Limonite	31.7 ± 2.2	164 ± 3	52.5 ± 3.1	5.4 ± 0.4	1.4
Long. 166° 58′ E	BNC3	7–11	Limonite	40.5 ± 0.6	151 ± 5	80.2 ± 1.2	5.2 ± 0.5	1.7
	BNC4	11–13	Limonite	11.3 ± 0.3	190 ± 5	27.5 ± 1.3	5.7 ± 0.7	1.5
	BNC5	13–14	Limonite and asbolite	8.0 ± 1.3	131 ± 2	24.6 ± 1.0	6.5 ± 0.3	1.7
	BNC6	14–17	Limonite and asbolite	9.5 ± 0.5	110 ± 3	21.5 ± 0.5	5.1 ± 0.6	3.2
	BNC7	17–20	Saprolite	6.3 ± 0.3	83 ± 2	21.0 ± 0.5	4.8 ± 0.7	1.6
Pamalea,	BIP1	11–13	Limonite and asbolite	14 ± 1.9	67 ± 3	52 ± 2	16 ± 13	1.4
Indonesia:	BIP2	13–17	Asbolite	8.5 ± 1.5	43 ± 5	45 ± 5	7 ± 1.2	2.5
Lat. 4° 10′ S Long. 121° 37′ E	BIP3	18–19	Quartz boxwork and saprolite	6.2 ± 1.9	18 ± 3	30 ± 3	5.3 ± 1.2	2.3

In order to check that significant differences in neutron self-shielding did not arise between the dilute aqueous solutions used as controls and the geological samples, some experiments were performed using portions of a given lateritic material that had been doped with known small additions of platinum or palladium. A comparison of the results shown in Table IV

TABLE IV

ANALYSIS OF NEW CALEDONIAN LATERITIC ORE BNC2 USING STANDARD ADDITIONS OF PLATINUM AND PALLADIUM

Mass of Pt added/μg	Specific activity of ^{197}Pt normalised to a fixed time/ counts per 10 min per ng Pt	Mass of Pd added/μg	Specific activity of ^{109}Pd normalised to a fixed time/ counts per 10 min per ng Pd
0.190	1 025	0.045 8	41 195
0.252	986	0.061 2	43 632
0.342	1 034	0.120 6	42 105
0.534	992		

Content of undoped samples derived on the above basis: platinum, 164 ± 17 μg kg⁻¹; palladium, 51 ± 5 μg kg⁻¹.

with the corresponding results in Table III, obtained by comparison with aqueous standards, indicates satisfactory agreement.*

Discussion

A review by Gijbels[33] deals in detail with the importance of interfering nuclear reactions in neutron-activation analysis for platinum metals. In the determination of trace amounts of the elements in samples of other noble metals (n,p) and (n,α) reactions must be taken into account,[34] but in the analysis of rocks or soils such nuclear processes do not give rise to interference of any consequence. In geological materials containing uranium in considerable excess of palladium, interference with the determination of the latter element can arise from the nuclear reaction $^{235}U(n,f)^{109}Pd$. Gijbels[33] has shown that 1 p.p.m. of uranium gives rise in this manner to an apparent palladium content of 0.002 5 p.p.b. (parts per 10^9). Uranium has been determined in all the samples investigated in the present work and the fission product contribution to the palladium-109 activity measured can be ignored. However, in the determination of palladium in granitic rocks, where the uranium to palladium ratio is much higher, this interference must be taken into consideration.[35,36]

Conclusions

The radiochemical method developed in this investigation permits a fairly rapid determination of platinum, palladium, gold and iridium. Specificity is achieved and the procedure demonstrates the practical advantage of using liquid - liquid extractions in the separation of these elements.

Considering the fact that only sub-microgram amounts of the elements have been involved, the over-all precision of results obtained is acceptable.

The authors thank INCO Limited for supplying the samples of lateritic ores. They also thank the Science Research Council for sponsoring the irradiations in the nuclear reactor at Aldermaston. An SRC studentship to one of us (I.A.) and an award from the British Council (to S.A.) are acknowledged.

References

1. Crocket, J. H., *in* Wedepohl, K. H., *Editor*, "Handbook of Geochemistry," Springer-Verlag, Berlin, 1969, Section II–1.
2. Crocket, J. H., *in* Wedepohl, K. H., *Editor*, "Handbook of Geochemistry," Springer-Verlag, Berlin, 1974, Section II–4.
3. Beamish, F. E., "The Analytical Chemistry of the Noble Metals," Pergamon Press, Oxford, 1966.
4. Beamish, F. E., and Van Loon, J. C., "Recent Advances in the Analytical Chemistry of the Noble Metals," Pergamon Press, Oxford, 1972.
5. Beamish, F. E., and Van Loon, J. C., *Min. Sci. Engng*, 1972, **4** (4), 3.
6. Lederer, C. M., Hollander, J. M., and Perlman, I., "Table of Isotopes," Sixth Edition, John Wiley, New York, 1967.
7. Irving, H., *Q. Rev. Chem. Soc.*, 1951, **5**, 200.
8. Morris, D. F. C., and Ali Khan, M., *Talanta*, 1968, **15**, 1301.
9. Lewis, P. A., Morris, D. F. C., Short, E. L., and Waters, D. N., *J. Less-common Metals*, 1976, **45**, 193.
10. Morris, D. F. C., *Chemy Ind.*, 1973, 624.
11. Lloyd, K. W., and Morris, D. F. C., *Talanta*, 1961, **8**, 16.
12. Simon, F. O., and Millard, H. T., *Analyt. Chem.*, 1968, **40**, 1150.
13. Flanagan, F. J., *Geochim. Cosmochim. Acta*, 1973, **37**, 1189.
14. Laul, J. C., Case, D. R., Wechter, M., Schmidt-Bleck, F., and Lipschutz, M. E., *J. Radioanalyt. Chem.*, 1970, **4**, 241.
15. Rowe, J. J., and Simon, F. O., *Circ. U.S. Geol. Surv.*, 1968, No. 599.
16. Crocket, J. H., Keays, R. R., and Hsieh, S., *Geochim. Cosmochim. Acta*, 1967, **31**, 1615.
17. Nadkarni, R. A., and Morrison, G. H., *Analyt. Chem.*, 1974, **46**, 232.
18. Gilbert, E. N., Veriovkin, G. V., and Mikhailov, V. A., *J. Radioanalyt. Chem.*, 1976, **31**, 365.
19. Haffty, J., and Riley, L. B., *Talanta*, 1968, **15**, 111.
20. Crocket, J. H., and Skippen, G. B., *Geochim. Cosmochim. Acta*, 1966, **30**, 129.
21. Baedecker, P. A., Chou, C. L., and Wasson, J. T., "Proceedings 3rd Lunar Science Conference," *Geochim. Cosmochim. Acta*, 1972, **2**, Special Supplement, 1343.

* However, note that for analysis of certain geological materials that contain elements as major constituents with high neutron-absorption cross-sections, *e.g.*, lepidolite, such liquid controls are likely to be unsuitable.

22. Millard, H. T., and Bartel, A. J., *in* Brunfelt, A. O., and Steinnes, E., *Editors*, "Activation Analysis in Geochemistry and Cosmochemistry," Universitetsforlaget, Oslo, 1970, p. 353.
23. Ehmann, W. D., Baedecker, P. A., and McKown, D. M., *Geochim. Cosmochim. Acta*, 1970, **34**, 493.
24. Gillum, D. E., and Ehmann, W. D., *Radiochim. Acta*, 1971, **16**, 123.
25. Green, T. E., Law, S. L., and Campbell, W. J., *Analyt. Chem.*, 1970, **42**, 1749.
26. Greenland, L. P., Rowe, J. J., and Dinnin, J. I., *Prof. Pap. U.S. Geol. Surv.*, 1971, No. 750B, B-175.
27. Das, H. A., Janssen, R., and Zonderhius, J., *Radiochem. Radioanalyt. Lett.*, 1971, **8**, 257.
28. Baedecker, P. A., Schaudy, R., Elzie, J. L., Kimberlin, J., and Wasson, J. T., "Proceedings 2nd Lunar Science Conference," *Geochim. Cosmochim. Acta*, 1971, **2**, Special Supplement, 1037.
29. Gijbels, R., Millard, M. T., Desborough, G. A., and Bartel, A. J., *in* Brunfelt, A. O., and Steinnes, E., *Editors*, "Activation Analysis in Geochemistry and Cosmochemistry," Universitetsforlaget, Oslo, 1970, p. 359.
30. Gijbels, R., and Govaerts, A., *J. Radioanalyt. Chem.*, 1973, **16**, 7.
31. Fritze, K., and Robertson, R., *in* De Voe, J. R., *Editor*, "Modern Trends in Activation Analysis," *Natn. Bur. Stand.* (*U.S.*) *Spec. Publ.*, 1969, No. 312, Volume II, 1279.
32. Boldt, J. R., and Queneau, P., "The Winning of Nickel," Methuen, London, 1967.
33. Gijbels, R., *Talanta*, 1971, **18**, 587.
34. Morris, D. F. C., and Killick, R. A., *Talanta*, 1961, **8**, 129.
35. Crocket, J. H., Keays, R. R., and Hsieh, S., *J. Radioanalyt. Chem.*, 1968, **1**, 487.
36. Crocket, J. H., *in* Brunfelt, A. O., and Steinnes, E., *Editors*, "Activation Analysis in Geochemistry and Cosmochemistry," Universitetsforlaget, Oslo, 1970, p. 339.

Received *August* 11*th*, 1976
Accepted *September* 16*th*, 1976

Analyst, January, 1977, Vol. 102, pp. 25–28

A Selective Reagent for the Spectrophotometric Determination of Palladium

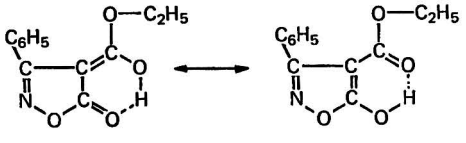
F. Corigliano, S. Di Pasquale and A. Ranieri

Istituto di Chimica Analitica, Università di Messina, 98100 Messina, Italy

A spectrophotometric method for the determination of trace amounts of palladium(II) with ethyl 3-phenyl-5-isoxazolone-4-carboxylate was developed. With this reagent, palladium forms a yellow complex, stable in the pH range 1–6.5, which is extractable into 4-methylpentan-2-ol from 0.05–4 M perchloric acid medium. The coloured complex adheres to Beer's law at 370 nm in aqueous and in 4-methylpentan-2-ol solutions, with molar absorptivities of 3.5×10^3 and 3.9×10^3 l mol^{-1} cm^{-1}, respectively. Other platinum-group metals do not interfere. In the presence of highly coloured ions, extraction with 4-methylpentan-2-ol eliminates all interferences. Cyanide ions interfere in the method.

Keywords: Palladium determination; spectrophotometry; ethyl 3-phenyl-5-isoxazolone-4-carboxylate reagent

Ethyl 3-phenyl-5-isoxazolone-4-carboxylate (EPI) (**I**) is a chelating agent that has already been used for the spectrophotometric determination of iron.[1] It reacts with palladium(II) to give a yellow complex, which is extractable into 4-methylpentan-2-ol from an acidic medium. Under suitable conditions, the reagent can be used for the spectrophotometric determination of palladium in aqueous solution and, by solvent extraction, in the presence of all other platinum metals.



I

Experimental

Reagents

Standard palladium solution, 10^{-2} M. Palladium(II) chloride was dissolved in perchloric or hydrochloric acid and the solution was standardised gravimetrically by precipitating palladium with dimethylglyoxime.[2]

Reagent solutions. A 0.5% solution of EPI in 95% ethanol was used for the determination in aqueous solution, and a 0.25% solution in 4-methylpentan-2-ol for the extraction method. The reagent itself was synthesised by the method of Stagno d'Alcontres *et al.*[3] and recrystallised from diethyl ether.

Other reagents. All other chemicals were of analytical-reagent grade. The solutions of other cations were prepared from their sulphates, nitrates or chlorides.

Procedure

Determination in aqueous solution

Place an aliquot of sample containing 30–300 μg of palladium in a 10-ml calibrated flask, add 1 ml of 0.5% EPI solution in ethanol, adjust the pH to 3–4 with sodium acetate solution and dilute to 10 ml with distilled water. Measure the absorbance at 370 nm against a reagent blank.

Determination by solvent extraction

Place an aliquot of sample, containing 13.5–135 μg of palladium, and 1 ml of 2 M perchloric acid in a glass-stoppered centrifuge tube and dilute to 5 ml with distilled water. Add

5.0 ml of the 0.25% solution of EPI in 4-methylpentan-2-ol, shake the tube for 5 min and centrifuge. Remove the organic layer and measure its absorbance at 370 nm against a 4-methylpentan-2-ol blank.

Results and Discussion

Spectral Properties and Reaction Conditions

Fig. 1 shows the absorption spectra of the yellow complex in aqueous solution and in 4-methylpentan-2-ol solution. The absorption maxima are at 340 and 350 nm, respectively, where the absorbance of the reagent solutions is not negligible. Hence, to ensure that the excess of reagent does not affect the readings, the wavelength of 370 nm is suggested for both determinations.

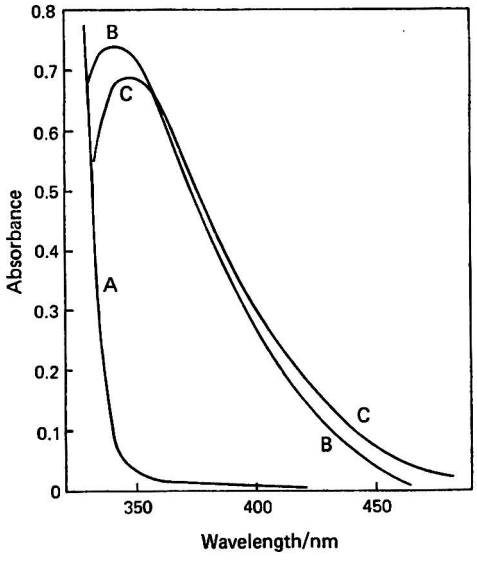


Fig. 1. Absorption spectra: A, reagent against 4-methylpentan-2-ol ([EPI] = 1×10^{-2} M); B, Pd - EPI complex against reagent in aqueous solution ([EPI] = 2×10^{-3} M); and C, Pd - EPI complex against reagent in 4-methylpentan-2-ol ([EPI] = 1×10^{-2} M). Pd concentration, 1.6×10^{-4} M.

Solutions containing fixed amounts of palladium and reagent were prepared and the effect of pH on the colour reaction was studied. In aqueous solution, it was noted that the absorbance at 370 nm was constant over the pH range 1–6.5. Use of hydrochloric instead of perchloric acid is without influence.

The yellow complex is extracted quantitatively into 4-methylpentan-2-ol from acidic solutions and the effect of various concentrations of perchloric acid on the absorbance of the extract is shown in Table I. The maximum occurs at hydrogen-ion concentrations of 0.05 M or higher. In this instance, the use of hydrochloric acid is not advantageous because it lengthens the extraction time.

The absorbance in aqueous and 4-methylpentan-2-ol solutions was studied as a function of the molar ratio of the reagent to palladium(II). A constant absorbance was obtained with a 4:1 ratio of reagent to palladium(II) in aqueous solution and 15:1 if the extraction method was used. For general purposes, a 10- or 50-fold molar excess of reagent, respectively, is therefore recommended.

The reaction between palladium and the reagent occurs immediately at room temperature and the colour of the complex is stable in both aqueous and 4-methylpentan-2-ol solutions.

The system obeys Beer's law for concentrations of palladium in the final prepared solution of up to 35 p.p.m. using an aqueous solution and up to 32 p.p.m. using a 4-methylpentan-2-ol

TABLE I

EFFECT OF CONCENTRATION OF PERCHLORIC ACID ON ABSORBANCE OF THE PALLADIUM COMPLEX IN 4-METHYLPENTAN-2-OL

Concentration of palladium(II) = 17.0 p.p.m.

Concentration of perchloric acid/M ..	4.0	1.0	0.1	0.05	0.04	0.02	0.01	0.006
Absorbance at 370 nm	0.628	0.632	0.634	0.620	0.525	0.431	0.294	0.098

solution. The sensitivities of the colour reactions according to Sandell[4] are 0.030 and 0.027 $\mu g\ cm^{-2}$ (at 370 nm), with molar absorptivities of 3.5×10^3 and $3.9 \times 10^3\ l\ mol^{-1}\ cm^{-1}$ in aqueous and 4-methylpentan-2-ol solution, respectively.

The precision of the procedures was checked by measuring the absorbance of eight samples, each containing a final palladium concentration of 14 p.p.m., through 1.0-cm cells. The mean absorbances were 0.462 in aqueous solution and 0.515 in 4-methylpentan-2-ol solution, with standard deviations of 0.004 and 0.006, respectively. The optimum concentration ranges for accurate determination, as deduced from Ringbom plots, are 3.0–30 and 2.7–27 p.p.m. in aqueous and 4-methylpentan-2-ol solution, respectively.

Composition and Stability Constant

Job's method of continuous variation and the molar ratio method indicate the formation of a 1:4 metal - ligand complex. The species extractable into 4-methylpentan-2-ol is $H_2Pd(HIS)_4$, as indicated by studies on partition equilibria.[5] The apparent stability constant and partition coefficient, calculated from spectrophotometric data, are 1×10^{23} and 4.5×10^3, respectively.[5]

Effect of Other Ions

Several ions were examined for their effect on the determination of palladium(II). The amounts of foreign ions reported in Tables II and III did not cause a deviation in absorbance of more than $\pm 2\%$ from that expected. The platinum-group metals do not form complexes with the reagent at room temperature. Platinum(II) and platinum(VI), however, give

TABLE II

TOLERANCE LIMITS IN THE DETERMINATION IN AQUEOUS SOLUTION OF 100.0 μg OF PALLADIUM IN THE PRESENCE OF FOREIGN IONS

Foreign ion	Tolerance limit/mg	Foreign ion	Tolerance limit/mg
Ni(II)	3.0	Pt(IV)	0.2
Co(II)	3.0	Rh(III)	0.2
Cu(II)	3.0	Ir(III)	0.2
Cr(III)	1.5	U(VI)*	6.0
Ba(II)	10.0	Ti(IV)	1.0
Zn(II)	10.0	Mo(VI)	1.5
Fe(III)*	5.0	V(V)	0.3

* Interference due to iron(III) and uranium(VI) was removed by adding 1 ml of 1% fluoride solution.

TABLE III

TOLERANCE LIMITS IN THE DETERMINATION BY SOLVENT EXTRACTION OF 80.0 μg OF PALLADIUM IN THE PRESENCE OF FOREIGN IONS

Foreign ion	Tolerance limit/mg	Foreign ion	Tolerance limit/mg
Rh(III)	2.3	Co(II)	8.0
Ir(III)	2.8	Cu(II)	5.0
Ru(III)	2.8	Ni(II)	5.0
Pt(IV)	3.0	Zn(II)	10.0
U(VI)	6.0	Ba(II)	10.0
Fe(III)	2.0	Cr(III)	10.0
Ti(IV)	3.0	V(V)	0.3

yellow complexes with the reagent in hot hydrochloric acid solutions. The interference due to the presence of highly coloured ions is best eliminated by extraction with 4-methylpentan-2-ol, as shown by the results in Table III. Iron(III) and uranium(VI) interfere in the aqueous solution procedure and addition of fluoride (1 ml of a 1% solution) as a masking agent is suggested. Their interference, however, is better tolerated by the extraction method, as determinations can be carried out at pH values below 1, where the complexes are dissociated.

Common anions such as borate, nitrate, phosphate, arsenate, sulphate, halide, acetate, tartrate, citrate and oxalate are tolerated at molar ratios of up to 1 000–5 000-fold relative to palladium. Cyanide ion interferes seriously.

Conclusions

Many colorimetric reagents for palladium have been reported and were reviewed by Beamish.[6,7] Considerable efforts have been made to find reagents with increased sensitivity. Nevertheless, methods with attractive sensitivities (*e.g.*, *p*-nitrosodiphenylamine, arsenazo III, "palladiazo" and ternary complexes)[8–12] usually suffer from serious interferences when additional steps (masking agents, separation procedures, buffering within narrow pH ranges) are not introduced. Whenever high sensitivity is not required (palladium contents higher than 1 p.p.m.), less sensitive but more practical methods are convenient. In this concentration range, the colorimetric method involving extraction into chloroform with dimethylglyoxime is, perhaps, the most simple and selective of those proposed to date.[13] The sensitivity of this method is constant in the pH range 1–6, but the maximum selectivity is achieved at a pH of about 1 (only gold and ruthenium interfere).[14] At this pH, however, more than one extraction step or large volumes of extractant are required for complete extraction of the palladium complex.[15]

EPI is almost three times more sensitive than dimethylglyoxime. In addition, palladium determination by means of the extraction procedure with EPI can be carried out at higher acidities without interferences (except cyanide) and with a high extraction coefficient,[5] which allows rapid, complete, single-step and low-volume extractions.

