

High-performance liquid chromatography of selected organic peroxides with oxidative amperometric detection

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Reversed-phase high-performance liquid chromatography with oxidative amperometric detection was optimized for the determination of several organic peroxides in drinking water under ideal conditions. The determinations were performed under isocratic conditions using acetonitrile and methanol as the organic modifiers with 0.05 M potassium phosphate buffer solution. The oxidative amperometric response of the organic peroxides was dependent on the concentration of organic modifier and the electrode potential. The optimum electrode potential ($E^{\text{ox}}_{\text{APP}}$), for the simultaneous determination of the organic peroxides was approximately $+1.150 \pm 0.05$ V versus the Ag/AgCl reference electrode. The maximum analytical signal for butan-2-one peroxide and *tert*-butyl hydroperoxide, when using acetonitrile, was obtained with 20% v/v organic modifier. For cumene hydroperoxide, the maximum analytical signal was achieved with approximately 35% v/v acetonitrile. The retention time of cumene hydroperoxide, on an octyldecylsilane column (250×4 mm id), decreased sharply from >100 to <10 min when the organic modifier concentration was varied from 5 to 50% v/v. The retention time of butan-2-one and *tert*-butyl hydroperoxide, under the same conditions, varied by <10 min. The calibration curves for the aliphatic peroxides and aromatic peroxide were linear from 2 to 200 ng and from 0.2 to 200 ng injected, respectively.

Keywords: Organic peroxides; hydroperoxides; hydrodynamic voltammograms; oxidative amperometric detection; reversed-phase liquid chromatography; glassy carbon sensor

The application of ozone as an alternative to the use of chlorine for drinking water pre-treatment and treatment has received increasing attention in recent years. Ozone offers numerous advantages for water treatment such as removal of taste and odor compounds, some dissolved inorganic materials and even color, which makes it attractive as a drinking water disinfectant. Perhaps more importantly, ozone treatment has been shown to decrease substantially the potential for trihalomethane formation and improve the flocculation of certain suspended particulate matter.^{1,2} Nevertheless, at present, the amount of information available with regard to the formation of ozone by-products during drinking water disinfection and the ultimate fate of these by-products is limited.^{3–5} Some of the known potential ozonation by-products that are of particular interest to drinking water researchers include aldehydes, ketones, organic acids, nitrosamines, chloramines, organic peroxides, quinones, bromate and chlorate.

The US Environmental Protection Agency's Office of Research and Development and Office of Water have jointly published a comprehensive research plan⁴ that describes, in part, the Agency's commitment to conduct substantial and comprehensive chemical research in the drinking water area. Of

particular concern to the Agency is human exposure to disinfection by-products that may be potential human carcinogens. As a consequence, the focus of part of this research effort has been the study of organic peroxides and hydroperoxides. In seeking to obtain information with regard to understanding the mechanisms associated with the ozonation process and formation of organic peroxides, and to develop sensitive and selective methods for this purpose, the approach has been to investigate the utility of electrochemical based techniques, as detection procedures, in conjunction with chromatographic methods of analysis. The literature contains little information with regard to determining organic peroxides using electrochemical based techniques except for polarographic (dc),^{6–22} biamperometric (or dead stop end-point),^{23,24} coulometric (controlled-potential),²⁵ etc., techniques employed more than a quarter century earlier. The high-performance liquid chromatographic (HPLC) determination of organic peroxides and hydroperoxides has been performed with various spectroscopic modes of detection such as UV,^{26–32} IR,³⁰ NMR,³⁰ mass spectrometric³⁰ and chemiluminescence.^{33,34} The relatively few publications dealing with liquid chromatography utilizing amperometric detection, in applications involving organic peroxides and hydroperoxides, have involved reductive mode detection approaches at gold amalgamated,^{35–37} dropping mercury,³⁷ glassy carbon,³⁸ and platinum³⁹ electrodes and, more recently, electrocatalysis at iron phthalocyanine chemically modified electrodes.⁴⁰

In this work, we determined the optimum conditions for the reversed-phase HPLC separation of butan-2-one peroxide, *tert*-butyl hydroperoxide and cumene hydroperoxide accompanied by oxidative amperometric detection. To our knowledge, this is the first report on the oxidative amperometric detection and determination of organic peroxides and hydroperoxides at an unmodified solid (glassy carbon) sensor following a chromatographic separation. Several reversed-phase column types and eluent systems were evaluated. The most important chromatographic variable was the ratio of organic modifier to water, which ranged from as low as 5% to 50% v/v solutions. The effect of this experimental variable on the retention times, retention factors (k), separation factors (α) and amperometric responses (analytical signal) of the analytes was examined.