If maximum recovery is essential, it is important that no limits to the extractability with 4-methylpentan-2-ol have been observed for up to milligram amounts of palladium.

Thus, the method is highly selective for palladium extraction and determination and can be applied directly to the analysis of palladium-containing materials subsequent to the use of an acidic dissolution technique (*e.g.*, fuming with perchloric and sulphuric acids).

References

1. Corigliano, F., and Di Pasquale, S., *Talanta*, 1976, **23**, 545.
2. Charlot, G., "Les Methodes de la Chimie Analytique," Masson, Paris, 1966, p. 839.
3. Stagno d'Alcontres, G., Lo Vecchio, G., and Lamonica, G., *Gazz. Chim. Ital.*, 1961, **91**, 1005.
4. Sandell, E. B., "Colorimetric Determination of Traces of Metals," Third Edition, Interscience, New York, 1961.
5. Corigliano, F., Di Pasquale, S., and Ranieri, A., *Annali Chim.*, in the press.
6. Beamish, F. E., *Talanta*, 1965, **12**, 743.
7. Beamish, F. E., and Van Loon, J. C., "Recent Advances in the Analytical Chemistry of the Noble Metals," Pergamon Press, Oxford, 1972.
8. Ryan, D. E., *Analyst*, 1951, **76**, 167.
9. Marheuke, E. R. R., and Sandell, E. B., *Analytica Chim. Acta*, 1963, **28**, 259.
10. Sen Gupta, J. G. G., *Analyt. Chem.*, 1967, **39**, 18.
11. Pérez-Bustamante, J. A., and Burriel-Martí, F., *Analytica Chim. Acta*, 1967, **37**, 49 and 62.
12. Dagnall, R. M., El-Ghamry, M. T., and West, T. S., *Talanta*, 1968, **15**, 1353.
13. Nielsch, W., *Z. Analyt. Chem.*, 1954, **142**, 30.
14. Davis, W. F., *Talanta*, 1969, **16**, 1330.
15. Fraser, J. G., Beamish, F. E., and McBryde, W. E. A., *Analyt. Chem.*, 1954, **26**, 495.

Received *May 18th*, 1976
Accepted *July 26th*, 1976

Analyst, January, 1977, Vol. 102, pp. 29–34

Determination of Pyrimethamine, Ethopabate and Sulphaquinoxaline in Poultry Feeding Stuffs by High-performance Liquid Chromatography Using a Weak Cation-exchange Column Packing

G. B. Cox and K. Sugden

Department of Industry, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, SE1 9NQ

A method based on high-performance liquid chromatography, using a weak cation-exchange column packing, is described for the simultaneous determination of pyrimethamine, ethopabate and sulphaquinoxaline in poultry feeding stuffs. The method is applicable to mash and pelleted feeds containing at least 1 mg kg^{-1} of pyrimethamine and ethopabate and to mash feeds containing at least 5 mg kg^{-1} of sulphaquinoxaline. A comparison is made between this and other methods of analysis of these prophylactics and, with the exception of sulphaquinoxaline in pelleted feeds, good agreement is obtained. An alternative procedure is described for the determination of sulphaquinoxaline in pelleted feeds.

Keywords: *Prophylactics determination*; *poultry feeding stuffs*; *high-performance liquid chromatography*

Pyrimethamine, ethopabate and sulphaquinoxaline are coccidiostats that are added to poultry feeding stuffs at levels of the order of 5, 5 and 60 mg kg^{-1}, respectively. Official methods of analysis exist for ethopabate[1] and sulphaquinoxaline[2,3] and a procedure for the determination of pyrimethamine has also been published.[4] These procedures involve time-consuming clean-up and derivative formation techniques and, moreover, each coccidiostat has to be determined individually, as no method permits their simultaneous determination. This paper describes a method involving the use of high-performance liquid chromatography (HPLC) for the simultaneous determination of pyrimethamine, ethopabate and sulphaquinoxaline in mash feeds and of pyrimethamine and ethopabate in pelleted feeds. A method for the determination of sulphaquinoxaline in pelleted feeds is also described, but as it requires the use of a different extraction procedure, simultaneous analysis of all three drugs in such feeds is precluded.

Experimental

Materials

Ethanol, absolute.
Dimethylformamide.
Pyrimethamine BVetC.
Ethopabate BVetC.
Sulphaquinoxaline BVetC.

Pyrimethamine, ethopabate and sulphaquinoxaline standard solution. Dissolve 0.012 5 g of pyrimethamine, 0.012 5 g of ethopabate and 0.075 g of sulphaquinoxaline in ethanol and make the volume up to 50 ml in a calibrated flask.

Pyrimethamine, ethopabate and sulphaquinoxaline working solution. Dilute 1 ml of the standard solution to 50 ml with ethanol in a calibrated flask: 4 μl of this solution contain 20 ng each of pyrimethamine and ethopabate and 120 ng of sulphaquinoxaline.

HPLC mobile phase. Dissolve 68 g of potassium dihydrogen orthophosphate (analytical-reagent grade) in water. Add 100 ml of ethanol and 30 ml of 10% *V/V* analytical-reagent grade orthophosphoric acid and dilute to 1 000 ml with water in a calibrated flask.

Preparation of column packing material

Heat some silica gel (LiChrosorb SI 60, average particle size 5 μm) under reflux with 2 N hydrochloric acid for 2 h, filter, wash with water and dry it at 200 °C for 15 h. Next heat 5 g of the silica gel under reflux with 1.5 ml of 3-chloropropyltrichlorosilane in 50 ml of dry 1,4-dioxan for 4 h. Filter, wash with dry 1,4-dioxan and acetone and dry the product and heat it under reflux with 1.5 g of β-alanine in 50% aqueous alcohol for 4 h. Then filter, wash with water and dry the product.

Apparatus

The liquid chromatographic apparatus has been described elsewhere.[5] The variable-wavelength ultraviolet monitor was operated at 220 nm for all analyses except for the determination of sulphaquinoxaline in pelleted feeding stuffs, when a wavelength of 362 nm was employed. The weak cation-exchange material was packed into a stainless-steel column (150 mm $\times$ 4.6 mm i.d.) by use of a previously reported procedure.[6] The mobile phase was delivered at a flow-rate of 1 ml min^{-1}.

Procedure

Determination of pyrimethamine and ethopabate in mash and pelleted feeds and sulphaquinoxaline in mash feeds

Grind the sample to pass a British Standard 16-mesh sieve and mix it thoroughly. Weigh accurately approximately 10 g of ground sample, transfer it to a 100-ml flask and add, by means of a pipette, 20 ml of ethanol. Stopper the flask and shake it mechanically for 60 min, then filter the mixture through a Whatman No. 42 filter-paper and take 4 μl of the clear filtrate for chromatographic analysis.

Determination of sulphaquinoxaline in pelleted feeds

Grind the sample to pass a British Standard 16-mesh sieve and mix it thoroughly. Weigh accurately approximately 10 g of the prepared sample, transfer it to a 50-ml calibrated flask, add 30 ml of dimethylformamide and heat on a steam-bath for 30 min, stirring the mixture occasionally. Next remove the flask from the steam-bath, stopper it and shake it mechanically for a further 30 min. Make the contents of the flask up to volume with dimethylformamide and filter the mixture through a Whatman No. 42 filter-paper. Take 4 μl of the clear filtrate for chromatographic analysis.

Results and Discussion

Initially, chromatography was carried out on silica gel-based strong cation and anion exchangers that were synthesised in this laboratory. These columns were unsuitable for the simultaneous analysis of the coccidiostats, giving rise to inefficient chromatography and poorly resolved peaks. The weak cation-exchange material was developed especially for this work and was found to give the desired separation of components. The characterisation of this material is described elsewhere.[6] Care was taken to ensure that the feed extracts were clear and free from all solids after filtration. The presence of small particles of feed inadvertently deposited on top of the column was found to interfere with the efficiency of the chromatography and sensitivity was lost.

The concentration of the coccidiostats in the samples analysed was calculated by measurement of the chromatographic peak height after calibration of the detector response with the pyrimethamine, ethopabate and sulphaquinoxaline working solution. For all three drugs, the relationship between peak height and amount injected was rectilinear over the concentration range of interest.

Analysis of Mash Feeding Stuffs

In preliminary experiments several solvents were investigated for the simultaneous extraction of the three coccidiostats from feeding stuffs. Absolute ethanol was found to be a solvent suitable for the quantitative extraction of pyrimethamine, ethopabate and sulphaquinoxaline from mash feeds. The selection of detector wavelength was dictated by the spectroscopic properties of pyrimethamine and ethopabate, which exhibited sufficient absorption at 220 nm for their detection at the concentrations of interest. Although sulphaquinoxaline does not

show an absorption maximum at 220 nm, this wavelength was also satisfactory for the detection of the coccidiostat in mash feeds.

The method was first tested on samples of ground wheat and layers mash that had been previously medicated with pyrimethamine, ethopabate and sulphaquinoxaline. The recovery of the three prophylactics from both types of sample was satisfactory (see Tables I and II).

TABLE I

RECOVERY OF PYRIMETHAMINE, ETHOPABATE AND SULPHAQUINOXALINE FROM MEDICATED GROUND WHEAT

	Pyrimethamine		Ethopabate		Sulphaquinoxaline			
	Added/ mg kg^{-1}	Found/ mg kg^{-1}	Added/ mg kg^{-1}	Found/ mg kg^{-1}	Added/ mg kg^{-1}	Found/ mg kg^{-1}	Added/ mg kg^{-1}	Found/ mg kg^{-1}
	5	5, 5, 6, 5, 7, 5, 5, 4, 6, 4, 4, 4,	5	4, 5, 4, 4, 4, 5, 4, 5, 4, 4	60	55, 60, 55, 53, 53, 53, 53, 57, 54, 54, 62, 60	23	21, 21, 22, 23, 23, 25, 21, 21, 22, 22
Mean:		5		4		56		22
Standard deviation:	(12 results)	1.0	(10 results)	0.5	(12 results)	3.2	(10 results)	1.3

TABLE II

RECOVERY OF PYRIMETHAMINE, ETHOPABATE AND SULPHAQUINOXALINE FROM MEDICATED LAYERS MASH

	Pyrimethamine		Ethopabate		Sulphaquinoxaline	
	Added/ mg kg^{-1}	Found/ mg kg^{-1}	Added/ mg kg^{-1}	Found/ mg kg^{-1}	Added/ mg kg^{-1}	Found/ mg kg^{-1}
	5	6, 5, 5, 4, 6, 4, 5, 5, 4, 4, 5	5	4, 4, 4, 4, 4, 5, 4, 4, 4, 4, 5	60	53, 51, 55, 58, 54, 56, 55, 56, 56, 56, 54
Mean:		5		4		55
Standard deviation:	(11 results)	0.8	(11 results)	0.4	(11 results)	1.9

No interference from co-extracted material was observed, as is illustrated by chromatograms of unmedicated and spiked mashes in Figs. 1 and 2, respectively. Detection limits, 1 mg kg^{-1} for both pyrimethamine and ethopabate and 5 mg kg^{-1} for sulphaquinoxaline (based upon a 4-μl injection and calculated as three times the base-line noise), are significantly lower than the concentrations at which the drugs are normally added to feeding stuffs. Moreover, the methods of analysis for ethopabate[3] and sulphaquinoxaline[2] that are recommended by the Medicines (Animal Feeding Stuffs) (Enforcement) Regulations 1976 have limits of detection of 2 and 20 mg kg^{-1}, respectively. The HPLC method was then used for the analysis of various

TABLE III

DETERMINATION OF PYRIMETHAMINE, ETHOPABATE AND SULPHAQUINOXALINE IN COMMERCIAL MASH POULTRY FEEDING STUFFS

	Pyrimethamine found/mg kg^{-1}		Ethopabate found/mg kg^{-1}		Sulphaquinoxaline found/mg kg^{-1}	
Sample	HPLC method	Other method[4]	HPLC method	Official method[1]	HPLC method	Official method[2]
CGD diet A	5	4	—	—	67	65
	5	3	—	—	70	65
Poultry mash	—	—	—	—	25	24
	—	—	—	—	25	
Layers mash	—	—	5	6	—	—
	—	—	5		—	—
CDG diet B	—	—	6	7	—	—
	—	—	7		—	—

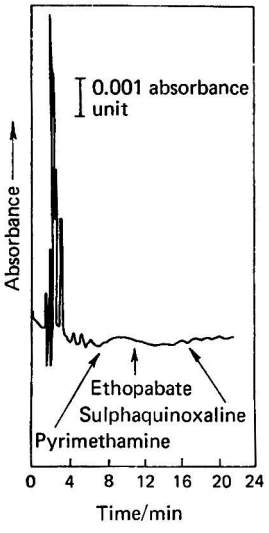


Fig. 1. Chromatogram of the ethanol extract of unmedicated layers mash. (Detector wavelength 220 nm. For other conditions, see text.)

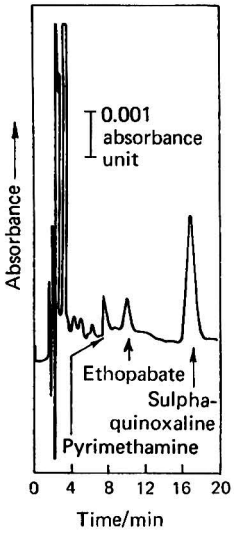


Fig. 2. Chromatogram of the ethanol extract of a poultry mash medicated with pyrimethamine (4 mg kg^{-1}), ethopabate (6 mg kg^{-1}) and sulphaquinoxaline (61 mg kg^{-1}). (Conditions as for Fig. 1.)

commercial mash feeds and the results were compared with those obtained by the established methods of analysis (Table III). The agreement between the methods was satisfactory.

Grass and fish meals are sometimes added to feeding stuffs at levels not normally exceeding 5 and 10% *m/m*, respectively. Analysis of a feed before and after the addition of the meals at these levels revealed that they had little influence upon the determination of the prophylactics (see Table IV). In order to check the possibility of chromatographic interference from other additives that may be present, a sample containing arsanilic acid (100 mg kg^{-1}), nitrovin (15 mg kg^{-1}), nifursol (56 mg kg^{-1}), dimetridazole (200 mg kg^{-1}), virginiamycin (0.01 UK megaunit kg^{-1}) and the vitamins A (14 000 I.U. kg^{-1}), D_3 (1 750 I.U. kg^{-1}) and E (5 I.U. kg^{-1}) was analysed. A small peak appeared shortly after that due to pyrimethamine but otherwise there was no interference. Pyrimethamine, ethopabate and sulphaquinoxaline

TABLE IV

DETERMINATION OF PYRIMETHAMINE, ETHOPABATE AND SULPHAQUINOXALINE IN A COMMERCIAL MASH FEEDING STUFF SPIKED WITH GRASS AND FISH MEALS

Sample	Pyrimethamine found/mg	Ethopabate found/mg	Sulphaquinoxaline found/mg
Poultry mash (untreated 5 g) ..	0.04	0.05	0.70
	0.04	0.05	0.67
Poultry mash (5 g) and grass meal (0.25 g, approximately 5%) ..	0.04	0.04	0.64
	0.04	0.05	0.64
	0.04	0.03	
	0.04	0.04	
Poultry mash (5 g) and fish meal (0.5 g, approximately 10%) ..	0.04	0.04	0.65
	0.05	0.04	0.64
	0.04	0.05	
	0.04	0.04	

are sometimes added to feeding stuffs in conjunction with amprolium so several feeds containing amprolium were analysed. The efficiency of extraction of this prophylactic was of the order of 60% and no interference with the determination of the other coccidiostats was observed. A method for the determination of amprolium by HPLC has previously been published.[7]

Analysis of Pellet Feeding Stuffs

The determination of pyrimethamine and ethopabate in pelleted poultry feeding stuffs is achieved satisfactorily by use of the procedure adopted for mash feeds. Some commercially available pelleted feeding stuffs were analysed by both this and the established methods of analysis. There was good agreement between the results (see Table V).

TABLE V

DETERMINATION OF PYRIMETHAMINE, ETHOPABATE AND SULPHAQUINOXALINE IN COMMERCIAL PELLETED POULTRY FEEDING STUFFS

	Pyrimethamine found/mg kg^{-1}		Ethopabate found/mg kg^{-1}		Sulphaquinoxaline found/mg kg^{-1}	
Sample	HPLC method	Other method[4]	HPLC method	Official method[1]	HPLC method	AOAC amended method[3]
Intensive growers A	—	—	2	2	30	34
	—	—	2	2	32	
Intensive growers B	—	—	3	3	46	54
	—	—	3	3	46	
Poultry feed	2	3	2	3	48	38
	3	3	2	3	54	40
	2		2			38
Baby chick feed			3	4	55	58
			3	4	59	

The extraction of sulphaquinoxaline from pelleted feeds using ethanol was found to be unsatisfactory. Other workers in this laboratory have found that the determination of sulphaquinoxaline in pelleted feeds is satisfactorily achieved by the amendment of the method of the Association of Official Analytical Chemists[3] to involve the use of hot dimethylformamide as the extraction solvent. The same extraction procedure has been adopted for the HPLC method reported here. With use of this extraction technique, however, other materials were extracted from the feed, which absorbed light at a wavelength of 220 nm and interfered with the determination of sulphaquinoxaline. In consequence, the detector was operated at 362 nm, a wavelength at which sulphaquinoxaline has an absorption maximum and at which the interference was minimised. Under these conditions a detection limit of 15 mg kg^{-1} of sulphaquinoxaline in feed was obtained. A chromatogram of the dimethylformamide extract of a feeding stuff is shown in Fig. 3. The commercial pelleted feeding stuffs mentioned in connection with analysis for pyrimethamine and ethopabate above were also analysed for sulphaquinoxaline content by this procedure and by the modified AOAC method. Comparison of the results obtained by using the two methods (Table V) shows there to be reasonable agreement.

Conclusion

The procedures described allow the determination of pyrimethamine, ethopabate and sulphaquinoxaline in poultry feeding stuffs. The detection limits of 1 mg kg^{-1} for pyrimethamine and ethopabate in mash and pelleted feeds and 5 mg kg^{-1} for sulphaquinoxaline in mash feeds and 15 mg kg^{-1} in pelleted feeds are well below the levels at which the drugs are normally added to the feeding stuff. A comparison of the proposed methods of analysis with the established procedures over a range of feeding stuffs shows there to be reasonable agreement between the results and that the HPLC method is a faster and simpler procedure.

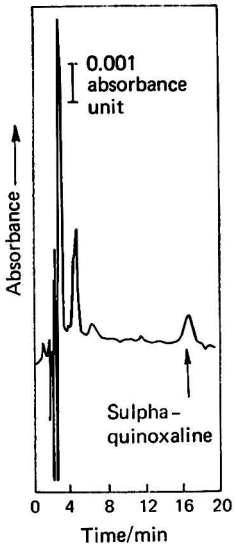


Fig. 3. Chromatogram of the dimethylformamide extract of a pelleted poultry feed medicated with sulphaquinoxaline (46 mg kg^{-1}). (Detector wavelength 362 nm. For other conditions see text.)

The authors thank Mrs. G. V. Alliston for carrying out the spectrophotometric analyses for the purpose of comparing the liquid chromatographic results and for useful discussions concerning the determination of sulphaquinoxaline. We also thank Spillers Ltd. and the Central Veterinary Laboratory of the Ministry of Agriculture, Fisheries and Food for supplying the samples and the Government Chemist for permission to publish this paper.

References

1. "The Medicines (Animal Feeding Stuffs) (Enforcement) Regulations 1976," HM Stationery Office, London, 1976, p. 46.
2. "The Medicines (Animal Feeding Stuffs) (Enforcement) Regulations 1976," HM Stationery Office, London, 1976, p. 59.
3. Szalkowski, C. R., *J. Ass. Off. Analyt. Chem.*, **1973**, **56**, 758.
4. Garber, J. B., Fink, D. W., and Szalkowski, C. R., *J. Ass. Off. Analyt. Chem.*, **1973**, **56**, 1161.
5. Cox, G. B., *J. Chromat.*, **1976**, **116**, 244.
6. Cox, G. B., Loscombe, C. R., Slucutt, M. J., Sugden, K., and Upfield, J. A., *J. Chromat.*, **1976**, **117**, 269.
7. Cox, G. B., and Sugden, K., *Analyst*, **1976**, **101**, 738.

Received *July 7th*, **1976**
Accepted *September 3rd*, **1976**

Analyst, January, 1977, Vol. 102, pp. 35–41

Simultaneous Determination of Phenobarbitone, Primidone and Phenytoin in Small Samples of Blood by Gas - Liquid Chromatography

A. M. Bruce and Hamilton Smith

Department of Forensic Medicine, University of Glasgow, Glasgow, G12 8QQ

Phenytoin, phenobarbitone and primidone in small samples of blood can be identified and measured simultaneously. The acidified blood is extracted with 3 ml of diethyl ether and 2 ml of the extract are evaporated to dryness. The residue is dissolved in 80 μl of diethyl ether containing methyltestosterone (15 mg per 100 ml) as the internal standard. An aliquot of this solution is injected on to an SE-30 column where the drugs are separated and detected by a flame-ionisation detector. Drugs commonly associated in treatment with these three and cholesterol, which is co-extracted, do not interfere.

Keywords: *Phenytoin*; *phenobarbitone*; *primidone*; *blood*; *gas - liquid chromatography*

A study of the treatment used in an epileptic colony revealed that most of the long-term patients were controlled with phenytoin. A further breakdown of the information showed that the use of two or more drugs was common. Of those receiving phenytoin treatment, 65% received phenobarbitone in addition and 28% received both phenobarbitone and primidone. The use of drugs other than these two with phenytoin was not usual. A proposed investigation of drug levels in blood therefore required the development of a method for the simultaneous determination of phenytoin, phenobarbitone and primidone. The volume of blood available was limited to a maximum of 1 ml because many successive samples were to be taken from patients. Gas chromatography was the technique selected because it is sensitive and rapid.

Gas chromatography of barbiturates, including phenobarbitone, has been widely reported in the literature.[1–7] These publications described the selection of columns and conditions and the separation and measurement of barbiturates and some related drugs. The determination of phenytoin by gas chromatography has also received much attention. Some workers, such as Parker *et al.*,[7] included it in a study with barbiturates while others, such as MacGee,[8] described it as a special topic. Primidone has received some attention but mostly in connection with its metabolic conversion into phenobarbitone.[9,10]

The equipment used for this study had independently controllable detector, injector and column ovens and was fitted with a flame-ionisation detector. All measurements were made with an electronic integrator. Early work showed that the wide variations in response of the detector to the three drugs at the various concentrations expected required the use of a wide-range amplifier.

Experimental

Choice of Column Packing

Glass columns of dimensions 5 ft × ¼ in were used. They were packed with the materials shown in Table I and tested by injection of blood and barbiturate standards in diethyl ether as described in Table II. The results indicated that of the column packing materials (liquid phases) tested, none was suitable for use in the determination of phenobarbitone by direct injection of blood. Although the limit of detection of phenobarbitone varied from one liquid phase to another, all columns had to be run at an attenuation that gave an unstable base line. In addition, many of the columns had to be used under conditions close to the maximum recommended temperature of the liquid phase and this temperature led to rapid deterioration of the column packing with a resultant drop in performance. Problems such

as tailing of peaks and failure of the solvent peak to return to base line before the phenobarbitone was eluted were common to several of the columns. In every instance the injection of 1 μl of blood gave many interfering peaks and led to rapid deterioration in the response to phenobarbitone on the column concerned. It was evident that no further trials of direct injection of blood would be useful and the investigation of columns was therefore restricted to tests with standard solutions of the drugs.

TABLE I

COLUMN PACKING MATERIALS USED

Liquid phase	Support*	Composition, *m/m*	Mesh size	Temperature range/°C	Maximum recommended temperature/°C	Activity	Conditioning temperature/°C
ApiezonL + dimer acid + trimer acid ..	Chromosorb W, AW DMCS	10 + 0.5 + 0.5 + 89	80–100	15–250	250	Non-polar	250
Silicone elastomer SE-30	Chromosorb W, AW DMCS	5 + 95	80–100; 60–80	50–240	250	Non-polar	245–250
Silicone OV-1 + trimer acid	Chromosorb W, AW DMCS	2 + 1 + 97	80–100	50–300	350	Non-polar	345
Silicone OV-17	Chromosorb G, AW DMCS	2.5 + 97.5	80–100	50–300	350	Semi-polar	345
FFAP	Chromosorb W, AW DMCS	15 + 85	60–80	50–250	250	Polar	245
Carbowax 20M (polyethylene glycol 20 000)	Chromosorb W, AW DMCS	20 + 80	80–100	50–220	220	Polar	220
Neopentyl glycol adipate + trimer acid ..	Chromosorb W, AW DMCS	3 + 0.75 + 96.25	80–100	50–200	200	Polar	200
Cyclohexanedimethanol succinate ..	Chromosorb W, AW DMCS	2 + 98; 4.5 + 95.5	80–100	50–250	250	Polar	245

* AW = acid washed; DMCS = treated with dimethyldichlorosilane.

Using these solutions, the more promising columns and some related ones, as shown in Table III, were tested. The best column packing was a 1 + 1 mixture of 5% SE-30 and 4.5% cyclohexanedimethanol succinate and retention times for various barbiturates when using this packing are given in Table IV. Unfortunately, preliminary experiments with blood extracts resulted in a peak close to and interfering with the phenytoin peak when the various mixtures of packings were tested. Subsequent analyses by mass spectrometry showed the interfering material to be cholesterol, and the 5% SE-30 column was selected for the remainder of the work. The conditions chosen from among the earlier trials were a column temperature of 230 °C and a nitrogen flow-rate of 100 ml min^{-1}. Blackmore[3] reported a variation in retention time if the amount of barbiturate injected on to this column was less than 1 μg. He also found considerable peak tailing but stated that *in situ* silanisation improved the resolution and recommended successive injections of 100 μg of diethylbarbituric acid before use to improve the performance of the column. In this study, the 5% SE-30 column performed very well provided that it was treated occasionally with 1,1,1,3,3,3-hexamethyldisilazane. This treatment reduced peak tailing and appeared to remove any adsorptive effects. The injection of diethylbarbituric acid was not necessary.

Interference by other drugs used in the treatment of epilepsy would be a major drawback in any method. This hazard was assessed by investigating the retention times of the drugs commonly used in the epilepsy treatment unit. The results given in Table V show that there is unlikely to be any interference from this source.

TABLE II

COLUMN PERFORMANCE

Column	Column temperature*/°C	Carrier gas	Direct injection of blood	Injection of barbiturate standard
Apiezon L	160 230 250	N_2	Many peaks and rapid deterioration	Temperatures near to maximum required for reasonable retention times. Column deterioration
5% SE-30	Various	N_2	Many peaks	Good response but tailed peaks
OV-1	160 170	N_2 and He		Very low sensitivity but short retention times
OV-17	210	N_2 and He	Poor detection and rapid deterioration	Unstable base line and poor peak shape; much tailing
FFAP	Various	N_2	Very many peaks	Detection poor
Carbowax 20M ..	150	N_2		Many small peaks
Neopentyl glycol adipate ..	195	N_2	Many peaks	Active sites on column require saturation with barbiturate. This bleeds off especially when a sample is injected
3% Cyclohexanedimethanol succinate	220 245 235 240	N_2 He 	Many peaks	Detection poor Unstable base line Improved detection Unstable base line
2% Cyclohexanedimethanol succinate	Various	N_2	Many peaks	Unstable base line but good response at 230 °C

* Detector temperature +30 °C, injector temperature +15 °C.

Extraction

The simplest extraction technique for blood involves shaking it with a suitable solvent; the solvent can then be analysed directly or subjected to a concentration procedure. The three drugs were tested in turn.

The direct extraction of 1 ml of blood was investigated using 1 ml of chloroform, diethyl ether, ethyl acetate, cyclohexanol, amyl alcohol or hexan-1-ol and mixing in a 10-ml glass-stoppered centrifuge tube for 30 s on a vortex mixer. Both chloroform and diethyl ether gave very good recoveries (greater than 90%) and diethyl ether was chosen because of the damaging effect that chloroform had on the column (spiking and unstable base line) and on the detector (corrosion) when repeated 10-μl injections were made. This technique worked

TABLE III

SELECTED COLUMN PERFORMANCE WITH STANDARD SOLUTIONS

Column	Column temperature/°C	Injection of standards
Apiezon L	210	Good response, short retention time for phenobarbitone but base line unstable
2% Cyclohexanedimethanol succinate ..	215–230	Good response but long retention time even at 230 °C
5% SE-30		Excellent response but tailed peaks. Useful short retention times for phenobarbitone and phenytoin
Various SE-30 + cyclohexanedimethanol succinate mixtures ..	225–250	Good response but phenytoin tended to have long retention times; 5% SE-30 + 4.5% cyclohexanedimethanol succinate (1 + 1) at 225 °C and a nitrogen flow-rate of 75 ml min^{-1} are excellent for the separation of barbiturates. A selection of retention times for these is given in Table IV. Under these conditions the retention time for phenytoin is 40 min; at 250 °C it is 13 min

TABLE IV

RETENTION TIMES FOR BARBITURATES

Column packed with a 1 + 1 mixture of 5% SE-30 and 4.5% cyclohexanedimethanol succinate: temperature, 225 °C; nitrogen flow-rate, 75 ml min^{-1}.

Drug	Retention time/min
Amylobarbitone	2.6
Hexobarbitone	2.8
Pentobarbitone	2.9
Vinbarbitone	3.4
Quinalbarbitone	3.5
Methylphenobarbitone	4.0
Cyclobarbitone	8.6
Phenobarbitone	14.0

well for phenobarbitone but was not so useful for the other drugs. Further experiments were carried out by acidifying the blood samples with hydrochloric and organic acids. The results showed that concentrated hydrochloric acid made extraction of the blood almost impossible owing to the thick mixture formed and the dilute acid or organic acids such as acetic or glucuronic acid gave a workable solution. Both for general convenience and owing to a preference not to add organic reagents that might complicate any subsequent process, 0.5 ml of 0.5 M hydrochloric acid was chosen for the purpose of acidification. This step gave no great improvement for phenobarbitone but was useful for the other drugs.

The remaining problem was the investigation of the mixing time on the vortex mixer and it was found that 15–30 s achieved maximum extraction.

The technique was then tested on blood samples. It was found that emulsions formed frequently and the extracted solution was too dilute for the measurement of phenytoin at

TABLE V

RETENTION TIMES ON 5% SE-30 OF DRUGS IN USE IN THE TREATMENT OF EPILEPSY

Drug	Trade-name	Classification	Retention time relative to phenytoin*	
			Column temperature 200 °C	Column temperature 230 °C
Acetazolamide	Diamox	Acidic	—	0.742
Amitriptyline	Tryptisol	Basic	0.464	0.618
Beclamide	Nydrane	Neutral	0.094	0.134
Carbamazepine	Tegretol	Basic	0.674	0.337, 0.843
Chlordiazepoxide	Librium	Basic	1.295	1.572
Chlorpromazine	Largactil	Basic	1.143	1.314
Diazepam	Valium	Neutral	0.954	1.179
Ethosuximide	Zarontin	Neutral	—	0.22, 0.51, 1.12
Ethotoin	Peganone	Neutral	0.127	0.224
Haloperidol	Serenace	Basic	—	—
Meprobamate	Equanil	Neutral	0.045 (0.121)	—
Methoin	Mesantoin	Neutral	0.104	0.169
Nitrazepam	Mogadon	Basic	—	2.942
Pericyazine	Neulactil	Basic	—	—
Pheneturide	Benuride	Neutral	0.045	0.056
Phenobarbitone	Luminal	Acidic	0.210	0.314
Phenytoin*	Epanutin	Acidic	1.000	1.000
Primidone	Mysoline	Acidic	0.576	0.741
Procyclidine	Kemadrin	Basic	0.433	0.565
Promazine	Sparine	Basic	0.759	0.865
Propantheline bromide	Pro-Banthine	†	0.857	0.891
Sulthiame	Ospolot	Acidic	1.348	1.618
Thioridazine	Melleril	Basic	—	8.55

* The retention time of phenytoin is 17.64 min at 200 °C and 4.45 min at 230 °C.

† Quaternary ammonium compound, water soluble.

therapeutic levels. These difficulties were resolved by increasing the volume of diethyl ether used in the extraction to 3 ml. The resulting mixture was agitated on a vortex mixer for 30 s and then centrifuged. A 2-ml volume of the ether solution was then removed and carefully evaporated to dryness on a water-bath at 60–70 °C. The residue was dissolved in 80 μl of diethyl ether containing an internal standard. The volume could be varied to suit the experimental requirements.

Selection of the Internal Standard

An internal standard was necessary for the accurate measurement of the drugs in the extract. A number of materials that were thought to be suitable were chromatographed and gave the results shown in Table VI. Methyltestosterone was selected for use because it gave a symmetrical peak with a retention time of 2.05 relative to that of phenytoin. Repeated injections of a standard solution in diethyl ether gave good reproducibility for the operator involved and caused no deterioration of the column. The standard ether solution was stable for many months when stored in the dark. The concentration of the standard can be adjusted as necessary but a value of 15 mg per 100 ml is useful.

TABLE VI

RETENTION TIMES RELATIVE TO PHENYTOIN OF POSSIBLE INTERNAL STANDARDS ON 5% SE-30

Column temperature 220 °C.

Compound	Retention time relative to phenytoin	Compound	Retention time relative to phenytoin
Methoin	0.16	Chlorprothixene	1.41
Ethotoin	0.19	Chlorpromazine	1.42
Heptabarbitone	0.34	Oestradiol	1.81
Methaqualene	0.55	Oestrone	1.85
Atropine	0.65	Methyltestosterone	2.05
Cocaine	0.66	Chloroquine	2.52
Codeine	1.01	Cinchocaine	2.74
Oxazepam	1.02	Nitrazepam	3.18
Diazepam	1.28	Cholesterol	7.10
Chlordiazepoxide	1.39		

The relationship between the response of the internal standard and that of the three drugs was checked to see if it remained constant for a given drug. One-millilitre aliquots of standard solutions of the drugs in water were extracted using the method developed and injected on to the SE-30 column at 230 °C. The ratio of the area of the drug peak to the area of the methyltestosterone peak was calculated and the results are shown in Table VII. The reproducibility was acceptable and methyltestosterone was chosen for the final investigations in this study.

TABLE VII

REPRODUCIBILITY OF THE RATIO OF PEAK AREAS OF DRUG AND INTERNAL STANDARD

Figures in parentheses are masses of drug dissolved in 100 ml of blood.

	Phenobarbitone	Primidone	Phenytoin
Number of tests	10 (0.49 mg)	4 (2.04 mg)	12 (1.56 mg)
Mean r*	0.336	0.344	1.314
Standard deviation	0.013 8	0.024	0.052
Number of tests	10 (10.6 mg)	4 (2.01 mg)	6 (0.78 mg)
Mean r*	7.885	0.331	0.624
Standard deviation	0.204	0.012	0.021
Number of tests	6 (2.12 mg)	4 (1.17 mg)	—
Mean r*	1.385	0.211	—
Standard deviation	0.043	0.017	—

* r is the ratio of the area of the drug peak to the area of the methyltestosterone peak.

Gas Chromatography

Solutions of phenobarbitone, primidone and phenytoin in blood in various concentrations were extracted using the technique developed and aliquots of the extract were injected on to the gas chromatograph at 230 °C in order to establish whether the ratio of the concentration to the area of the drug peak divided by the area of the methyltestosterone peak was linear. This was found to be so for phenobarbitone (up to 10 mg per 100 ml), primidone (up to 2.5 mg per 100 ml) and phenytoin (up to 1.5 mg per 100 ml). No trials were made above these limits and the linear relationship may well persist above them. It was noted in the earlier experiments when using a narrow-range flame-ionisation amplifier that for phenobarbitone the linear relationship did not hold. Further investigation showed that the curve found reflected the characteristics of the amplifier and that substitution of a wide-range amplifier resulted in a linear relationship.

Procedure

The extraction method was checked for the recovery of each drug and then tested by the determination of all three drugs in a known sample as described below. Separate samples of blood having known concentrations of phenobarbitone, primidone and phenytoin were analysed and the results are given in Table VIII. Apart from phenobarbitone, the recoveries were rather low but the reproducibility was acceptable. As a further test, a sample containing known amounts of the three drugs was analysed. The results listed in Table IX show that the complete method gives acceptable results. The coefficients of variation were 2.7% for phenobarbitone, 8.8% for primidone and 5.1% for phenytoin at a column temperature of 230 °C. It was decided to change the column temperature to the lowest value that gave reasonably short retention times. This temperature was found to be 225 °C and repeating the analysis at this setting gave coefficients of variation of 2.1% for phenobarbitone, 6.6% for primidone and 2.6% for phenytoin. The recommended temperature for the column was therefore changed to 225 °C. The method in its final form was as follows.

TABLE VIII

RECOVERY OF DRUGS FROM SEPARATE SAMPLES OF BLOOD

	Phenobarbitone	Primidone	Phenytoin
Known concentration/mg per 100 ml ..	2.12	2.01	2.24
Recovery, %	98.7	70.7	85.0
Standard deviation, %	3.9	3.3	1.6
Number of tests	5	5	10

A 1-ml sample of blood was acidified with 0.5 ml of 0.5 M hydrochloric acid in a 10-ml centrifuge tube with a ground-glass stopper. Diethyl ether (3 ml) was added and the contents were mixed for 30 s on a vortex mixer. The resultant solution was centrifuged and 2 ml of the ether layer were transferred to another tube and evaporated to dryness in a water-bath at 60–70 °C. The residue was taken up in 80 μl of diethyl ether containing methyltestosterone (15 mg per 100 ml) as the internal standard and 10-μl aliquots of the solution were injected

TABLE IX

SIMULTANEOUS DETERMINATION OF PHENOBARBITONE, PRIMIDONE AND PHENYTOIN

Drug concentrations expressed in milligrams per 100 ml of blood.

	Pheno-barbitone	Primidone	Phenytoin	Column tempera-ture/°C
Known concentration	1.95	2.70	1.08	—
Found concentration	1.92	2.73	1.02	230
Standard deviation	0.052	0.24	0.052	
Number of samples	5	5	5	
Found concentration	1.90	2.73	1.04	225
Standard deviation	0.040	0.18	0.027	
Number of samples	4	4	4	

into the gas chromatograph. The glass column (5 ft × ¼ in) was packed with 5% SE-30 on Chromosorb W (acid washed and treated with dimethyldichlorosilane). The column was treated occasionally with 1,1,1,3,3,3-hexamethyldisilazane in order to maintain its performance. The column oven was maintained at 225 °C, the injection port at 240 °C and the flame-ionisation detector at 255 °C. A nitrogen flow-rate of 100 ml min^{-1} was used with suitable hydrogen and air supplies to give a stable flame. It is not necessary to use exactly 80 µl of ether solution but the volume used each time must be the same. A micro-dispenser or a selected syringe can be used.

The method was tested by analysing several hundred blood samples and no difficulties were experienced. It was necessary, however, to evaporate the ether extract carefully in order to avoid losses.

Conclusion

A rapid gas-chromatographic method has been developed for the determination of therapeutic levels of phenobarbitone, primidone and phenytoin in small blood samples. The method has no interference from other common drugs used in an epileptic treatment unit or from cholesterol, which was co-extracted from the blood.

Retention times are listed for a selection of barbiturates, anti-epileptic drugs and some others of general interest to those treating epileptics.

A. M. Bruce thanks Quarrier's Homes, who provided financial assistance during this study.

References

1. Berry, D. J., *J. Chromat.*, **1973**, **86**, 89.
2. Blackmore, D. J., and Jenkins, R. W., *J. Forens. Sci. Soc.*, **1968**, **8**, 34.
3. Blackmore, D. J., *Perkin-Elmer Analyt. News*, **1968**, No. **3**, 1.
4. Bogan, J., and Smith, H., *J. Forens. Sci. Soc.*, **1967**, **7**, 37.
5. Leach, H., *Perkin-Elmer Analyt. News*, **1968**, No. **3**, 4.
6. MacGee, J., *Clin. Chem.*, **1971**, **17**, 587.
7. Parker, K. D., Wright, J. A., Halpern, A. F., and Hine, C. H., *J. Forens. Sci. Soc.*, **1968**, **8**, 125.
8. MacGee, J., *Analyt. Chem.*, **1970**, **42**, 421.
9. Bogan, J., Rentoul, E., and Smith, H., *J. Forens. Sci. Soc.*, **1965**, **5**, 97.
10. Bogan, J., and Smith, H., *J. Pharm. Pharmac.*, **1968**, **20**, 64.

Received *May* **10***th*, **1976**
Accepted *September* **3***rd*, **1976**

Indirect Determination of Phenelzine in Urine

B. Caddy and A. H. Stead

Forensic Science Unit, Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, University of Strathclyde, Glasgow, G1 1XW

The problems associated with the determination of phenelzine in biological fluids are described. Conversion of phenelzine, *in situ*, into 2-phenylethanol and the gas-chromatographic assay of the latter compound provide an easy, precise and specific method for the determination of phenelzine in urine.

Keywords: *Phenelzine determination*; *urine*; *monoamine oxidase inhibitor*; *gas chromatography*

The metabolism of biogenic amines such as serotonin, adrenaline, noradrenaline, dopamine and tyramine is inhibited by a group of drugs referred to as monoamine oxidase inhibitors (MAOIs). This inhibition results in an increase in the level of these neurotransmitters in the brain, which, in turn, causes a stimulation of the central nervous system. This effect has led to the MAOIs being widely used for the alleviation of neurotic and psychotic depressions. However, when MAOIs are taken in overdose, or are administered with other drugs or certain types of foodstuffs, toxic symptoms may arise. These symptoms are well documented.[1–6] It is essential, therefore, that analytical procedures are developed to provide data for the help of the individual patient. These analytical procedures must be specific and, above all, sensitive, as the irreversible inhibition of the enzyme persists after most of the drug and/or its metabolites have disappeared.[7,8]

One of the more commonly used MAOIs, phenelzine, has been problematic in that it is unstable under basic conditions[9–11] and cannot be extracted directly from biological fluids for quantitative work. While enzymatic,[9] gas - liquid chromatographic,[12] potentiometric,[13] colorimetric[14,15] and titrimetric[16,17] procedures have been proposed for the identification of phenelzine and for its quantitative determination, none of these methods can be used both qualitatively and quantitatively in analyses for the drug in body fluids.