The glassy carbon sensor's oxidation potential ($E^{\text{ox}}_{\text{APP}}$), an important parameter for controlling the magnitude of the analytical response, was optimized for the simultaneous detection of the selected alkyl and aromatic organic peroxides and hydroperoxides. The effect of the potential on the oxidative amperometric response of the organic peroxides will be discussed.

Experimental

Apparatus

HPLC was performed with Models 200 and 200A liquid chromatographs (Bioanalytical Systems, West Lafayette, IN, USA). Each instrument is a completely integrated system

equipped with ternary gradient solvent delivery and a Rheodyne Model 7125 injection valve (six ports) with 10 μl –2 ml removable sample loops; however, it was fitted with a 20 μl sample loop for this work. The amperometric detector consisted of (a) a dual glassy carbon 0.069 cm^2 working electrode^{41,42} (MF-1000), (b) an Ag/AgCl reference electrode (MF-2021, Model RE-4), (c) a stainless-steel counter/auxiliary electrode and (d) a 0.002 in thin-layer gasket (MF-1046). The Model 200 liquid chromatograph was controlled by the BAS 200 System Director, a microprocessor-based membrane keypad unit. The system, output was displayed on a dual pen BAS RYT (strip-chart) recorder (MF-8075). The Model 200A liquid chromatograph was controlled by a Gateway (North Sioux City, SD, USA) Model 2000 386SX/33 computer system, employing the Microsoft (Seattle, WA, USA) Windows 3.1 operating system, BAScontrol and ChromGraph. The system output was displayed on (a) a Gateway 2000, Crystal Scan 1572 FS video display terminal, (b) a Panasonic (Model KX-P2180) Impact Dot Matrix Printer (Kyushu Matsushita Electric, software (BAS) Osaka, Japan) and (c) a Linear Scientific (Reno, NV, USA) dual-pen strip chart recorder (Model 1201).

Chromatography

The reversed-phase analytical columns evaluated were as follows: (a) Hibar LiChrosorb RP-18 (ODS, C_{18} , 5 μm), 250 \times 4.0 mm id (EM Separations Technology, Gibbstown, NY, USA); (b) Hibar LiChrosorb RP-8 (octyl, C_8 , 5 μm), 250 \times 4.0 mm id (EM Separations Technology); (c) Spherisorb (hexyl, C_6 (5 μm), 250 \times 4.6 mm id (Alltech, Deerfield, IL, USA); (d) PRP-1 (10 μm) 250 \times 4.1 mm id (Hamilton, Reno, NV, USA) and (e) Biophase^{II} MF-6032 (C_8 , 5 μm , 200 \times 4.0 mm id (Bioanalytical Systems). When the system was idle for more than a few days it was flushed with 30–40% v/v acetonitrile solution and the columns were flushed and stored in the same solution. Note: the experimental results presented in this paper consist mostly of data generated with the Spherisorb column in place.

The mobile phases were acetonitrile–water (5 + 95 to 50 + 50 v/v) and methanol–water (5 + 95 to 50 + 50 v/v) including 0.05 M phosphate buffer, pH \approx 7. The flow rate was 1.0 ml min^{-1} unless stated otherwise and the column back-pressures at this flow rate ranged from 2000 to 3900 lb in^{-2} . The column back-pressure was generally higher for the methanol–water than the acetonitrile–water eluents. The pump(s) performed at optimum efficiency when the flow rate was 1.0 ml min^{-1} . The volume of standard and/or sample materials injected during all experimental phases (amperometric–chromatographic behavior/response characterization and optimization and quantitative determinations) was 20 μl .

The order of elution of organic peroxide analytes was established by making successive injections of standard solutions of each analyte individually. Mixtures of these organic peroxides/hydroperoxides were subsequently chromatographed to verify their separation at various modifier ratios.

Hydrodynamic voltammetry

Response–potential curves (hydrodynamic voltammograms and/or chromatographic voltammograms) were also obtained by performing HPLC experiments with oxidative amperometry under constant potential conditions manually stepped sequentially from about +0.60 to +1.200 V *versus* Ag/AgCl. Known amounts of organic peroxide analytes were injected after the potential (E_{APP}) was applied and the resultant current had reached a steady-state value. The measured signal corresponding to either P_{MAX} or the peak area was plotted against E_{APP} (V) *versus* Ag/AgCl.