A method that can be used to overcome the problem of the instability of phenelzine is to derivatise the molecule *in situ*, prior to extraction. This approach has been adopted by Caddy *et al.*,[18] who prepared the acetonide after liberation of the phenelzine salt with sodium carbonate. Levels of 2 μg ml^{-1} in urine were detectable despite the conversion being of the order of only 20%.

An alternative approach to the problem is to enhance the decomposition of phenelzine under controlled conditions and to analyse the decomposition product(s). By the oxidation of phenelzine to styrene and/or ethylbenzene with Fehling's solution, Gelbicova-Ruzickova *et al.*[19] were able to determine the drug in urine. However, rigid control over the oxidation conditions was required and the products of oxidation suffered the disadvantage that they are difficult to derivatise. This disadvantage becomes important when applying the method to plasma samples. This paper describes the preliminary investigation, as applied to urine, of the application of iodate oxidation of a hydrazine[20] for the determination of phenelzine.

Experimental

Reagents

Phenelzine sulphate. W. R. Warner and Co. Ltd., Eastleigh, Hants.
Sulphuric acid, 50% *V/V*.
Potassium iodate solution, 5% *m/V*.
Sodium hydroxide solution, 4% *m/V*.
Diethyl ether.
Sodium sulphate, anhydrous.
Dodecan-1-ol. Reagent grade.
2-*Phenylethanol.* Reagent grade.
Hydrochloric acid, concentrated.

All of the reagents were of AnalaR grade unless otherwise stated, and were used without further purification.

Oxidation of Phenelzine Sulphate

To phenelzine sulphate (approximately 200 mg), dissolved in 5 ml of water, add 2 ml of 50% *V/V* sulphuric acid and approximately 5 ml of 5% *m/V* potassium iodate solution and shake the mixture vigorously. Filter to remove any precipitated iodine and extract the filtrate with diethyl ether. Separate the organic layer, wash it once with 4% *m/V* sodium hydroxide solution to remove further iodine, dry it over anhydrous sodium sulphate and evaporate it to dryness under reduced pressure.

The brown oily residue was distilled to yield 52 mg (theoretical yield 114 mg) of a colourless liquid, b.p. 120 °C at 0.1 mmHg. The product was identified by elemental analysis (found, C 78.5, H 8.0%; calculated for $C_8H_{10}O$, C 78.7, H 8.2%), infrared spectroscopy [$\nu_{max.}$ peaks: 3530 (–OH), 2930 (–CH_2– stretching), 1605, 1500 (C=C aromatic), 1458 (–CH_2– deformation), 1049 (–O–H in-plane deformations), 915, 860, 750, 705 (aromatic)]; mass spectrometry [*m/e*: 122 (parent ion), 91 (base peak, McClafferty rearrangement), 65 (fragmentation of base peak, ejection of C_2H_2)] and nuclear magnetic resonance spectroscopy (identical with an authentic sample).

Standard Solutions

Phenelzine sulphate. Prepare an aqueous solution containing 500 μg ml^{-1} (50 mg in 100 ml) of phenelzine sulphate. Dilute 10 ml of this solution to 100 ml (50 μg ml^{-1}), then further dilute 2–10 ml of the solution obtained to 50 ml to give working solutions containing 2–10 μg ml^{-1}.

Dodecan-1-ol. Prepare an approximately 400 μg ml^{-1} solution of dodecan-1-ol in diethyl ether (2 drops of the alcohol in 100 ml). Dilute 5 ml of this solution to 100 ml to give a working solution containing approximately 20 μg ml^{-1}.

2-Phenylethanol. Prepare a 500 μg ml^{-1} (50 mg in 100 ml) solution of 2-phenylethanol in diethyl ether. Dilute 10 ml of this solution to 100 ml (50 μg ml^{-1}), then further dilute 2–10 ml of the solution obtained to 50 ml to give working solutions containing 2–10 μg ml^{-1}.

These stock solutions of phenelzine sulphate and dodecan-1-ol are stable for 1 week and at least 3 weeks, respectively, when stored at room temperature, after which time they may deteriorate. Storage at 4 °C prolongs the lifetime of the phenelzine sulphate solution to 4 weeks before noticeable breakdown occurs. Working solutions are prepared as required.

Preparation of Calibration Graphs for Phenelzine Sulphate in Aqueous Solution

To 4 ml of an aqueous solution of phenelzine sulphate (containing 2–10 μg ml^{-1}) in a 20-ml glass-stoppered test-tube, add 1 ml of a 20 μg ml^{-1} solution of dodecan-1-ol in diethyl ether and 4 ml of diethyl ether. To this mixture, add 2 ml of 50% *V/V* sulphuric acid and shake the tube vigorously by hand for 5 s. Add 2 ml of 5% *m/V* potassium iodate solution and shake for a further 10 s. Allow the layers to separate, transfer the organic layer into a capillary-based centrifuge tube and very carefully evaporate it to about 1.0 ml in a water-bath at 35 °C before concentrating the remainder at room temperature under a stream of air to a final volume of approximately 50 μl. Examine this solution by gas - liquid chromatography using the conditions given below.

A Perkin-Elmer F-11 gas chromatograph fitted with a flame-ionisation detector and a 2 m × 2 mm i.d. glass column packed with 10% *m/m* FFAP on Gas-Chrom Q (80–100 mesh) was used. The operating conditions were: column temperature, 150 °C; injection block temperature, 170 °C; and nitrogen flow-rate, 40 ml min^{-1}. Samples of 1 μl were injected and a graph of relative peak height (2-phenylethanol/dodecan-1-ol) against concentration was constructed.

Under these conditions, 2-phenylethanol had a retention time of 7.13 min and dodecan-1-ol 9.07 min.

This procedure was repeated eight times in order to evaluate the method statistically.

Preparation of Calibration Graphs for Phenelzine Sulphate in Urine

Add suitable volumes of an aqueous solution of phenelzine sulphate to samples of drug-free

urine such that final concentrations of the drug salt in the urine are in the range 2–10 μg ml^{-1}.

To 4 ml of each urine sample in a 20-ml glass-stoppered test-tube, add 2 ml of 50% *V*/*V* sulphuric acid and approximately 10 ml of diethyl ether and shake the tube vigorously by hand for 10 s. Allow the layers to separate and discard the organic layer. Add 1 ml of a 20 μg ml^{-1} solution of dodecan-1-ol in diethyl ether to the urine, followed by 4 ml of diethyl ether and 2 ml of 5% *m*/*V* potassium iodate solution, and shake vigorously for 10 s. Proceed with the extraction and the gas - liquid chromatographic analysis as described for aqueous solutions.

This procedure was repeated five times for statistical evaluation of the results.

Determination of the Percentage Recovery of 2-Phenylethanol Extracted from Urine

Add suitable volumes of an aqueous solution of 2-phenylethanol to samples of drug-free urine such that final concentrations of the alcohol in the urine are in the range 2–10 μg ml^{-1}. The addition of the oxidising agent, the extraction and the analysis are carried out as described under Preparation of Calibration Graphs for Phenelzine Sulphate in Urine.

Determination of the Percentage Conversion of Phenelzine into 2-Phenylethanol

Use 4 ml of aqueous solutions of 2-phenylethanol in the concentration range 2–10 μg ml^{-1} instead of solutions of the drug salt and follow the method for phenelzine sulphate as described above. The gradient of the straight line produced after plotting the peak-height ratio against the concentration of 2-phenylethanol in micrograms per millilitre is compared with that obtained from a standard phenelzine sulphate calibration graph produced as described above.

Results and Discussion

Oxidation of Phenelzine Sulphate

The major and only identifiable product produced when phenelzine (**I**) is oxidised with iodate under acidic conditions is 2-phenylethanol (**II**). The production of this compound may be rationalised in the manner shown in Fig. 1.

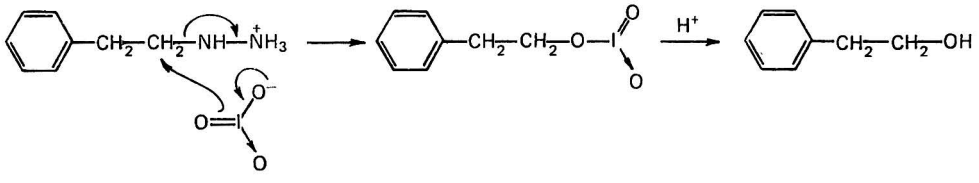


Fig. 1. Schematic diagram for the oxidation of phenelzine with iodate under acidic conditions.

Preparation of Calibration Graphs for Aqueous Solutions of Phenelzine Sulphate

Although in their studies on the reduction of iodate with hydrazine Hasty[20] and Marzadro and de Carolis[21] reported that optimum conditions are achieved if the acid concentration lies within the range 50–100%, no measurable effect was observed on the oxidation of phenelzine with iodate using acid volumes of 1–5 ml at concentrations of 50–100%. Very poor yields were obtained when the oxidation was carried out at neutral pH and, because of the instability of phenelzine and the fact that iodate is not reduced to iodide under alkaline conditions, the assay was not attempted at alkaline pH. Variations in the concentration and volume of the iodate solution of 1–10% and 1–5 ml, respectively, also had no measurable effect. The conditions described above for the oxidation of aqueous phenelzine sulphate were therefore chosen wholly on practical considerations.

The choice of extracting solvent for 2-phenylethanol was subject to the requirements of high affinity for the alcohol combined with a low degree of extraction of the iodine produced in the reaction. An initial study of recoveries from aqueous solutions suggested that both diethyl ether and chloroform were suitable for the extraction step, and the former was chosen on economic considerations.

After extraction into the organic solvent it was found possible, by careful evaporation, to concentrate the solutions of 2-phenylethanol up to 100-fold (4 ml down to approximately

50 μl) without loss of the alcohol or the internal standard. Violent boiling of the ether, too vigorous an evaporation procedure with air or evaporation to dryness led to inconsistent results owing to the appreciable volatility of 2-phenylethanol. It was noted that when the concentrated ether extract of the alcohol was allowed to stand at room temperature for more than 10 min before injection on to the gas chromatograph less reproducible results were also obtained.

Previous references to the gas-chromatographic analysis of 2-phenylethanol[22–24] have suggested a wide range of stationary phases of widely differing polarities. Of those phases examined, FFAP (10% *m/m* on 80–100-mesh Gas-Chrom Q) was selected as it gave the most symmetrical peak shapes, high sensitivity and a more efficient separation. A typical chromatogram from the analysis of aqueous solutions of phenelzine sulphate is shown in Fig. 2.

Decomposition of 2-phenylethanol or the internal standard, or both, leading to irreproducible results, was noted when the injection block temperature was increased much above 200 °C.

Precision of the Oxidation Procedure

The ratios of peak heights given by 2-phenylethanol and dodecan-1-ol, recorded from the chromatogram, were plotted against the concentration, in micrograms per millilitre, of phenelzine sulphate added.

The standard calibration graph, comparing the amount of drug salt originally added with the amount of phenelzine detected as 2-phenylethanol, was found to be linear over the range of standards used. It was reproducible for one set of standards if evaporation of the diethyl ether in the stock solution of internal standard was prevented.

The results of eight replicate analyses of aqueous phenelzine sulphate solutions of concentration 2–10 μg ml^{-1} are shown in Table I.

Statistical analysis of the curve indicated that the oxidation procedure was acceptable for the assay of phenelzine sulphate in aqueous solutions.

TABLE I

REPRODUCIBILITY OF YIELDS OF 2-PHENYLETHANOL FOLLOWING THE OXIDATION OF AQUEOUS PHENELZINE SULPHATE SOLUTIONS WITH IODATE, AND THE ASSOCIATED REGRESSION LINE

Phenelzine sulphate concentration/μg ml^{-1}	Peak-height ratio*	Standard deviation	Coefficient of variation, %
2	0.242	0.033	13.64
4	0.435	0.040	9.20
6	0.613	0.030	4.89
8	0.799	0.027	3.38
10	1.011	0.011	1.09

All data: $y = 0.063 + 0.094x$, where y = peak-height ratio of 2-phenylethanol to dodecan-1-ol and x = concentration (μg ml^{-1}).
Standard deviation of regression coefficient = 0.001 4.
Standard deviation of intercept = 0.008 1.
Residual error = 0.000 9.
F value = 4 516.

* Values are the means of eight determinations.

Preparation of Calibration Graphs for Phenelzine Sulphate in Urine

In order to establish the oxidation procedure as a means of analysis for the drug present in urine samples, phenelzine sulphate was added to blank urines, oxidised, and the resultant 2-phenylethanol extracted and determined by gas - liquid chromatography.

It was considered necessary to establish that the presence, if any, of other compounds in the urine would not adversely affect the precision of the determination. No interfering substances were detected in a preliminary ether wash of five different samples of urine but this step was retained as a necessary safeguard against interferences. The blank urines did not give any interfering compounds from the oxidation of normal urinary constituents during the full assay procedure.

Emulsions sometimes formed in the course of extraction of the acidified urine sample with diethyl ether, particularly in the initial clean-up procedure. It was found that bubbling air through the emulsions with a Pasteur pipette was sufficient to break them up.

A typical chromatogram from the determination of phenelzine sulphate in urine is shown in Fig. 3.

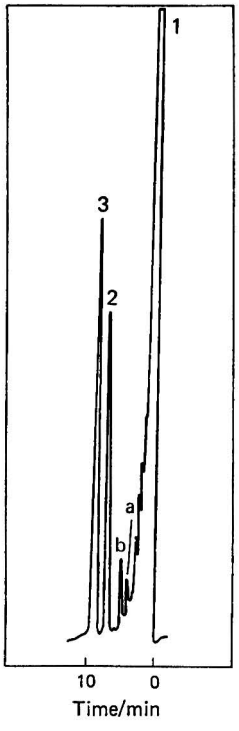


Fig. 2. Gas chromatogram of the aqueous extract: 1, solvent front; 2, 2-phenylethanol; 3, dodecan-1-ol (internal standard); a and b, unidentified products arising from the oxidation of phenelzine sulphate.

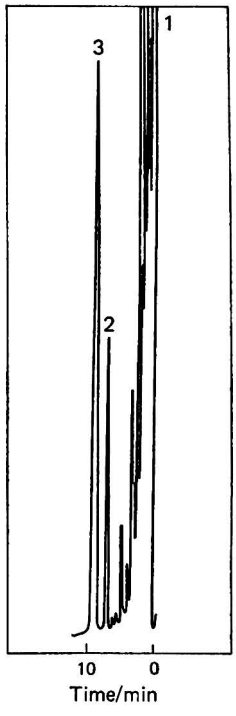


Fig. 3. Gas chromatogram of the urinary extract: 1, solvent front; 2, 2-phenylethanol; 3, dodecan-1-ol (internal standard).

Precision of the Oxidation Procedure

Calibration graphs of peak-height ratio against concentration, in micrograms per millilitre, of phenelzine sulphate added to urine were plotted. Very good linearity and reproducibility over the range 2–10 μg ml^{-1} was obtained. The results of five replicate determinations (Table II) indicated that the oxidation procedure was suitable for the determination of phenelzine sulphate present in urine.

Statistical analysis of the curve indicated that the procedure was satisfactory for the assay of phenelzine sulphate in urine and was capable of yielding significant results.

Statistical evaluation of the standard curves from both aqueous and urinary solutions of phenelzine sulphate by comparison of their variances showed that at both $P = 0.05$ and 0.01 the two could be considered identical. This result has practical significance in that aqueous standards can be used for an assay of the drug in urine samples.

Percentage Recovery of 2-Phenylethanol extracted from Urine

A number of 2–10 μg ml^{-1} 2-phenylethanol solutions, prepared in drug-free urine, were analysed in duplicate by the procedure described and the results compared with those for a similar series of solutions of 2-phenylethanol in diethyl ether. It was found that the amount of 2-phenylethanol extracted lies between 87.8 and 94.0% with a mean, calculated from the

TABLE II

REPRODUCIBILITY OF YIELDS OF 2-PHENYLETHANOL FOLLOWING THE OXIDATION OF PHENELZINE SULPHATE IN URINE WITH IODATE, AND THE ASSOCIATED REGRESSION LINE

Phenelzine sulphate concentration/μg ml^{-1}	Peak-height ratio*	Standard deviation	Coefficient of variation, %
2	0.204	0.031	15.10
4	0.395	0.027	6.76
6	0.612	0.029	4.69
8	0.784	0.021	2.63
10	1.017	0.019	1.87

All data: $y = 0.018 + 0.098x$ (y and x as in Table I).
Standard deviation of regression coefficient = 0.0015.
Standard deviation of intercept = 0.0087.
Residual error = 0.0007.
F value = 4 512.

* Values are the means of five determinations.

regression lines of the two graphs, of 92.8% and a coefficient of variation of 2.9% (Table III).

Extraction into chloroform made no difference to the recovery of the alcohol but introduced manipulative problems during the preliminary wash.

The recovery of 2-phenylethanol by a single extraction into approximately 5 ml of diethyl ether was not improved by use of a series of extractions. Emulsions formed very readily if small volumes of diethyl ether (less than 2 ml) were used, resulting in a low recovery, and after four successive extractions using approximately 5 ml of diethyl ether in each, no appreciable increase in yield in the pooled extracts was noted. The higher degree of concentration in the latter instance gave rise to an increase in concomitantly extracted residues, leading to an increased possibility of interference in the assay.

TABLE III

RECOVERIES OF 2-PHENYLETHANOL FROM URINE

2-Phenylethanol concentration added/μg ml^{-1}	Peak-height ratio (unextracted)	Peak-height ratio (extracted)	2-Phenylethanol concentration found/μg ml^{-1}	Relative standard deviation/ μg ml^{-1}	Recovery, %	Coefficient of variation, %
2	0.385	0.338	1.75	0.12	87.8	6.9
4	0.740	0.661	3.57	0.18	89.3	5.0
6	1.129	1.002	5.32	0.24	88.7	4.5
8	1.490	1.401	7.52	0.17	94.0	2.3
10	1.867	1.713	9.18	0.26	91.8	2.8

For each determination, the alcohol was extracted by vigorous shaking by hand for approximately 10 s. The recovery was not improved by shaking by hand for up to 1 min. Tilt-shaking for 30 min was shown to be as effective but, although this procedure minimised the formation of emulsions, shaking by hand was adopted as the method of choice as only one sample could be prepared at a time.

An examination of the pH dependence of the extraction showed that adjustment of the pH by addition of concentrated sodium hydroxide solution, even when the mixture was cooled in an ice-bath, invariably led to losses in recovery. Although this finding could represent a pH dependence in extraction, evaporation loss at the zone of addition of the alkali is more likely as severe losses could be incurred by the addition of only small volumes of sodium hydroxide solution.

Percentage Conversion of Phenelzine into 2-Phenylethanol

A calibration graph, prepared from the results of duplicate determinations at different concentrations, for 2-phenylethanol extracted from aqueous solution was compared with a standard phenelzine sulphate calibration graph. The conversion of phenelzine sulphate into 2-phenylethanol was calculated from the regression lines of the two graphs and was found to lie between 50.5 and 59.4% of the theoretical value with a mean of 57.4% and

a coefficient of variation of 8.0% (Table IV). There was no evidence in any of the standard graphs to suggest that the yield changed over the concentration range examined. Despite the non-quantitative yields of 2-phenylethanol, the reproducibility at any given concentration was good.

TABLE IV

CONVERSION OF PHENELZINE SULPHATE INTO 2-PHENYLETHANOL

Phenelzine sulphate and 2-phenylethanol concentrations/ $\mu g\ ml^{-1}$	Peak-height ratio (phenelzine sulphate)	Peak-height ratio (2-phenyl-ethanol)	Recovery of drug as the alcohol,* %	Relative standard deviation, %	Coefficient of variation, %
2	0.060	0.209	50.5	4.6	9.1
4	0.123	0.401	53.6	4.0	7.5
6	0.182	0.623	51.1	5.2	10.2
8	0.274	0.869	55.1	2.5	4.5
10	0.340	1.002	59.4	3.5	5.9

* Including a correction factor for differences in relative molecular mass; 1 μg of phenelzine sulphate will give 0.572 μg of 2-phenylethanol under ideal conditions.

Lower Limit of Detection

The method recommended by Wilson[25] was used to calculate the limit of detection for the analysis. From results collected during the analysis of drug-free urine samples, it was calculated that an original concentration of 0.29 $\mu g\ ml^{-1}$ of phenelzine sulphate extracted from 4 ml of urine can be determined by the proposed procedure. This value was readily confirmed by preparing diluted standards, with suitable dilution of the internal standard, when a gas-chromatographic peak corresponding to 0.05 $\mu g\ ml^{-1}$ of the drug could easily be seen.

Rate of Analysis

As the gas-chromatographic retention times under the operating conditions are 7.13 and 9.07 min for 2-phenylethanol and the internal standard, respectively, any sample can be run in under 15 min and consequently the rate of analysis was judged to be adequate for the assay of the drug.

Stability of Phenelzine Sulphate in Urine

There was no loss of drug from urine samples stored at 4 °C in sealed glass containers with no preservatives over a period of 3 weeks.

Specificity of the Analysis

No interferences from drug-free urine samples analysed by the iodate oxidation were noted. The preliminary ether wash from acidified urine will remove any acidic or neutral drugs that may be present, and no available basic drugs will give rise to 2-phenylethanol after undergoing the oxidation procedure, ensuring that the assay is specific for phenelzine and those of its metabolites which contain the hydrazine group. Other pharmaceutically active hydrazine derivatives, *e.g.*, nialamide and isoniazid, will be oxidised by iodate but their oxidation products will not interfere in the determination of 2-phenylethanol.

Conclusion

The proposed method for the determination of phenelzine in urine, involving oxidation with iodate under acidic conditions, extraction of the oxidation product, 2-phenylethanol, and determination of the latter by gas - liquid chromatography, is rapid and sensitive and is capable of determining 0.05 $\mu g\ ml^{-1}$ of the drug in urine. A level of 2 $\mu g\ ml^{-1}$ has been determined with a relative standard deviation of $\pm 6.0\%$ and good linearity was found over the concentration range studied. No interfering gas-chromatographic peaks arising from urinary constituents were encountered and the method should not be subject to interference from drugs administered simultaneously with phenelzine.

One of the authors (A.H.S.) acknowledges support from the Medical Research Council during the tenure of which this work was carried out.

References

1. Stockley, I. H., *Pharm. J.*, 1969, **203**, 147.
2. Stockley, I. H., *Pharm. J.*, 1969, **203**, 174.
3. Tipton, K. F., *in* Aldridge, W. N., *Editor*, "Mechanisms of Toxicity," Macmillan, London, 1971, p. 13.
4. Longo, V. G., "Neuropharmacology and Behaviour," W. H. Freeman and Co., San Francisco, 1972, p. 59.
5. Goodman, L. S., and Gilman, A., *Editors*, "The Pharmacological Basis of Therapeutics," Fourth Edition, Macmillan, New York, 1970, pp. 184 and 733.
6. Curry, A. S., *in* "Advances in Forensic and Clinical Toxicology," CRC Press, Cleveland, Ohio, 1972, p. 153.
7. Colvin, L. B., *J. Pharm. Sci.*, 1969, **58**, 1433.
8. Juchau, M. R., and Horita, A., *in* di Carlo, F. J., *Editor*, "Drug Metabolism Reviews," Volume 1, Marcel Dekker, New York, 1972–73, p. 71.
9. Curry, A. S., and Mercier, M., *Nature, Lond.*, 1970, **228**, 281.
10. Schlitt, L., Rink, M., and von Stackelberg, M., *J. Electroanalyt. Chem.*, 1967, **13**, 10.
11. Clarke, E. G. C., *Editor*, "Isolation and Identification of Drugs," Volume 1, Pharmaceutical Press, London, 1969, p. 482.
12. Pellerin, F., and Makkawi, H., *Annls Pharm. Fr.*, 1971, **29**, 421.
13. Pinzauti, S., dal Piaz, V., and la Porta, E., *J. Pharm. Sci.*, 1974, **63**, 1446.
14. Dospeux, P. F., *Chim. Analyt.*, 1971, **53**, 251.
15. Asmus, E., and Ganzke, J., *Z. Analyt. Chem.*, 1973, **265**, 325.
16. Soliman, R., and Belal, S. A., *J. Drug. Res.*, 1974, **6**, 7.
17. Barakat, M. Z., Abou-el-Makarem, M., and Abd el-Raoof, M., *Analyt. Chem.*, 1974, **46**, 777.
18. Caddy, B., Tilstone, W. J., and Johnstone, E. C., *Br. J. Clin. Pharmac.*, 1976, **3**, 633.
19. Gelbicova-Ruzickova, J., Novak, J., and Chundela, B., *Biochem. Med.*, 1971, **5**, 537.
20. Hasty, R. A., *Mikrochim. Acta*, 1973, **6**, 925.
21. Marzadro, M., and de Carolis, A., *Rc. Ist. Sup. Sanità*, 1963, **26**, 629; *Chem. Abstr.*, 1964, **60**, 13882b.
22. Fishbein, L., and Zielinski, W. L., *J. Chromat.*, 1967, **28**, 418.
23. Engan, S., *Brygmesteren*, 1969, **26**, 23.
24. Kahn, J. H., and Connor, H. A., *J. Ass. Off. Analyt. Chem.*, 1972, **55**, 1155.
25. Wilson, A. L., *Analyst*, 1961, **86**, 72.

Received *September 6th*, 1976
Accepted *September 17th*, 1976

SHORT PAPERS

Dissolution of Mercury(II) Sulphide by Digestion with Sulphuric Acid - Nitric Acid and Oxidation with Potassium Permanganate

A. M. Ure

Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen, AB9 2QJ,

Keywords: Mercury(II) sulphide dissolution; atomic-absorption spectrophotometry; mercury determination; minerals

Recent statements[1,2] to the effect that digestion with sulphuric acid - nitric acid (2 + 1) mixture at 60 °C with subsequent oxidation by potassium permanganate[3] fails adequately to dissolve mercury(II) sulphide raise doubts about the usefulness of such procedures in the preparation of soil, rock and sediment samples for the atomic-absorption determination of mercury by the reduction - aeration technique. The use of aqua regia[1] in order to overcome this difficulty causes a loss of mercury as the volatile chloride.[4] The proposed use[2] of a small addition of hydrochloric acid in a modification of Iskandar's procedure[3] gave complete recovery of 1 000 p.p.m. of mercury as mercury(II) sulphide, but it was only tested by these workers at high levels and using a flame method. No evidence of inadequate recovery at the parts per million level was presented because the small standard additions required could not be weighed accurately. A comparison of this procedure using additional hydrochloric acid with the unmodified Iskandar procedure for soils and sediments showed that the methods gave identical values, this being attributed by the authors[2] to the absence of mercury(II) sulphide in the samples tested.

A test of the conclusions regarding the solubility and recovery of mercury(II) sulphide has been made with samples containing mercury(II) sulphide at the levels (less than 1 p.p.m.) at which it is likely to occur in rocks and soils, using a (1 + 1) sulphuric acid - nitric acid digestion procedure[5] based on that of Iskandar[3] and including an overnight oxidation with potassium permanganate. Synthetic samples containing mercury(II) sulphide, either as the chemical reagent or as natural cinnabar (85% HgS), were prepared by mixing about 1 mg (accurately weighed to ± 1 μg with an electric microbalance) with 1.00 g of pure, finely ground (to a particle size of less than 150 μm) quartzite (silica) by hand-grinding them in an agate mortar. By subsequent 10-fold dilutions with silica powder, and thorough hand-mixing in the mortar for 30 min at each dilution, synthetic rock samples containing mercury as mercury(II) sulphide were prepared with mercury contents of about 0.07, 0.2 and 2 p.p.m. While the accuracy with which the mercury content is known may not be better than 10–20%, this is sufficient to test whether the 80–90% failure in recovery,[1] reported for the digestion procedure, is correct. The samples were, in addition, analysed following an oxygen-flask combustion procedure,[5] which showed no interference from sulphide and which should be capable of dealing with samples containing mercury(II) sulphide.

The results are shown in Table I and confirm that an effective recovery of mercury as mercury(II) sulphide is obtained at these concentrations. This conclusion is supported by the fact that other workers[6,7] have shown that mercury(II) sulphide can be dissolved by use of concentrated sulphuric acid and potassium permanganate. In addition, a large number of comparative analyses of soils, peats and rocks by the digestion and the oxygen flask combustion procedures[5] have shown no disagreement, although mercury(II) sulphide is a highly probable component.

The apparent failure of oxidative digestion procedures with mercury(II) sulphide may be explained, in part, by the fact that where mercury occurs as sulphide, there is a strong possibility that other sulphides, and in particular iron(II) sulphide, will occur in relatively large amounts. It has been shown (unpublished work) that oxidative digestion converts the iron(II) to the iron(III) state, in which form it preferentially oxidises the tin(II) chloride

used at the reduction - aeration stage. As a result, the reduction of mercury compounds to the elemental mercury, which is essential in atomic-absorption determination, is impeded or prevented with samples the iron content of which is greater than 20%. The iron content of rocks and soils is generally too low for this interference to occur, but in ores and minerals the effect could be important.

TABLE I

RECOVERY OF MERCURY FROM SIMULATED ROCK SAMPLES CONTAINING MERCURY(II) SULPHIDE BY OXIDATIVE DIGESTION AND BY OXYGEN-FLASK COMBUSTION PROCEDURES

Form of mercury present	Theoretical mercury content, p.p.m.	HNO_3 - H_2SO_4 - $KMnO_4$ digestion: mercury content,* p.p.m.	Oxygen-flask combustion: mercury content,* p.p.m.
Mercury(II) sulphide†	0.21	0.29	0.25
Cinnabar‡	0.07	0.07	0.08
Cinnabar	0.14	0.18	0.21
Cinnabar	0.80	0.90	0.70

* Method of Ure and Shand.[5]
† Laboratory-reagent grade mercury(II) sulphide.
‡ Supplied by courtesy of the Department of Geology, Aberdeen University.

Conclusion

It has been shown, by using the atomic-absorption reduction - aeration technique, that mercury as mercury(II) sulphide can be recovered effectively, in the amounts likely to occur in soils and rocks, by sulphuric acid - nitric acid - potassium permanganate oxidative digestion. It is suggested that interference at the reduction - aeration stage by iron(III), naturally present or formed by oxidation during digestion, may be a partial explanation of an apparent failure to dissolve mercury(II) sulphide.

References

1. Jacobs, L. W., and Keeney, D. R., *Envir. Sci. Technol.*, 1974, **8**, 267.
2. Agemian, H., and Chau, A. S. Y., *Analyst*, 1976, **101**, 91.
3. Iskandar, I. K., Syers, J. K., Jacobs, L. W., Keeney, D. R., and Gilmour, J. T., *Analyst*, 1972, **97**, 388.
4. Jonasson, I. R., Lynch, J. J., and Trip, L. J., *Geol. Surv. Pap. Can.*, 1973, paper 73–21.
5. Ure, A. M., and Shand, C. A., *Analytica Chim. Acta*, 1974, **72**, 63.
6. Tomicek, O., *Chem. Ztg*, 1925, **49**, 281.
7. Kolthoff, I. M., Sandell, P. J., and Sandell, E. B., "Analytical Chemistry of the Elements," Volume 3, Part II, Interscience, New York, 1961, p. 254.

Received *July 5th*, 1976
Accepted *August 24th*, 1976

Determination of Silica in Rocks and Minerals by a Combined Gravimetric and Atomic-absorption Spectrophotometric Procedure

J. N. Walsh
Department of Geology, King's College, University of London, Strand, London, WC2R 2LS

Keywords: *Silica determination*; *minerals*; *gravimetry*; *atomic-absorption spectrophotometry*

The determination of silica in a rock or mineral remains one of the most serious problems in the analysis of silicates. In most rock or mineral samples analysed silica is the most abundant oxide and in many instances it is more abundant than all of the other elements

combined. For this reason the accuracy required is of a different order of magnitude from that required for the other elements. Silica contents of rocks and minerals are normally in the range 40–75% and are quoted to two decimal places.

There are a number of direct instrumental methods for the determination of silica, *e.g.*, X-ray fluorescence, atomic-absorption spectrophotometry and colorimetry. However, it is debatable whether or not these methods provide the same degree of accuracy as the classical gravimetric method. The purpose of this paper is to give details of a method adapted from the gravimetric method of Washington.[1] The original gravimetric procedure involved a double evaporation with hydrochloric acid and removal of the insoluble silica by filtration. In the proposed method only one evaporation is carried out and the silica remaining in solution is determined by atomic-absorption spectrophotometry.

The method is similar to that proposed by Jeffery and Wilson,[2] in which the silica remaining in solution after one evaporation is determined colorimetrically, but the proposed method is more rapid and is not restricted to the analysis of rocks containing less than 20% of iron(III) oxide.

New values obtained for a number of standard rocks, covering a wide range of compositions, are presented in an attempt to assess the accuracy of the method.

Procedure

One gram of powdered rock is weighed accurately into a platinum or palau (gold - palladium alloy) crucible and mixed carefully with approximately 6 g of anhydrous sodium carbonate. The mixture is fused at 900 °C for 1 h and then allowed to cool. The fusion cake is transferred into a 250-ml platinum dish, covered with a watch-glass in order to avoid loss of material through effervescence and dilute hydrochloric acid is added. The resulting solution is evaporated to dryness on a water-bath in order to make the silica insoluble. After cooling the dish and contents, the soluble salts are re-dissolved in 100 ml of 20% hydrochloric acid and the silica is filtered off through a 12.5 cm diameter Whatman No. 40 filter-paper. The silica residue is washed eight times with warm 5% hydrochloric acid, transferred to a 35-ml platinum crucible and then dried and ignited at 1 100 °C until a constant mass is recorded. Ten drops of 25% sulphuric acid and 15 ml of hydrofluoric acid are then added and the silica is evaporated off as volatile silicon tetrafluoride on a hot-plate in a fume cupboard. The crucible is then heated slowly over a Bunsen burner in order to drive off excess of sulphuric acid, the contents are re-ignited at 1 100 °C and the crucible is re-weighed. The loss in mass is presumed to be due to the insoluble silica.

A small amount of silica (experience has shown this to be about 2–6 mg for most rock compositions) remains in solution after the evaporation and filtration. This amount is determined by means of atomic-absorption spectrophotometry. The filtrate is diluted to 500 ml and sprayed directly into the nitrous oxide - acetylene atomising flame. The instrument settings are not critical provided that a fuel-rich flame is used in order to obtain the necessary sensitivity for silicon.

A standard solution containing 0.05 mg ml^{-1} of silica, which is equivalent to 2.5% of silica in a rock, is prepared and its absorbance measured.

Finally, the value for the amount of silica determined gravimetrically is added to the value for the small amount in the filtrate that has been determined by atomic-absorption spectrophotometry, to give the total silica content of the rock or mineral.

Results

As has occurred in other studies,[3–5] no serious interferences were found in the atomic-absorption spectrophotometric determination of silica.

The amount of residual silica found in solution after one evaporation (expressed as a percentage by mass in the rock), for the standard rocks analysed, is given in Table I. This table also gives the absorbance values obtained for silica in the rocks and the standard solution. No scale expansion was used and only one standard solution is normally necessary as the calibration graph is linear over the working range (0–0.05 mg ml^{-1} of silica).

These amounts of silica were added to the amounts obtained gravimetrically to give the values shown in Table II. The accuracy of the method can be assessed from the close agreement between the values obtained and the quoted values. The agreement is greatest for

TABLE I

SILICA REMAINING IN SOLUTION AS DETERMINED BY ATOMIC-ABSORPTION SPECTROPHOTOMETRY AFTER A SINGLE EVAPORATION WITH HYDROCHLORIC ACID

Absorbance for 0.05 mg ml^{-1} standard solution of silica (equivalent to 2.5% of silica in rock) is 0.135.

Rock*	Absorbance	Silica, %
W-1	0.023	0.43
BCR-1	0.031	0.57
AGV-1	0.024	0.44
GSP-1	0.025	0.46
G-2	0.017	0.31
NIM-G	0.025	0.47
NIM-S	0.020	0.37
NIM-N	0.024	0.44
NIM-L	0.031	0.57
NIM-D	0.017	0.31

* US Geological Survey and National Institute for Metallurgy standard rocks. See footnote to Table II.

the US Geological Survey standard rocks, for which the quoted values are compiled from a much larger number of determinations than are the values for the newer NIM standard rocks. The values given in Table II were obtained on a routine basis and are single determinations, not the best of several attempts.

TABLE II

PERCENTAGE OF SILICA IN ROCK SAMPLES AS DETERMINED BY THE PROPOSED COMBINED PROCEDURE AND COMPARED WITH QUOTED VALUES

	Silica, %	
Rock*	Combined procedure	Quoted value*
W-1	52.66	52.64
BCR-1	54.45	54.50
AGV-1	59.07	59.00
GSP-1	67.40	67.38
G-2	69.09	69.11
NIM-G	75.62	75.59
NIM-S	63.66	63.72
NIM-N	52.61	52.43
NIM-L	52.48	52.52
NIM-D	39.05	38.86

* Values obtained by Flanagan[6] for US Geological Survey samples W-1 (diabase), BCR-1 (basalt), AGV-1 (andesite), GSP-1 (granodiorite), G-2 (granite) and National Institute for Metallurgy (South Africa) samples NIM-G (granite), NIM-S (syenites), NIM-N (norite), NIM-L (lujavrite), NIM-D (dunite).

Conclusion

The proposed method offers a considerable improvement in speed and probably some improvement in accuracy over the original gravimetric method as it is known that small amounts of silica can remain in solution even after two evaporations with hydrochloric acid. The method is more rapid than the combined gravimetric and colorimetric method of Jeffery and Wilson.[2]

References

1. Washington, H. S., "Chemical Analysis of Rocks," John Wiley, New York, 1930.
2. Jeffery, P. G., and Wilson, A. D., *Analyst*, 1960, **85**, 478.
3. Van Loon, J. C., and Parissis, C. M., *Analyst*, 1969, **94**, 1057.
4. Lee, R. W., and Güven, N., *Chem. Geol.*, 1975, **16**, 53.
5. Whiteside, P. J., "Atomic Absorption Data Book," Pye Unicam, Cambridge, 1975.
6. Flanagan, F. J., *Geochim. Cosmochim. Acta*, 1973, **37**, 1189.

Received *July 21st*, 1976
Accepted *September 16th*, 1976

Method for the Extraction of Organochlorine Compounds from Quail Eggs

Grahame L. Wardall

Ministry of Agriculture, Fisheries and Food, Pest Infestation Control Laboratory, Tolworth, Surbiton, Surrey, KT6 7NF

Keywords: *Organochlorine compounds*; *quail eggs*; *extraction*; *gas - liquid chromatography*

A need arose in this laboratory for a rapid method for extraction of organochlorine compounds from the eggs of Japanese quail (*Coturnix coturnix japonica*) that had been fed experimentally with various organochlorine pesticides. Published methods did not meet our criteria for rapidity,[1] reproducibility[2] or purity of the final extract,[3] and an alternative method was therefore sought. The proposed method combines clean-up and extraction procedures, thus saving time, and produces an extract that is clean enough for electron-capture gas - liquid chromatography without affecting the long-term sensitivity of the apparatus.

Experimental

Apparatus

A Pye, Series 104, chromatograph equipped with a nickel-63 electron-capture detector was used, together with a 5 ft × 4 mm i.d. silanised glass column packed with a 1 + 1 mixture of 2% OV-101 and 2% OV-210 on 100–120-mesh Diatomite C pre-treated with 0.2% Epikote 1001 resin.

The chromatography tube had the dimensions 35 × 2 cm, with a medium sinter and tap. A 14 cm diameter glass mortar and pestle was used to grind the samples.

Reagents

Hexane. AnalaR. Re-distilled, the fraction boiling at 69 °C being used.

Sodium sulphate. AnalaR. Dried at 400 °C for 24 h.

Celite 545.

Basic alumina, 100–200 mesh, Type H. Peter Spence & Sons. Activity 3.5 (by Desaga - Giulini test kit).

All reagents and glassware were checked for electron-capturing impurities during the course of blank extractions of control quail eggs.

Preparation of Sample

Grind the egg (5–10 g) with 20 g of sodium sulphate until a fine dry powder is formed; occasionally more sodium sulphate is necessary. Chop any large pieces of shell finely with scissors and then grind. Add 5 g of Celite 545 and mix evenly. The resulting powder can now be passed to the extraction stage or can be stored in stoppered bottles with no apparent effect on the recovery.

Half fill the chromatography column with re-distilled hexane. Pack 5 g of alumina into the bottom of the column, then add the egg powder, taking care to remove any air bubbles

by tapping the column. Attach a reservoir of solvent. Open the tap and collect 100 ml of the eluate in a calibrated flask; all of the compounds tested have been found to be eluted in this volume.

Gas Chromatography

Analyse the eluate without further treatment by using the OV-101 - OV-210 column maintained at 180 °C, with a detector temperature of 300 °C, an injection temperature of 210 °C and a flow-rate of carrier gas (oxygen-free nitrogen) of 50 ml min^{-1}.