Reagents and chemicals

HPLC-grade acetonitrile and methanol (OPTIMA) were obtained from Fisher Scientific (Fairlawn, NJ, USA). In-house distilled water was passed through a four-bowl (two Ion-Ex cartridge filters, one carbon cartridge, one Organex-Q cartridge, one Millipak 0.22 μm filter for final filtration) Milli-Q Water System (Millipore, Bedford, MA, USA). The organic peroxides, butan-2-one peroxide (ethyl methyl ketone peroxide), $[\text{CH}_3\text{COCH}(\text{CH}_3)\text{O}]_2$, and hydroperoxides, *tert*-butyl hydroperoxide $[(\text{CH}_3)_3\text{COOH}]$ and cumene hydroperoxide $[\text{C}_6\text{H}_5\text{C}(\text{CH}_3)_2\text{OOH}]$ (Aldrich, Milwaukee, WI, USA) were used as received. Certified ACS grade potassium dihydrogenphosphate and dipotassium hydrogenphosphate (Fisher Scientific) were used, without additional purification, to prepare buffered eluent solutions of pH \approx 7. High purity helium, 99.995% (Matheson Gas Products, Secaucus, NJ, USA) was used to purge/deoxygenate the chromatographic eluent solutions.

Caution. Organic peroxides may be carcinogens and some are potentially mutagenic and teratogenic substances.^{41,42} Pure standard material (liquid and solid) and stock standard solutions of these compounds should be handled with suitable protection for the skin, eyes, nasal passages, clothing, *etc.*

Acetonitrile is an extremely toxic organic solvent and constitutes an environmental hazard if proper care is not exercised when disposing of chromatographic waste.^{43–45}

Results and discussion

Hydrodynamic voltammetry

Fig. 1(a)–(d) show hydrodynamic voltammograms (HDVs) constructed from chromatographic data generated with dual cell–parallel configuration oxidative amperometric detection in 20% v/v acetonitrile and 0.05 M phosphate buffer and 35% v/v methanol and 0.05 M phosphate buffer at pH \approx 7, respectively. The optimum potential for chromatographic analysis was determined by hydrodynamic voltammetric measurements on successive injections of standard aliquots containing the individual peroxides and hydroperoxides and mixtures of these compounds. The applied potential (E_{APP}) was varied in 50 mV anodic step increments, at each electrode, and the corresponding maximum oxidative current or peak height and peak area response was subsequently measured at each potential.

The analytical signals for butan-2-one peroxide and *tert*-butyl hydroperoxide increased with the positive potential steps from about +0.600 to +1.200 V *versus* Ag/AgCl. On the other hand, the analytical signals for the oxidation of cumene hydroperoxide, over the same potential range, initially increased with the positive potential step. However, the peak height and area responses for cumene hydroperoxide subsequently decreased with higher applied anodic potentials, beyond about +1.175 \pm 0.025 V *versus* Ag/AgCl, resulting in peak-shaped HDVs [Fig. 1(a)–(d)]. In phosphate buffered acetonitrile [Fig. 1(a) and (b)], the two alkyl peroxides appear to be approaching a plateau or perhaps a peak in their response; however, it was not possible to acquire definitive response characterization data successfully beyond the limits indicated. The responses of the two alkyl peroxides [Fig. 1(c) and (d)], in buffered methanol eluent, tended to increase with successive anodic potential steps, giving little indication as to whether a plateau or a peak-shaped waveform may be produced.

Organic peroxides and hydroperoxides, like a number of other organic compounds, may generally be predicted thermodynamically to be oxidized at potentials accessible at unmodified solid electrode surfaces, such as glassy carbon.⁴⁶ In some instances, however, kinetically inhibiting electrochemical processes may be encountered. The alkyl peroxide and

hydroperoxide appear to be barely electro-oxidizable at the lower potentials, *i.e.*, $\geq +0.60$ V and $\leq +0.900$ V *versus* Ag/AgCl [Fig. 1(a)–(d)], as compared with cumene hydroperoxide, on the unmodified sensor surface. Further, the redox process appears to be more facile, over the available potential range, in phosphate buffered acetonitrile than in phosphate buffered methanol, as indicated by the enhanced oxidative response in this eluent system [compare Fig. 1(a) and (b) with Fig. 1(c) and (d)]. Beyond about +1.200 V *versus* Ag/AgCl, however, the system background current or baseline response also increased and the sensors took longer to reach a steady-state current value where there was minimal drift in the baseline signal. In addition, some distortion of the analyte peaks occurred, accompanied by interfering unidentified spurious peaks. Therefore, from the above-mentioned HDVs, the apparent optimum oxidation potential for the simultaneous detection and quantitative determination of the organic peroxides and hydroperoxides was about +1.150 V *versus* Ag/AgCl. Even though, as shown in Fig. 1(a)–(d), the signals for *tert*-butyl hydroperoxide and butan-2-one peroxide increase with higher positive potentials, the signal-to-noise ratio and selectivity of the detection are maximized at the lower oxidation potential(s).

Reversed-phase liquid chromatography

Effect of mobile phase composition

Fig. 2(a) and (b) show chromatograms for the elution of butan-2-one and *tert*-butyl hydroperoxide and cumene hydroperoxide, respectively, under reversed-phase isocratic elution conditions. The retention order under the specified conditions is butan-

2-one peroxide < *tert*-butyl hydroperoxide < cumene hydroperoxide. The elution of cumene hydroperoxide, because of its greater degree of hydrophobicity, with 10% v/v phosphate buffered methanol is prohibitively long [Fig. 2(b)], exceeding 1 h. As the percentage of organic modifier is increased, the retention time of cumene hydroperoxide decreases rapidly [Fig. 2(b)]. As a consequence, the chromatography appears to favor gradient elution for simultaneous separation and determination, and because of the potential minimization of the peak widths and elution times. However, gradient elution has the disadvantages of requiring longer equilibration times and incompatibility with (thin-layer) amperometric detection.

The effect of the amount of acetonitrile and methanol in the mobile phase on retention behavior of the organic peroxides and hydroperoxides was evaluated, and the results are presented, in part, in Fig. 3(a)–(c) (retention factors) and Fig. 4(a)–(c) (retention times) for C₁₈ and C₆ reversed-phase columns, respectively. The retention factors [see Fig. 3(a)–(c)], $k = (t_R - t_M)/t_M$, where t_R is the retention time of the peak of interest and t_M is the mobile phase hold-up time, and the separation factors, $\alpha = k_2/k_1$, were measured. Optimum chromatographic conditions were sought by varying the concentration of the organic modifiers and subsequently comparing the quality of the separation and the relative duration of the analysis by calculation of the retention and separation factors.

The logarithm of the retention factors for the organic peroxides and hydroperoxides decreases linearly with increasing percentage of acetonitrile and methanol modifiers [Fig. 3(a)–(c)]. However, the individual slopes are different, particularly noticeable between the aromatic and alkyl analytes, which is indicative of a change in selectivity on varying the percentage of the organic modifiers. The experimentally