Results and Discussion

Recoveries were measured by spiking control eggs by addition to the powder and by injection into the whole egg of 50-μl volumes with a Hamilton syringe. Three eggs were spiked at each of five concentrations of the compounds in hexane; the concentrations used were chosen to approximate to the residues expected in the experimental eggs (1–10 μg). The solvent was allowed to evaporate from the powder before continuing the analysis. No difference was detectable in the recoveries from the two methods of spiking (Table I). Four chlorinated biphenyls were also tested for recovery, with the following results (mean values): decachlorobiphenyl, 63%; 2,4,6,4'-tetrachlorobiphenyl, 61%; 2,4,6,2'-tetrachlorobiphenyl, 60%; and 4,4'-dichlorobiphenyl, 80%. The method has been applied successfully to over 200 laboratory samples.

TABLE I

RECOVERIES OF PESTICIDES FROM SPIKED QUAIL EGGS

Compound spiked*	Recovery, % Range	Mean
pp'-DDE	86–98	91.4
pp'-DDD	85–101	93.0
pp'-DDT	80–98	90.8
op'-DDT	92–99	96.0
HEOD	75–94	82.6
DDMU	77–95	84.5

**pp'*-DDE = 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene; *pp'*-DDD = 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane; *pp'*-DDT = 1,1,1-trichloro-2,2-bis(4-chlorophenyl)-ethane; *op'*-DDT = 1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane; HEOD = 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-*exo*-1,4-*endo*-5,8-dimethanonaphthalene (dieldrin); DDMU = 1-chloro-2,2-bis(4-chlorophenyl)ethylene.

For experiments involving many routine analyses, the continual use of an electron-capture detector with crude extracts can have serious effects on the life of the column and the sensitivity of the detector, and the high albumen and water contents of the eggs can cause particular difficulty. Extracts prepared by the proposed method have had no noticeable effect on the standing currents of the detector and the life of the column was greatly improved. Combined with speed of extraction and reproducible recovery, these advantages have led to our adoption of this method for several large-scale biological experiments, in which the order of magnitude of the residues was required. With some samples, a ten-fold concentration was achieved with no effect on the chromatograph, so increasing the sensitivity to 0.01 mg kg^{-1}.

I acknowledge discussions with and help from Mr. S. Bailey and the technical assistance of Mrs. H. P. Wardall.

References

1. Holden, A. V., and Marsden, K., *J. Chromat.*, 1969, **44**, 481.
2. Maunder, M. J. de F., Egan, H., Godly, E. W., Hammond, E. W., Roburn, J., and Thomson, J., *Analyst*, 1964, **89**, 168.
3. Taylor, A., *Analyst*, 1962, **87**, 824.

Received *May 17th*, 1976
Accepted *August 2nd*, 1976

Determination of the Anthelmintic Diethylcarbamazine in Tissue and Plasma by Gas - Liquid Chromatography

J. A. Bogan

Department of Veterinary Pharmacology, University of Glasgow Veterinary School, Veterinary Hospital Bearsden Road, Bearsden, Glasgow, G61 1QH

Keywords: Diethylcarbamazine determination; anthelmintic; tissue; plasma; gas - liquid chromatography

Diethylcarbamazine (1-diethylcarbamoyl-4-methylpiperazine; Banocide; Hetrazan) has been used as an anthelmintic in man and animals for about 30 years. It is used in man for the treatment of filariasis and is widely used in domesticated animals against lungworms, especially in cattle, but has also been used against a number of other helminth parasites. No method is available for the determination of diethylcarbamazine residues in tissue or plasma and the only report on the kinetics of diethylcarbamazine is that of Brown,[1] concerning [^{14}C]diethylcarbamazine in man. Because of the requirements of the Medicines Act 1968, it is necessary to determine the residues of drugs in animals to be used as food and a method for the determination of diethylcarbamazine in tissue and plasma is the subject of this paper.

Experimental

Reagents

All reagents were of AnalaR grade.

Hexane. Redistilled, boiling range 67.5–69.5 °C.

Apparatus

Gas chromatograph, Pye 104. A 0.3 m × 4 mm i.d. column containing DC-200 (10% *m/m*) on 80–100-mesh Gas-Chrom Q was used, with column temperature 150 °C, detector at 200 °C and injection at 150 °C. The carrier gas was nitrogen at a flow-rate of 60 ml min^{-1}.

Detector. A flame-ionisation detector with hydrogen at 60 ml min^{-1} and air at 250 ml min^{-1} was used.

The column was pre-conditioned for 1 week at 225 °C. Repeated injections of 2 μl of a 1 000 μg ml^{-1} solution of diethylcarbamazine in hexane were made until a reproducible response was obtained to 20 μl of a 10 μg ml^{-1} solution. This reproducibility was obtained

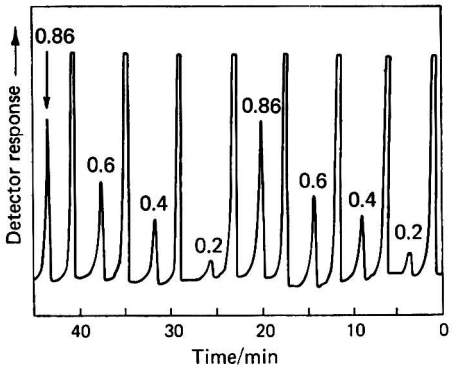


Fig. 1. Gas-chromatographic response to repeated injections of various amounts of diethylcarbamazine. The numbers indicate amount of sample injected in micrograms.

after conditioning for about 3 d. After this conditioning period the peak shape improved and reproducible responses were obtained after a single conditioning injection of 2 μl of a 1 000 μg ml^{-1} solution every 3 h. Under these conditions diethylcarbamazine has a retention time of 2.8 min. Typical responses are shown in Fig. 1 for successive 20-μl injections of 10, 20, 30 and 43 μg ml^{-1} solutions of diethylcarbamazine.

Partition Coefficients of Diethylcarbamazine

Standard aqueous solutions of diethylcarbamazine were prepared and adjusted to 1 N with hydrochloric acid and to 1 and 5 N with sodium hydroxide solution. Aliquots of these solutions were shaken for 10 min with equal volumes of redistilled hexane. The partition coefficients of diethylcarbamazine between hexane and the aqueous solutions were calculated from the amount recovered in the hexane layer as determined by means of gas - liquid chromatography. The values were 0, 0.15 and 0.93 for partition between hexane and 1 N hydrochloric acid, 1 N sodium hydroxide and 5 N sodium hydroxide solution, respectively.

Procedure

On the basis of the partition coefficients, it was decided to use the following method for extraction of diethylcarbamazine from aqueous solutions obtained from meat samples.

Tissue

A sample of meat is macerated in a Waring blender and a 50-g portion of the macerated sample is extracted for 2 h in a Soxhlet apparatus with 200 ml of water to which 10 ml of 1 N hydrochloric acid have been added. The extract is then concentrated to 10 ml on a hot-plate (diethylcarbamazine is stable under these conditions) and the 10-ml sample cooled and extracted with five 20-ml portions of hexane. The hexane washes are discarded and the aqueous solution is made alkaline by carefully adding solid sodium hydroxide (2.0 g) to a concentration of approximately 5 N, prior to extraction with two 20-ml portions of hexane. The hexane extracts are combined and evaporated under nitrogen to a volume of 0.5 ml. Twenty-microlitre aliquots of this solution are used for injection. If there is no detectable diethylcarbamazine in the 0.5-ml sample, the sample is concentrated to 0.2 ml and 20 μl of this solution are injected. Beyond this level of concentration the background contamination becomes too great and obscures the diethylcarbamazine peak.

The minimum detectable concentration of diethylcarbamazine in the injected sample is 2 μg ml^{-1}, which is equivalent to a concentration of 0.008 μg g^{-1} in meat.

With fat, the aqueous Soxhlet extract could not be concentrated to 10 ml owing to extracted fats and the extract was therefore first concentrated to 50 ml and washed with five 20-ml portions of hexane before further concentration to 10 ml and subsequent extraction.

Plasma

One millilitre of plasma is diluted with 3 ml of water and 1 ml of 1 N hydrochloric acid. The aqueous solution is washed with five 10-ml portions of hexane and the hexane layer discarded. The aqueous layer is then made alkaline, by adding solid sodium hydroxide to a concentration of 5 N, prior to extraction with two 10-ml portions of hexane. The hexane layers are combined and concentrated to 0.2 ml. Twenty-microlitre aliquots are used for injection.

The minimum detectable concentration of diethylcarbamazine in the injected sample is 2 μg ml^{-1}, which is equivalent to a concentration of 0.4 μg ml^{-1} in plasma. Typical chromatographic responses from positive samples are shown in Fig. 2.

Results

Recoveries of Diethylcarbamazine from Meat and Fat

Small aliquots of a solution of diethylcarbamazine in hexane were added to 50-g samples of macerated meat (muscle free of fat) and fat so as to give concentrations of 0.05, 0.10, 0.20, 0.30 and 0.40 μg g^{-1} of diethylcarbamazine. Recoveries varied between 64 and 87% for duplicate samples of meat at each concentration and between 55 and 80% for single samples of fat at each concentration. The mean recovery was 72% from meat and 66% from fat. Results of diethylcarbamazine determinations in meat and fat were therefore adjusted for these recoveries.

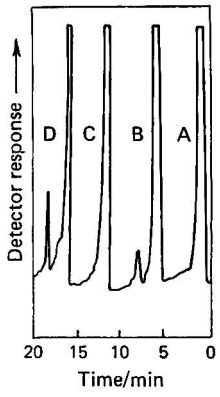


Fig. 2. Gas-chromatographic responses to A, blank muscle sample taken through procedure; B, muscle sample from dosed sheep; C, blank plasma sample taken through procedure; and D, plasma sample from orally dosed calf.

Residues of Diethylcarbamazine in Tissue

The study of residues indicated that diethylcarbamazine has a short half-life and in order to confirm this result diethylcarbamazine was administered to one 1-year-old calf orally, to a level of 25 mg kg^{-1}, and to another 1-year-old calf intramuscularly to the same level. The concentrations of diethylcarbamazine in plasma shown in Table I indicate that diethylcarbamazine has a short half-life in cattle.

TABLE I

VARIATION OF CONCENTRATION OF DIETHYLCARBAMAZINE (μg ml^{-1}) IN PLASMA IN CATTLE WITH TIME AFTER ADMINISTRATION

Time of sampling/min	Route	
	Oral	Intramuscular
0	n.d.*	n.d.
10	1.2	2.7
30	2.8	3.4
60	9.6	2.5
120	1.0	0.8
300	n.d.	n.d.
480	n.d.	n.d.

* n.d. = none detected, *i.e.*, 0.4 μg or less per millilitre of plasma.

Using the gas-chromatographic method described above and a column containing 3% OV-17 on Diatomite CQ under the same chromatographic conditions, the diethylcarbamazine recovered from plasma and tissue samples was found to have the same retention time and peak shape as those of authentic diethylcarbamazine.

Discussion

Problems with the Gas-chromatographic Analysis

As diethylcarbamazine has no suitable ultraviolet absorption or fluorescence, a gas-chromatographic method of analysis was sought and initially there was great difficulty in

finding a suitable stationary phase. A variety of columns of polar, non-polar and intermediate nature were tried, *viz.*, 3% SE-30 on Diatomite CQ, 10% DC 200 on Gas-Chrom Q, 3% OV-17 on Diatomite CQ, 10% PEG 20M on Gas-Chrom Q and 6% Pentasil-350 on Gas-Chrom Q. On all the column packings, the peak shape for diethylcarbamazine was tailed and a further problem was that variable responses were obtained for equal amounts of the compound. It was found that each column required a long pre-conditioning period (about 1 week) and that in order to obtain consistent responses the column had to be saturated with diethylcarbamazine. Of the column packings tried, 10% DC 200 on 80–100-mesh Gas-Chrom Q was found to give the most consistent performance. The performance was not improved by reducing the amount of stationary phase or by reducing the column temperature, which suggests that the loss of diethylcarbamazine on the column is caused by interaction with the support material and not by absorption on the stationary phase or by thermal breakdown.

Metabolism of Diethylcarbamazine

No evidence that diethylcarbamazine is metabolised was obtained by use of this method. Brown[1] has shown that in rats and monkeys 70% of ^{14}C-labelled diethylcarbamazine is excreted in 24 h in urine with the piperazine ring intact. Although diethylcarbamazine is lipid-soluble it is also extremely water-soluble and it is probable, therefore, that a large proportion will be excreted in urine unchanged.

The author thanks Robert Young & Co. Ltd. for financial support for this study and Miss R. Brockie and Mrs. M. Hossack for technical assistance.

Reference

1. Brown, H. W., *Clin. Pharmac. Ther.*, 1960, **1**, 87.

Received *February 27th*, 1976
Accepted *April 22nd*, 1976

Determination of Piperazine in Solutions Containing 1,1,4,4-Tetramethylpiperazinium Diiodide, Thiamine and Pyridoxine

G. C. Kondos*

Research Laboratories, Chropei Pharmaceutical Company, Piraeus, Greece

Keywords: *Piperazine determination*; *1,1,4,4-tetramethylpiperazinium diiodide*; *thiamine*; *pyridoxine*; *column chromatography*

There is an extensive literature on methods for the quantitative determination of piperazine,[1–28] but the analysis is difficult in the presence of 1,1,4,4-tetramethylpiperazinium diiodide, thiamine and pyridoxine, as they are all heterocyclic organic bases that often give the same colour reactions as piperazine and they, or their derivatives, are extracted together with the piperazine into an alkaline solution after acetylation.

This difficulty was enhanced in the presence of 1,1,4,4-tetramethylpiperazinium diiodide which, being homologous with piperazine, gives the same colour reactions,[4,6,21] especially the reaction with trinitrophenol,[25–28] which is the usual method for the gravimetric determination of piperazine.

This paper describes a method for the gravimetric determination of piperazine as diacetylpiperazine in an aqueous solution of 1,1,4,4-tetramethylpiperazinium diiodide, thiamine and pyridoxine in the presence of small amounts of thiourea and 1,1,1-trichloro-2-methylpropan-2-ol. A chromatographic method is used for the quantitative acetylation of piperazine to diacetylpiperazine, which is separated from the related accompanying compounds by chromatography in a glass column packed with Celite. The method can be applied to pharmaceutical preparations that are used for treatment of rheumatic diseases, neuritis, spondylo-

* Present address: Centre of Higher Technical Education, 95 Dinokratus Street, Athens, Greece.

arthritis, etc. It can also be used for animal feeds medicated with piperazine alone or in admixture with 1,1,4,4-tetramethylpiperazinium diiodide and other organic bases. The melting-point of diacetylpiperazine recrystallised from chloroform has been determined and confirmed by a mixed melting-point determination, but the value was below that given in the literature.[7,29,30]

Experimental

The following test solution was prepared by dissolving the substances in water and diluting to 100 ml: thiamine hydrochloride, 2.5 g; pyridoxine hydrochloride, 1.0 g; 1,1,4,4-tetramethylpiperazinium diiodide, 2.0 g; piperazine hexahydrate, 1.0 g; thiourea, 0.1 g; and 1,1,1-trichloro-2-methylpropan-2-ol, 0.1 g.

Determination of Piperazine Gravimetrically as Diacetylpiperazine in an Alkaline Solution

A 50-ml portion of the test solution was placed in a 250-ml beaker and 100 ml of a 12.5% *m/V* solution of sodium hydrogen carbonate were added. After the evolution of carbon dioxide had ceased the pH of the solution was 7.6 and 4 ml of acetic anhydride were added slowly in 1-ml portions, allowing 5 min, with stirring, before each addition for the carbon dioxide to be evolved. The pH of the solution was 7.1. The contents of the beaker were transferred quantitatively into a 250-ml separating funnel and extracted with 80-, 60-, 40- and 20-ml volumes of chloroform. The chloroform solution was dried with sodium sulphate and evaporated in a dish placed on a water-bath in an air stream, followed by 3 h at 50 °C under a pressure of 1 mmHg. The theoretical conversion factors were $F = 1.141\ 1$ to piperazine hexahydrate and 0.505 5 to piperazine.

The product was a mass of large crystals, melting-point 90–100 °C, in a yield of 110% owing to the presence of pyridoxine. Repetition of the above procedure gave a similar mass of large crystals, melting-point 90–97 °C, yield 113%.

Determination of Piperazine Gravimetrically as Diacetylpiperazine in an Alkaline Solution in the Absence of Pyridoxine

The procedure described above was followed exactly except that pyridoxine hydrochloride was omitted from the test solution and the product was recrystallised from benzene, giving a yield of 270.0 mg of derivative (65% of theoretical), melting-point 139 °C. A repeat determination gave a yield of 280.0 mg (68% of theoretical) in the form of large crystals, melting-point 140 °C.

This procedure was repeated a further eight times to obtain the results given in Table I. It was concluded that piperazine could not be acetylated quantitatively in the presence of water and that pyridoxine was extracted from an alkaline solution by chloroform together with the diacetylpiperazine. A method of acetylation using Celite as a solid diluent was developed.

TABLE I

YIELD OF DIACETYLPIPERAZINE FROM TEST SOLUTION IN THE ABSENCE OF PYRIDOXINE USING A GRAVIMETRIC METHOD

A 50-ml volume of test solution, containing 500 mg of piperazine hexahydrate and with pyridoxine hydrochloride omitted, was taken.

Mass of diacetylpiperazine/mg	Melting-point/°C	Yield, %
275	140	67
270	139	65
280	140	68
270	140	65
265	139	63.5
270	138	65
275	138	67
265	139	63.5

Chromatographic Method of Acetylation of Piperazine on Celite as Diluent

Mix, in a beaker, 25 ml of the test solution, 25 g of Celite 525 (Koch-Light Laboratories Ltd.) and 5 g of sodium hydrogen carbonate and knead the mixture thoroughly. Then add

2 ml of acetic anhydride and continue the kneading for 5 min. Prepare a glass chromatographic column (300 × 40 mm) containing 5 g of Celite 545 and above it 20 g of Celite 545 well kneaded with 20 ml of 1 N sulphuric acid for retention of pyridoxine or any other bases. Transfer the mixture to the column, using a further 5 g of Celite 545 to remove trace amounts of material from the beaker.

Tamp each of the above four layers after its addition in order to produce a column with a flow-time of 30 min for 200 ml of chloroform.

In order to effect transfer of trace amounts of diacetylpiperazine from the beaker add 200 ml of chloroform to the beaker and use this to elute the diacetylpiperazine from the chromatographic column. Evaporate the chloroform solution in a tared dish by heating the dish on a water-bath in an air stream. After the complete removal of the solvent, dry the residue at 50 °C under a pressure of 1 mmHg, for 3 h or until of constant mass (see Note). The melting-point of the product was 138–140 °C. Dissolve the contents of the dish in a methanol - diethyl ether (1 + 1) mixture and transfer it on to a column of the same dimensions (300 × 40 mm), containing 60 g of either Celite 545 or neutral alumina according to Brockmann. This second purification through the chromatographic column is not considered essential as it is intended only to remove, by simple absorption, unweighable amounts of impurities in order to obtain the theoretical melting-point of 142.5 °C, which serves as an identification for diacetylpiperazine. The identity of this material can be confirmed by carrying out a mixed melting-point determination with an authentic specimen of diacetylpiperazine.

NOTE: Use a dish with a weighed glass cover to avoid loss of 1,1,1-trichloro-2-methylpropan-2-ol by spitting.

The above procedure was repeated nine times starting with the same volume of the test solution and the results are given in Table II.

TABLE II

YIELD OF DIACETYLPIPERAZINE FROM TEST SOLUTION BY THE PROPOSED METHOD

A 25-ml volume of test solution, containing 250 mg of piperazine hexahydrate, was taken.

Mass of diacetylpiperazine/mg	Mass of piperazine hexahydrate calculated from mass of diacetylpiperazine/mg	Melting-point/°C	Yield, %
216.9	247.5	140	99.00
216.8	247.4	140	98.96
217.9	248.6	139	99.44
218.0	248.8	140	99.52
217.8	248.5	138	99.40
217.7	248.4	138	99.36
218.2	249.0	139	99.60
218.0	248.8	140	99.52
217.8	248.5	140	99.40

TABLE III

YIELD OF DIACETYLPIPERAZINE FROM A SOLUTION OF PIPERAZINE HEXAHYDRATE BY THE PROPOSED METHOD

A 25-ml volume of a 1% *m*/*V* solution of piperazine hexahydrate was taken.

Mass of diacetylpiperazine/g	Mass of piperazine hexahydrate calculated from mass of diacetylpiperazine/g	Melting-point/°C	Yield, %
0.217 9	0.248 6	140	99.46
0.217 2	0.247 8	139	99.14
0.217 8	0.248 5	140	99.41
0.217 1	0.247 7	140	99.09
0.219 4	0.250 4	138	100.14
0.219 0	0.249 9	139	99.96
0.219 1	0.250 0	140	100.00
0.218 3	0.249 1	139	99.64
0.217 9	0.248 6	140	99.45
0.217 0	0.247 6	140	99.05

Determination of Known Amounts of Piperazine

A 1% *m/V* solution of piperazine hexahydrate (USP XVIII) was prepared. A 25-ml portion of the solution was taken by pipette and treated according to the proposed method. The results of 10 replicate determinations are given in Table III.

No weighable amounts of residue were obtained when the test was carried out in the absence of piperazine, or on a solution of 1,1,4,4-tetramethylpiperazinium diiodide.

References

1. Mann, F. G., and Senior, A., *J. Chem. Soc.*, 1954, 4476.
2. Cavier, R., *Annls Pharm. Fr.*, 1955, **13**, 539.
3. Catogan, J. G., *J. Chem. Soc.*, 1955, 2971.
4. Rogers, E. W., *Br. Med. J.*, 1958, **2**, 1576.
5. Maynard, W. R., Jr., *J. Ass. Off. Agric. Chem.*, 1959, **42**, 610.
6. Beckman, H. F., and Feldman, L., *J. Agric. Fd Chem.*, 1960, **8**, 227.
7. Toldy, E., Csillag, F., Bobak, T., and Gyenes, I., *Magy. Kém. Foly.*, 1961, **67**(4), 180.
8. Pankratz, R. E., *J. Pharm. Sci.*, 1961, **50** 175.
9. Berisso, B., *Mikrochim. Acta*, 1962, 71.
10. Shah, R. C., Raman, P. V., and Mehta, M. M., *J. Pharm. Sci.*, 1965, **54**, 432.
11. Balandin, A. A., Ferapontov, V. A., Karpeiskaya, E. I., and Gorshkova, L. S., *Dokl. Akad. Nauk SSSR*, 1966, **170**, 589.
12. Loucks, M. F., and Nauer, L., Jr., *J. Ass. Off. Analyt. Chem.*, 1967, **50**, 268.
13. Friche, F. L., and Walters, S. M., *J. Ass. Off. Analyt. Chem.*, 1966, **49**, 1230.
14. Fürst, W., and Manneststätter, E., *Arch. Pharm., Berl.*, 1968, **301**, 596.
15. Ramotowski, S., and Srczosniak, M., *Dissnes. Pharm., Warsz.*, 1968, **20**, 453.
16. Spell, H. L., *Analyt. Chem.*, 1969, **41**, 902.
17. Eisenbraud, J., and Hauprich, H. E., *Arch. Pharm., Berl.*, 1970, **303**, 208.
18. Sile, M. K., Avot, A. A., Shimanskaya, M. V., and Stratin'sh, G. Yu., *Latv. PSR Zinàt. Akad. Vest., Kim. Ser.*, 1969, (5), 567; *Referat. Zh. Khim.* 19*GD*, 1970, (8), Abstr. No. 8 G259.
19. Knotz, F., *Scientia Pharm.*, 1970, **38**, 26.
20. Stewart, J. T., and Lotti, D. M., *Analytica Chim. Acta*, 1970, **52**, 390.
21. Ganesku, J., and Popescu, A., *Revue Roum. Chim.*, 1970, **15**, 1343.
22. McLean, J. D., and Daniels, O. L., *J. Ass. Off. Analyt. Chem.*, 1971, **54**, 555.
23. Mikhailova, T. A., Tavolski, D. Z., and Krolova, V. K., *Zav. Lab.*, 1972, **38**, 153.
24. Wang, R. T., Ven, H. C., and Lou, C. C., *Chemistry, Taipei*, 1971, (4), 107.
25. Wang, R. T., Ven, H. C., and Tsai, Y. H., *Chemistry, Taipei*, 1971, (1), 29.
26. "United States Pharmacopeia," XVIIIth Revision, Mack Publishing Co., Easton, Pa., 1970, p. 508.
27. "British Pharmacopoeia 1973," H.M. Stationery Office, London, 1973, p. 371.
28. "The British Pharmaceutical Codex 1975," The Pharmaceutical Press, London, 1975, p. 384.
29. Schmid, W. C., *Ber. Dt. Chem. Ges.*, 1891, **24**, 3241.
30. Herz, C., *Ber. Dt. Chem. Ges.*, 1897, **30**, 1585.

Received *November 11th*, 1975
Accepted *July 5th*, 1976

Spectrophotometric Determination of Methisazone

M. B. Devani, C. J. Shishoo and Hema J. Mody

Department of Pharmaceutical Chemistry, Lallubhai Motilal College of Pharmacy, Ahmedabad 9, India

Keywords: *Methisazone determination*; *spectrophotometry*; *2,3-dichloro-1,4-naphthoquinone reagent*

1-Methylindole-2,3-dione-3-thiosemicarbazone (methisazone) is used as a prophylactic against smallpox[1,2] and in the treatment of eczema vaccinatum[3,4] and gangrenosa vaccinia.[5,6] Deavin and Mitchell[7] have described the physical and chemical properties of methisazone and an iodimetric procedure has been proposed for its determination. The present work was undertaken to devise a simple colorimetric method for the determination of methisazone in dosage forms. Recently, 2,3-dichloro-1,4-naphthoquinone has been employed in the detection of various thio compounds.[8] The possibility of using this reaction for the colorimetric determination of methisazone has been investigated. The reaction conditions, such as concentration of ammonia, concentration of reagent and time of reaction, have been standardised and the results are shown to be in good agreement with those obtained by use of an iodimetric procedure.[7]

Experimental and Results

Apparatus

All spectral measurements were carried out on a Spectronic 20 spectrophotometer (Bausch and Lomb) equipped with four matched 10-ml cells with a 1-cm light path.

Reagents and Materials

Methisazone (Aldrich) and absolute ethanol were employed. All other reagents were of analytical-reagent grade.

Ethanolic ammonia solution, 12.5% *m/V*. Dry ammonia gas was passed into absolute ethanol at −5 °C until the mass had increased by 20%. The solution was then appropriately diluted with absolute ethanol.

2,3-*Dichloro*-1,4-*naphthoquinone reagent solution*. The reagent (65.0 mg) was dissolved in absolute ethanol and the solution diluted to 250 ml. It was then stored in a refrigerator.

Standard solution of methisazone, 0.3 mg ml^{-1}, *in absolute ethanol*. Freshly prepared solutions were used.

Determination of Wavelength of Maximum Absorbance

The standard solution of methisazone (4.0 ml) was mixed with 2.0 ml of ethanolic ammonia solution in a 25-ml calibrated flask. The mixture was allowed to stand for 20 min at room temperature and 12.0 ml of 2,3-dichloro-1,4-naphthoquinone solution were added. The reaction mixture was allowed to stand for 10 min before the final volume was adjusted to the mark with absolute ethanol. The absorbance was measured in the range 500–600 nm against a blank, which consisted of 12.0 ml of 2,3-dichloro-1,4-naphthoquinone reagent solution plus 2.0 ml of ethanolic ammonia solution diluted to 25.0 ml with absolute ethanol. The wavelength of maximum absorbance was found to be 560 nm (Fig. 1).

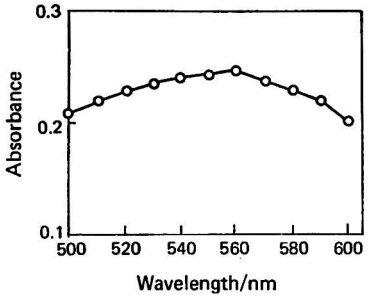


Fig. 1. Absorbance of the reaction product of methisazone standard solution (4 ml) with 12.0 ml of the reagent solution in the presence of 2.0 ml of ethanolic ammonia solution in 25.0 ml of reaction mixture.

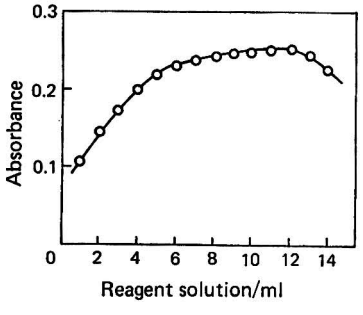


Fig. 2. Effect of the concentration of 2,3-dichloro-1,4-naphthoquinone reagent solution on the absorbance at 560 nm of the product formed on reaction with methisazone standard solution (4 ml) when using 2.0 ml of ethanolic ammonia solution in 25.0 ml of reaction mixture.

Factors that Affect the Reaction of Methisazone with 2,3-Dichloro-1,4-naphthoquinone Reagent

In the preliminary experiments, the determination of methisazone was attempted under the experimental conditions described previously for the determination of thiacetazone with 2,3-dichloro-1,4-naphthoquinone reagent.[9] However, the colour intensity of the product thus obtained was not measurable, and optimum reaction conditions were therefore sought.

Concentration of 2,3-*dichloro*-1,4-*naphthoquinone reagent*

The absorbance at 560 nm of the coloured product formed by the reaction of 4.0 ml of the methisazone standard solution with 2,3-dichloro-1,4-naphthoquinone increased as the

concentration of the reagent increased. The maximum absorbance was obtained in the presence of 12.0 ml of the reagent solution and decreased slightly on further increase in the volume of reagent solution (Fig. 2).

Concentration of ammonia

Methisazone standard solution (4.0 ml) was mixed with various amounts of ethanolic ammonia solution, the mixture was allowed to stand for 20 min at room temperature (see below) and then 12.0 ml of 2,3-dichloro-1,4-naphthoquinone solution were added. Maximum colour intensity was obtained in the presence of 2.0 ml of ethanolic ammonia solution (Fig. 3).

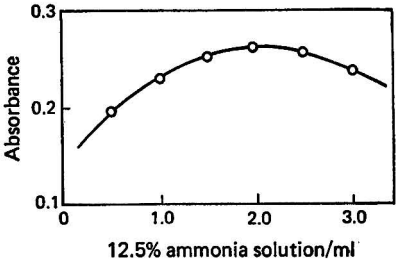


Fig. 3. Effect of the concentration of ethanolic ammonia solution on the absorbance at 560 nm of the product formed on reaction of methisazone standard solution (4 ml) with 12.0 ml of the reagent solution in 25.0 ml of reaction mixture.

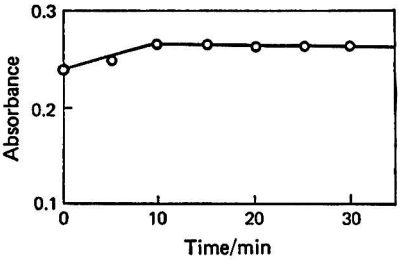


Fig. 4. Rate of development of colour after reaction of methisazone standard solution (4 ml) with 12.0 ml of the reagent solution when using 2.0 ml of ethanolic ammonia solution in 25.0 ml of reaction mixture.

Time of reaction between methisazone and ammonia

Maximum colour intensity developed on keeping the reaction mixture containing 4.0 ml of methisazone standard solution and 2.0 ml of ethanolic ammonia solution for 20 min before adding 12.0 ml of 2,3-dichloro-1,4-naphthoquinone reagent solution. In the subsequent reaction, the mixture was allowed to stand at room temperature for different time intervals. The colour intensity was found to reach a maximum after 10 min and to remain constant for about 3 h (Fig. 4).

Comparison with Iodimetric Method

Pure methisazone samples were analysed by the present method and an iodimetric method. The results are given in Table I, and show good agreement.

TABLE I

DETERMINATION OF METHISAZONE BY THE PROPOSED AND AN IODIMETRIC METHOD

	Proposed method			Iodimetric method[7]		
Sample No.	Amount taken/mg	Amount recovered/mg	Recovery, %	Amount taken/mg	Amount recovered/mg	Recovery, %
1	19.7	19.5	99.0	102.0	101.0	99.1
2	20.0	20.0	100.0	105.5	103.4	98.0
3	36.2	36.0	99.4	103.7	102.3	98.7
4	40.0	40.0	100.0	100.0	98.2	98.2
5	28.3	28.0	98.9	100.0	99.9	99.9
6	31.2	31.0	99.4	99.0	99.0	100.0
7	42.5	42.0	98.8	105.5	104.2	98.8
8	24.5	24.5	100.0	107.0	105.9	99.0
	Average ..		99.4	Average ..		99.0
	Standard deviation		±0.51	Standard deviation		±0.71

Analysis of Methisazone in Suspension (Marboran)

A suspension containing 30.0 mg of methisazone was accurately weighed and dissolved in warm ethanol. After cooling, it was transferred into a 100-ml calibrated flask and the final volume was adjusted to the mark with ethanol. The solution (4.0 ml) was pipetted into a 25-ml calibrated flask and treated as described above.

The amount of methisazone contained in the sample was determined by reference to a calibration graph. It was found that a graph of absorbance at 560 nm *versus* concentration of methisazone in the reaction mixture was a straight line passing through the origin for concentrations in the range 12.0–84.0 μg ml^{-1} when using 12.0 ml of the reagent solution and 2.0 ml of ethanolic ammonia solution in 25.0 ml of reaction mixture. Results for the analysis of sachets of Marboran suspension are given in Table II.

TABLE II

DETERMINATION OF METHISAZONE IN SUSPENSION

Sample No.	Labelled amount per sachet/g	Recovery per sachet*	
		Proposed method/g	Iodimetric method[7]/g
1	3.0	3.0 ± 0.02	3.0 ± 0.02
2	3.0	3.0 ± 0.02	3.0 ± 0.02

* Recovery ± standard deviation calculated from 10 determinations.

Analysis of Synthetic Mixtures Containing Methisazone

Four mixtures with the compositions given in Table III were prepared. A portion of the mixture equivalent to 30.0 mg of methisazone was accurately weighed. Four portions of 20.0 ml of warm absolute ethanol were used to extract the methisazone from the powder and each extract was filtered through a Whatman No. 40 filter-paper. The residue on the filter-paper was then washed with 10 ml of ethanol. The filtrate and the washings were combined in a 100-ml calibrated flask and, after cooling, the final volume was adjusted to the mark with ethanol. The solution (4 ml) was pipetted into a 25-ml calibrated flask and treated as described above.

TABLE III

RECOVERY OF METHISAZONE FROM VARIOUS EXCIPIENTS BY THE PROPOSED METHOD

Amount of methisazone in sample/g	Excipients	Recovery, %*
1.5	Talc (50.0 mg), magnesium stearate (25.0 mg) and starch (500.0 mg)	100.2
2.0	Talc (50.0 mg), magnesium stearate (25.0 mg) and starch (500 mg)	100.0
1.5	Starch (500.0 mg), gelatin (25.0 mg), magnesium stearate (25.0 mg) and talc (50.0 mg)	100.2
1.5	Starch (375 mg), gelatin (50.0 mg), magnesium stearate (25.0 mg) and talc (50.0 mg)	99.7

* Average recovery in five experiments.

The usual tablet diluents and excipients were found not to interfere in the analysis by the proposed method (Table III).

Conclusion

A colorimetric method has been established for the determination of methisazone in the concentration range 12.0–84.0 μg ml^{-1}. The method is rapid and has a reproducibility of ±0.51%. It has been applied successfully to the analysis of sachets. The results compare favourably with those obtained by an iodimetric method.[7]

The authors express their sincere thanks to Dr. C. S. Shah, Principal, Lallubhai Motilal College of Pharmacy, for providing the facilities to carry out this work. They are also indebted to the Wellcome Foundation Ltd., Dartford, Kent, for the gift of Marboran sachets.

References

1. White, D. C., *Med. J. Aust.*, 1968, *ii*, 368.
2. Stuart-Harris, C., *Br. Med. J.*, 1971, *i*, 387, 446.
3. Adels, B. R., and Oppe, T. E., *Lancet*, 1966, *i*, 19.
4. Mainwarning, D., *Br. Med. J.*, 1962, *i*, 1412.
5. Barlow, A. J. E., *Br. Med. J.*, 1962, *i*, 1144.
6. Daly, J. J., and Jackson, E., *Br. Med. J.*, 1962, *ii*, 1300.
7. Deavin, J. C., and Mitchell, D. E., *J. Pharm. Pharmac.*, 1965, **17**, Suppl., 56S.
8. Devani, M. B., Shishoo, C. J., and Shah, M. G., *Analyst*, 1973, **98**, 759.
9. Shishoo, C. J., Devani, M. B., and Shah, M. G., *Analyst*, 1973, **98**, 762.

Received *January 26th*, 1976
Amended *June 16th*, 1976
Accepted *July 19th*, 1976

Communication

Material for publication as a Communication must be on an urgent matter and be of obvious scientific importance. Rapidity of publication is enhanced if diagrams are omitted, but tables and formulae can be included. Communications should not be simple claims for priority: this facility for rapid publication is intended for brief descriptions of work that has progressed to a stage at which it is likely to be valuable to workers faced with similar problems. A fuller paper may be offered subsequently, if justified by later work.

Manuscripts are not subjected to the usual examination by referees and inclusion of a Communication is at the Editor's discretion.

Observations on the Direct Determination of Lead in Complex Matrices by Carbon Furnace Atomic-absorption Spectrophotometry

Keywords: *Lead determination*; *atomic-absorption spectrophotometry*; *electrothermal atomisation*

There have been numerous instances recorded of interference in the determination of lead in biological fluids, sea water and river waters using electrothermal atomisers. This interference is usually due to one or more of the following effects: (1) the presence of variable amounts of both cations and anions, *e.g.*, magnesium and sulphate, which inhibit the atomisation process, resulting in variable lead sensitivity; (2) non-specific absorption or light scattering by molecular vapours of the salt matrix; and (3) the loss of lead as volatile halide during ashing in the presence of high salt concentrations. The first type of interference has usually been overcome by the method of standard additions, but this method is prone to errors. Also, the varying sensitivity with samples of different origin complicates any estimates of over-all precision. Regan *et al.*[1] added ascorbic acid in order to prevent alkaline earth metal interference.

With some types of sample it has been possible to isolate the lead atomisation peak from the non-specific absorption peak. With more complex samples the use of background correction has allowed results to be obtained provided that the background absorption is within the range of the particular instrument used. Ediger *et al.*[2] reduced high salt background problems by adding ammonium nitrate.

The losses of lead as the halide can be avoided by careful attention to the ashing temperature, but this method is not always compatible with conditions necessary for the removal of organic matter. There have been various references to matrix modifications as a means of reducing interference in atomic-absorption spectrophotometry with electrothermal atomisation. For lead, the use of ammonium phosphate or orthophosphoric acid has been advocated.[3,4]

This communication describes a study of the effects of the addition of orthophosphoric acid on cation and anion interference in the determination of lead.

The equipment used was an Instrumentation Laboratory IL 151 atomic-absorption spectrophotometer with an IL 455 carbon furnace. Automatic deuterium-lamp background correction was used where indicated. (The effects described in this work have also been observed when using a Perkin-Elmer HGA 70 carbon tube furnace.) Peak-height measurements were carried out without range expansion. Peak-area measurements were effected for 4 s at 5× range expansion, initiated by the power programmer at the start of the atomisation stage. An Activion lead hollow-cathode lamp was used and the monochromater was peaked on the 283.3-nm lead line.

As our main interest was the determination of lead in urine, the interfering substances studied were those found in this type of sample. The concentration of orthophosphoric acid (2 + 98) was calculated to give a 100% excess over that required for the stoicheiometric replacement of the mean levels of chloride and sulphate in urine.

A 500-μl volume of water was placed in a 2-ml plastic cup, then 50 ng of lead as nitrate were added followed by the interfering substance. A 1-ml volume of water or orthophosphoric acid (2 + 98) diluent was added and the contents were mixed. A 10-μl volume of this solution was pipetted into the carbon furnace. The heating programme was optimised for the water mixture, *i.e.*, drying for 30 s at 100 °C, ashing for 35 s at 450 °C and atomisation for 5 s at 2 500 °C (maximum temperature reached). When orthophosphoric acid was added it was found that for maximum sensitivity a higher ashing temperature (700–900 °C) was optimum and was used. Under these conditions, no loss of lead occurred below 1 000 °C. The results of these experiments are shown in Table I.

TABLE I

VARIATION OF LEAD SENSITIVITY FACTORS (EQUAL TO 1.00 FOR 0.1 μg ml^{-1} OF LEAD ALONE) WITH MAJOR INTERFERING SALTS FOUND IN URINE AND THE EFFECT OF ADDITION OF ORTHOPHOSPHORIC ACID

Conditions	Peak-height sensitivity factor		Peak-area sensitivity factor	
	Water diluent	2% H_3PO_4 diluent	Water diluent	2% H_3PO_4 diluent
With 400 μg ml^{-1} $CaCl_2$	1.02	1.12	0.97	0.96
With 475 μg ml^{-1} $MgCl_2$	0.24	1.10	0.32	0.99
With 3 300 μg ml^{-1} Na_2SO_4	0.88	1.05	0.61	0.94
With all three of the above ..	0.46	1.15	0.27	0.93

All readings were taken with background correction and the small lead blanks from the reagents were allowed for. Duplicate injections were carried out and agreed within 5% on peak height and within 3% on peak area. The much improved freedom from interference in the orthophosphoric acid system with both peak height and peak area is evident from the results.

A sample of urine (sp. gr. 1.024) of low known lead content was treated as above. The water system gave a peak-area sensitivity factor of 0.26, which is in agreement with that obtained from the system containing all of the interfering ions. The urine treated with orthophosphoric acid gave very erratic results and no meaningful readings could be taken. As this behaviour indicated that the background correction was overloaded, the urine - orthophosphoric acid was re-run using the deuterium lamp only and gave a peak background absorbance of 2.5. This high value must be due to the volatilisation of sodium phosphates formed from the sodium chloride present in the urine. An alternative additive to orthophosphoric acid was sought.