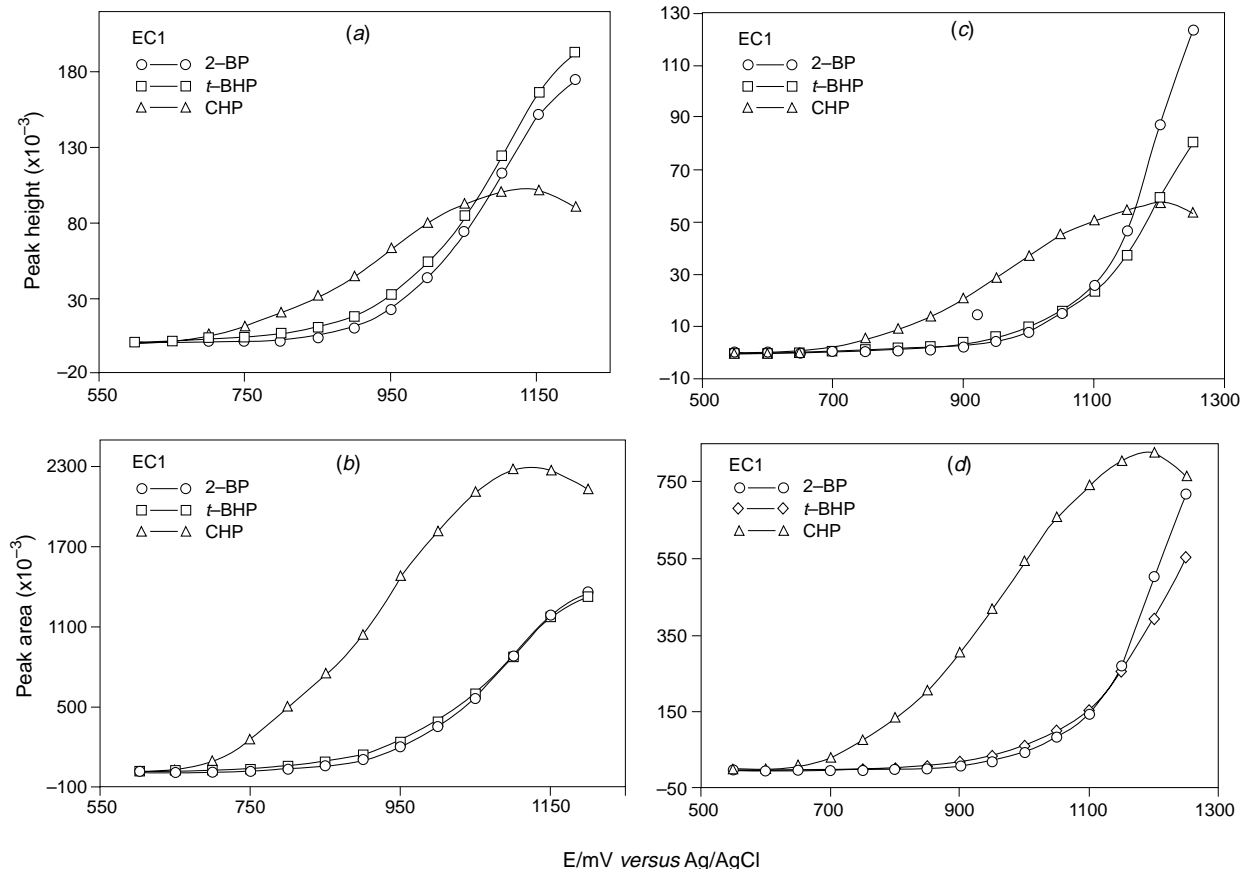


Fig. 1 Hydrodynamic voltammograms. Effect of potential (E_{APP}) on the peak height (a and c) and peak area (b and d). Conditions: eluent, 20% v/v acetonitrile (a and b) and 35% v/v methanol (c and d) and 0.05 M phosphatebuffer; electrode, unmodified glassy carbon; column, Spherisorb RP-6 (5 μ m), 25 \times 0.46 cm id; flow rate, 1 ml min⁻¹; amount of analyte, 100 ng each; E_{APP} , variable.

determined separation factors (α) indicate that whereas the selectivity changed with increasing modifier concentration, the conditions for separation remained favorable. The separation factor was determined to be >1 in all instances, and hence resolution even for the alkyl peroxides never appeared to be problematic. At organic modifier concentrations $>40\%$, for the $C_{18}, MeCN$ and $C_6, MeOH$ columns, the factor shows an unexpected increase, which cannot be explained satisfactorily. However, for the $C_{18}, MeCN$ column, the calculated separation factors over the entire range of modifier concentrations show no distinctive trend and are inconsistent. We proffer the following observation as perhaps a contributing factor to the described behavior. Extensive use of the columns, over extended periods of time, under the specified conditions, presented us with ever increasing analyte retention times. Remedies applied to correct the condition were unsuccessful and, as a consequence, the column instability that we encountered, particularly with the C_{18} columns, and with the pH as a potential contributing factor, could not be resolved.

Fig. 4(a)–(c) shows the effect of modifier concentration on the retention times of the organic peroxide analytes on the C_{18} and C_6 columns. Increasing concentrations of both acetonitrile and methanol exerted a dramatic effect on the retention behavior of cumene hydroperoxide. In Fig. 4(a), the retention time for cumene hydroperoxide is >1 h for the minimum modifier concentration and decreases to about 5 min for a modifier concentration of 50% v/v. Butan-2-one peroxide and *tert*-butyl hydroperoxide exhibit retention times barely exceeding 10 min even for the minimum organic modifier concentration used. Over the range of modifier concentrations studied, the