In the course of a series of experiments, ammonium molybdate was tried without success owing to the low sensitivity obtained. On repeating the work with orthophosphoric acid and urine it was found that under the same conditions as used previously, reproducible results were then obtained. The background absorption was measured and gave a peak value of 0.9 absorbance.

As this effect appeared to be associated with the use of molybdate on the graphite tube, this work was repeated using a new tube and the above results were confirmed. It was found that it was necessary only to pre-treat the tube with ammonium molybdate solution, carry out the normal atomisation programme and then analyse the urine - orthophosphoric acid mixture. The sample of urine was dosed with lead and treated as described for the interference experiments. A peak-area sensitivity factor of 0.95 (compared with orthophosphoric acid - water) was found for the urine under these conditions. The peak-height absolute sensitivity (for 0.0044 absorbance) was 0.5×10^{-11} g of lead.

In order to quantify this effect, an experiment was carried out in which injections of ammonium molybdate followed by atomisation were alternated with injections of urine - orthophosphoric acid starting with a new tube. During the urine atomisation the background absorption was monitored using the deuterium lamp.

The chart recordings are illustrated in Fig. 1. The integrated background absorption levelled out at 5% of the value obtained with the untreated tube. The mechanism of this effect has not

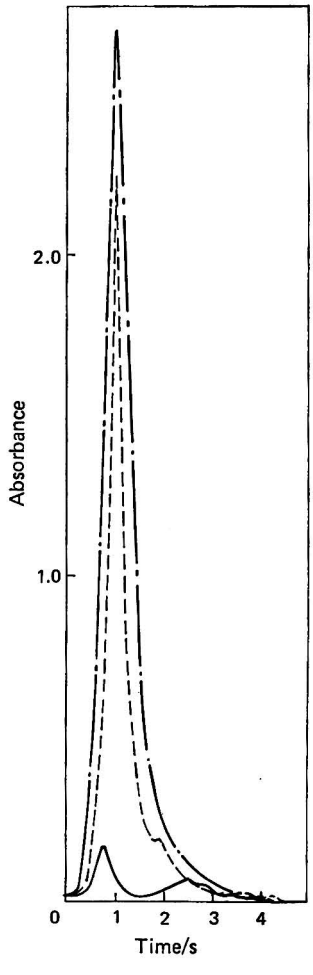


Fig. 1. Urine background absorbance after repeated treatment of the carbon tube with 20 μl of 1% ammonium molybdate solution: —·—·—, untreated tube; — — — —, after two treatments; and ————, after ten treatments.

been studied exhaustively but it is possible that it involves the reduction of the alkali metal phosphate to the phosphide with molybdenum or molybdenum carbide acting as a catalyst. Any background absorption, either molecular or atomic, would then be shifted into the far ultraviolet region.

Once treated, the carbon tube remained active without further addition of molybdate. However, an examination of the tube using a molybdenum hollow-cathode lamp during an atomisation cycle showed that there was a small loss of molybdenum. For this reason, it was decided that in future work a small amount (0.05%) of ammonium molybdate should be incorporated in the orthophosphoric acid diluent. More efficient methods of treating the tube with molybdenum have been sought and so far molybdophosphoric acid appears to have some advantages over ammonium molybdate.

The pre-treatment of carbon furnace tubes with molybdenum has been used by Henn[5] in the determination of selenium, although the role of molybdenum seems to have been primarily that of decreasing the volatility of the selenium.

To summarise the results of the above study, the addition of orthophosphoric acid permits the removal of chloride and sulphate as the free acids during the drying and ashing stages. The interference of sulphate and chloride and also that of alkaline earth metals are thus prevented. It also allows a higher ashing temperature (up to 1 000 °C) to be used without loss of lead, which results in more efficient removal of organic matter. Moreover, the pre-treatment of the carbon tube with molybdenum modifies the phosphate salt matrix so that the non-specific absorption during the lead atomisation is sufficiently low to allow automatic background correction to be applied.

The application of these procedures to the determination of lead in blood and urine have given very promising results and it is intended to publish specific methods at a later date.

References

1. Regan, J. G. T., and Warren, J., *Analyst*, 1976, **101**, 220.
2. Ediger, R. D., Peterson, G. E., and Kerber, J. D., *Atom. Absorption Newsl.*, 1974, **13**, 61.
3. Matousek, J. P., and Brodie, K. G., *Analyt. Chem.*, 1973, **45**, 1606.
4. Pardhan, S. I., and Ottaway, J. M., *Proc. Analyt. Div. Chem. Soc.*, 1975, **12**, 291.
5. Henn, E. L., *Analyt. Chem.*, 1975, **47**, 428.

Received *November 25th*, 1976

D. J. Hodges

Research and Development Department,
The Associated Octel Company Limited,
Ellesmere Port, Merseyside, L65 4HF

Book Reviews

BIBLIOGRAPHY OF PAPER AND THIN-LAYER CHROMATOGRAPHY 1970–1973 AND SURVEY OF APPLICATIONS. Edited by K. MACEK, I. M. HAIS, J. KOPECKÝ, V. SCHWARZ, J. GASPARIČ and J. CHURÁČEK. *Journal of Chromatography Supplementary Volume No. 5*, 1976. Pp. xviii + 744. Amsterdam, Oxford and New York: Elsevier Scientific Publishing Company. 1976. Price Dfl200; $76.95.

This book is really the fifth of a series of bibliographical compilations on the subject of paper and thin-layer chromatography that stretch back to 1944. The first two books of the series are relatively difficult to obtain and are in German, while the third and fourth were also published as supplementary volumes of the *Journal of Chromatography*. The period covered in this fifth volume is from 1970 to 1973, and this book will probably be the last in the present format as in future, starting with 1974 papers, bibliographies in book form will be limited to particular branches of chromatographic separations or expanding fields of application.

As one would expect with the very thorough and internationally respected Czechoslovak team who assembled the references, it is extremely large and detailed, containing about 5 600 references plus an author index and a listing of the substances chromatographed. Obviously, such a bibliography is impossible to review in the normal way, except to say that it is eminently usable, being broken down into a very large number of sub-sections, the scope of each being clearly defined in the 5-page introduction. Equally, there is a 3-page introduction in order to help one to elucidate under what name a particular compound may be listed in the "substances chromatographed" index. These introductions will repay handsomely for reading because of the greater ease with which one can then use the bibliography.

The whole book is a massive 744 pages long; it should be kept by libraries but used by those interested in chromatographic separations as an entry into the literature of the early 1970s on the subject. A valuable compilation; the reviewer admires the team's tenacity and efficiency.

G. NICKLESS

UNDERSTANDING AND OPTIMISING ELECTRON MICROSCOPE PERFORMANCE. By S. K. CHAPMAN. Pp. iv + 92. Beaconsfield, Buckinghamshire: Perkin-Elmer EM Publications. 1976.

This short paperback is essentially a series of practical operating tips for users of a transmission electron microscope. The author has gathered together the products of his many years of practical experience on a variety of microscopes.

A simplified description is given of the basic theory of transmission electron microscopy and of the functions of the various systems of a microscope. The major part of the book provides invaluable practical information on setting up and optimising instrumental conditions. There are also short chapters on photography for electron microscopy and on instrument evaluation.

This book will provide a valuable addition to the usual microscope operating manual, as it contains many useful practical tips collected in a readily accessible format. DENNIS E. HENN

ANALYTICAL METHODS IN OCEANOGRAPHY. A SYMPOSIUM SPONSORED BY THE DIVISION OF ANALYTICAL CHEMISTRY AT THE 168TH MEETING OF THE AMERICAN CHEMICAL SOCIETY, ATLANTIC CITY, N.J. SEPTEMBER 10–11, 1974. Edited by THOMAS R. P. GIBB, Jr. *Advances in Chemistry Series*, 147. Pp. x + 238. Washington, D.C.: American Chemical Society. 1975. Price $26.50.

Public concern with marine pollution has, in recent years, led most Western nations to introduce legislation controlling the discharge and dumping of toxic chemicals into estuaries and the sea. The monitoring of the levels of such materials in sea water and in marine biota has now become one of the major tasks of marine chemists. The very low concentrations at which pollutants can become ecologically significant (in parts per billion or below) and the ever-increasing diversity of compounds to be determined place great demands on analytical technology. The collection of samples frequently presents a major problem either because of contamination arising from the sampler and hydrographic wire, or even from the survey ship, or because of loss of the substance to be determined by adsorption on to the sampler or storage vessels. The analysis itself will usually involve a pre-concentration stage followed by a determination carried out by one of the

many highly sensitive instrumental techniques now available. These various facets of the examination of sea water are treated in this book.

Despite its comprehensive title, the book is highly restricted in its coverage and is confined almost entirely to sampling methods and to techniques for the determination of the commoner trace metals (atomic-absorption spectroscopy and anodic-stripping voltammetry only), transuranic elements and hydrocarbons in sea water. A number of other topics of less general relevance to analytical chemistry are also discussed, *e.g.*, the dissolution of silica and the occurrence of fluorite in sediments in an area subject to fluoride pollution. Although most of the 18 chapters are fairly commonplace, four do merit comment. Bates and Macaskill provide a thought-provoking chapter on the measurement of pH in sea water and suggest that it might be possible to eliminate salt bridge effects, which hinder the theoretical interpretation of such measurements. The determination of trace elements without the need for pre-concentration is explored in two chapters. Segar and Cantillo have examined the direct application of atomic-absorption techniques using a heated graphite atomiser. Although their results are encouraging, their method is sufficiently sensitive only for the analysis of inshore waters. An approach based on anodic-stripping voltammetry has been adopted by Zirino and Lieberman for the automatic determination of copper, zinc, cadmium and lead. Stolzberg describes a systematic study of the use of the ammonium tetramethylenedithiocarbamate - isobutyl methyl ketone system for the pre-concentration of trace elements. He points out that extraction is efficient only over a narrow pH range and stresses the necessity for using rigorously purified reagent. In his opinion, the extraction technique is suitable for use only with coastal waters.

Because of its relatively high price and restricted coverage, this is not a book which every marine chemist will wish to purchase, but it is one which should be available in libraries. It is unfortunately rather uneven in its coverage and it is disappointing to find so little reference to both *in situ* analysis and the determination of the dissolved organic components. These seem likely to be the major growth areas of the future and will present the marine analyst with some of his most challenging problems.

J. P. RILEY

PROGRESS IN DRUG METABOLISM. Volume 1. Edited by J. W. BRIDGES and L. F. CHASSEAUD. Pp. xiv + 286. London, New York, Sydney and Toronto: John Wiley & Sons. 1976. Price £9.80; $19.75.

Many countries emphasise safety evaluation of potent new medicines, and assessment of their metabolic fate is commonly required in submissions to regulatory agencies. There is a need for a selective and critical review that will inform the specialist versed in a different discipline without blinding him with jargon or detail; certainly regulatory assessors will not look kindly on any series that re-distils esoteric minutiae to a muddy condensate.

The first chapter of this book reviews the current contribution of mass spectrometry (MS), which is fast becoming the favoured tool for metabolic investigations when it is combined with gas chromatography (GC). Although GC - MS applications have frequently been reviewed, for the sake of the non-specialist 5 pages are devoted to describing the principles and data handling. Dr. Millard contrasts primary information on unknown metabolites obtained from qualitative MS with the much greater selectivity and sensitivity of single-ion monitoring. Numerous examples of metabolite application are discussed for the compromise technique of mass fragmentography, *i.e.*, the judicious simultaneous monitoring of selected ions with multiple detectors. Alternative, less drastic, means of ionisation (chemical ionisation and field ionisation and desorption) are also described. The value of high-resolution MS is somewhat cavalierly dismissed and there is an unfortunate typographic intrusion of a zero, implying a cost magnitude of £1 million for HRMS equipment.

Administering nominally inert drugs for activation *in vivo* to a potent form is a useful means of avoiding certain side-effects or of increasing target-cell specificity. This expedient is particularly valuable in the use of cytotoxic drugs which can be activated to a potent antagonist in a malignant tissue site. Dr. Conners discusses, with appropriate emphasis on methods of measurement, latent alkylating agents which can be activated by various means: reduction (mostly from azo compounds), esterases and other hydrolytic enzymes, microsomal action and pH adjustment. Other examples of latent antimetabolites include well known cytotoxic antibiotics.

Dr. Garner reviews the role of epoxides as metastable intermediates in the cellular oxidation of foreign and also endogenous substances. The resultant epoxide may have enhanced toxicity or represent the biologically active form or may even be carcinogenic, in which field most studies

seem to have been carried out. These two chapters naturally lead one to consider enzyme induction resulting from stimulation to metabolise alien substances such as pesticides, food additives or drugs, and thereby perturbing regular drug metabolism. Hunter and Chasseaud discuss human clinical consequences for microsomal enzymes. Some clinical analyses of liver enzyme levels are presented but the bulk of the chapter resides in the discussion of difficulties in demonstrating real differences in biological levels. The authors proceed to examine a very heterogeneous, albeit not too lengthy, list of inducers and alleged inhibitors. The appropriate reservations are made concerning route of administration, dosage, ambivalent metabolism and inter-patient variation.

The extent of protein binding is a key parameter in drug distribution, and knowledge of the factors which modify this relation is essential to consideration of the therapeutic role and possible hazard of a new drug or a reformulation or a derivatisation of an old one. Bridges and Wilson review present theoretical and biochemical knowledge with special reference to the binding of different classes of drug to albumin. Methodology is mentioned but, reasonably enough, not elaborated.

One might carp that this new series is not all "progress": a fair amount of material is in the form of a judicious review of prior art; but it is none the less valuable for that so far as a non-specialist reader is concerned. Each chapter has specific "conclusions" and the book has a good index. Overall, this volume is a promising beginning to a potentially valuable series.

G. F. PHILLIPS

FORTRAN IV IN CHEMISTRY. AN INTRODUCTION TO COMPUTER-ASSISTED METHODS. By G. BEECH. Pp. x + 303. London, New York, Sydney and Toronto: John Wiley & Sons. 1975. Price £8.75.

The author rightly points out that in the last 10 years there has been a great increase in the use of computer-assisted techniques in science teaching. In this book, he sets out to provide a series of specific computing tasks associated with chemical problems that might be encountered at undergraduate level. These examples are aimed at providing the centre-piece of an undergraduate ancillary course on computers. Some discussion of the mathematics and programming techniques is also given and it is aimed to give a professional approach to the programming. It is also stated that the programming listings are ready to operate on other installations but transportability of all of these programs is doubtful. In fact, the programming methods seem unsophisticated and there are many errors in the programs and the text which the author acknowledges as his responsibility. These detract a great deal from this work.

The book's ideal use should be as an undergraduate reading text, but the various errors make it doubtful for this area. In many areas the program listings could be better replaced by a program flow sheet. The policy of discussing program details such as the particular card number has overstretched the collating ability of the sub-editors and author. PETER B. STOCKWELL

FOOD ADDITIVES AND CONTAMINANTS COMMITTEE REVIEW OF THE LEAD IN FOOD REGULATIONS 1961. MINISTRY OF AGRICULTURE, FISHERIES AND FOOD. Pp. 49. London: HM Stationery Office. 1975. Price 65p.

SURVEY OF LEAD IN FOOD: FIRST SUPPLEMENTARY REPORT. WORKING PARTY ON THE MONITORING OF FOODSTUFFS FOR HEAVY METALS FIFTH REPORT. MINISTRY OF AGRICULTURE, FISHERIES AND FOOD. Pp. 34. London: HM Stationery Office. 1975. Price 50p.

These two pamphlets should be read together. The title of the first conforms with the usual practice of the Food Standards Committee in setting out the purpose of the work but it is more than a mere review of standards. It gives reasons for the standards suggested and the appendix gives a balanced review and literature survey on lead and toxicology.

The second pamphlet is what it says it is and as such it is well done, although clearly so much unpublished information has accumulated on this subject that it cannot hope to be comprehensive.

Both documents are essential reading for chemists in the food industry. In addition, if they were read in the objective way in which they were written they could afford valuable material for those people who exaggerate the dangers of an undoubtedly dangerous contaminant.

J. MARKLAND

Simultaneous Determination of Phenobarbitone, Primidone and Phenytoin in Small Samples of Blood by Gas - Liquid Chromatography

Phenytoin, phenobarbitone and primidone in small samples of blood can be identified and measured simultaneously. The acidified blood is extracted with 3 ml of diethyl ether and 2 ml of the extract are evaporated to dryness. The residue is dissolved in 80 μl of diethyl ether containing methyltestosterone (15 mg per 100 ml) as the internal standard. An aliquot of this solution is injected on to an SE-30 column where the drugs are separated and detected by a flame-ionisation detector. Drugs commonly associated in treatment with these three and cholesterol, which is co-extracted, do not interfere.

Keywords: *Phenytoin*; *phenobarbitone*; *primidone*; *blood*; *gas - liquid chromatography*

A. M. BRUCE and HAMILTON SMITH

Department of Forensic Medicine, University of Glasgow, Glasgow, G12 8QQ.

Analyst, 1977, **102**, 35–41.

Indirect Determination of Phenelzine in Urine

The problems associated with the determination of phenelzine in biological fluids are described. Conversion of phenelzine, *in situ*, into 2-phenylethanol and the gas-chromatographic assay of the latter compound provide an easy, precise and specific method for the determination of phenelzine in urine.

Keywords: *Phenelzine determination*; *urine*; *monoamine oxidase inhibitor*; *gas chromatography*.

B. CADDY and A. H. STEAD

Forensic Science Unit, Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, University of Strathclyde, Glasgow, G1 1XW.

Analyst, 1977, **102**, 42–49.

Dissolution of Mercury(II) Sulphide by Digestion with Sulphuric Acid - Nitric Acid and Oxidation with Potassium Permanganate

Short Paper

Keywords: *Mercury(II) sulphide dissolution*; *atomic-absorption spectrophotometry*; *mercury determination*; *minerals*

A. M. URE

Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen, AB9 2QJ

Analyst, 1977, **102**, 50–51.

Determination of Silica in Rocks and Minerals by a Combined Gravimetric and Atomic-absorption Spectrophotometric Procedure

Short Paper

Keywords: *Silica determination*; *minerals*; *gravimetry*; *atomic-absorption spectrophotometry*

J. N. WALSH

Department of Geology, Kings College, University of London, Strand, London, WC2R 2LS.

Analyst, 1977, **102**, 51–54.

Method for the Extraction of Organochlorine Compounds from Quail Eggs

Short Paper

Keywords: *Organochlorine compounds*; *quail eggs*; *extraction*; *gas - liquid chromatography*

GRAHAME L. WARDALL

Ministry of Agriculture, Fisheries and Food, Pest Infestation Control Laboratory, Tolworth, Surbiton, Surrey, KT6 7NF.

Analyst, 1977, **102**, 54–55.

Determination of the Anthelmintic Diethylcarbamazine in Tissue and Plasma by Gas - Liquid Chromatography

Short Paper

Keywords: *Diethylcarbamazine determination*; *anthelmintic*; *tissue*; *plasma*; *gas - liquid chromatography*

J. A. BOGAN

Department of Veterinary Pharmacology, University of Glasgow Veterinary School, Veterinary Hospital, Bearsden Road, Bearsden, Glasgow, G61 1QH.

Analyst, 1977, **102**, 56–59.

Determination of Piperazine in Solutions Containing 1,1,4,4-Tetramethylpiperazinium Diiodide, Thiamine and Pyridoxine

Short Paper

Keywords: *Piperazine determination*; 1,1,4,4-*tetramethylpiperazinium diiodide*; *thiamine*; *pyridoxine*; *column chromatography*

G. C. KONDOS

Research Laboratories, Chropei Pharmaceutical Company, Piraeus, Greece.

Analyst, 1977, **102**, 59–62.

Spectrophotometric Determination of Methisazone

Short Paper

Keywords: *Methisazone determination*; *spectrophotometry*; 2,3-*dichloro*-1,4-*naphthoquinone reagent*

M. B. DEVANI, C. J. SHISHOO and HEMA J. MODY

Department of Pharmaceutical Chemistry, Lallubhai Motilal College of Pharmacy, Ahmedabad 9, India.

Analyst, 1977, **102**, 62–66.

Observations on the Direct Determination of Lead in Complex Matrices by Carbon Furnace Atomic-absorption Spectrophotometry

Communication

Keywords: *Lead determination*; *atomic-absorption spectrophotometry*; *electrothermal atomisation*

D. J. HODGES

Research and Development Department, The Associated Octel Company Limited, Ellesmere Port, Merseyside, L65 4HF.

Analyst, 1977, **102**, 67–69.

ANALAO 102 (1210) 1–72 (1977) January 1977

ISSN 0003–2654

THE ANALYST

THE ANALYTICAL JOURNAL OF THE CHEMICAL SOCIETY

CONTENTS

Printed by Heffers Printers Ltd, Cambridge, England

Entered as Second Class at New York, USA, Post Office