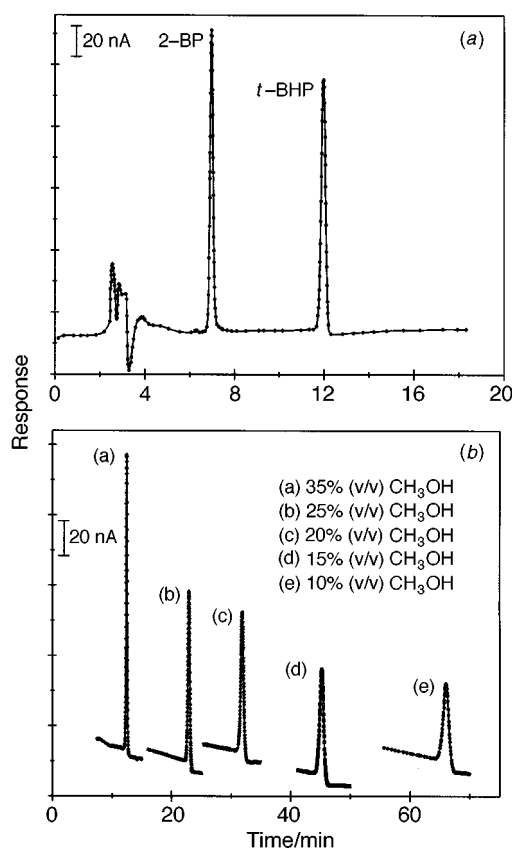


Fig. 2 Reversed-phased liquid chromatograms. Conditions: eluent, 5% v/v methanol (a), 10–35% v/v methanol (b), and 0.05 M phosphate buffer; electrode, unmodified glassy carbon; E_{APP} , +1250 mV versus Ag/AgCl; flow rate, 1 ml min⁻¹; sample volume, 20 μ l; column, Spherisorb RP-6 (5 μ m), 25 \times 0.46 cm id; analytes, butan-2-one peroxide, *tert*-butyl hydroperoxide and cumene hydroperoxide, 100 ng each.

retention times for either butan-2-one peroxide or *tert*-butyl hydroperoxide varied by less than 7 ± 2 min and the peroxides were at all times clearly resolved.

The amperometric response of the organic peroxides and hydroperoxides with acetonitrile as the organic modifier is shown in Fig. 5. The amperometric response for the alkyl organic peroxide and hydroperoxide increased with increasing modifier concentration up to about 20% v/v and subsequently decreased in a fairly linear fashion with further increases in the concentration of acetonitrile. Likewise, the amperometric oxidative response of cumene hydroperoxide appeared to increase linearly with increasing organic modifier concentration up to approximately 35% v/v. The response beyond the indicated maximum also appeared to decrease linearly with increasing organic modifier concentration.

The information obtained from Figs. 3–5 reveals that it is possible simultaneously to separate, detect and determine the organic peroxides under isocratic elution conditions in a single chromatographic run. Employing acetonitrile as the modifier, in laboratory confirmatory experiments, we selected concentrations in the range 25–35% v/v and obtained acceptable amperometric responses and reasonable retention times, in particular for cumene hydroperoxide. As noted previously the change in the retention times of the alkyl organic peroxides was insignificant with increasing modifier concentration.

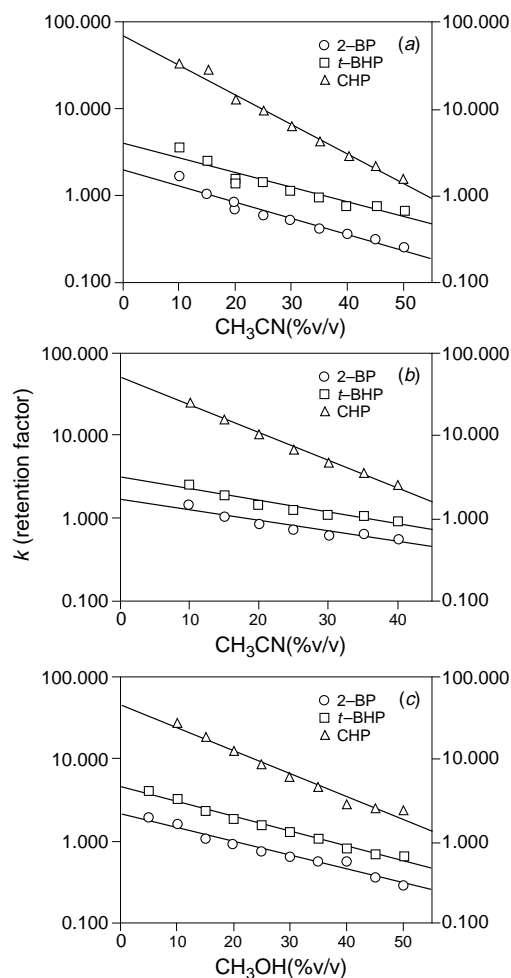


Fig. 3 Effect of organic modifier concentration on the retention factor of the organic peroxides. Analytes, butan-2-one peroxide, *tert*-butyl hydroperoxide and cumene hydroperoxide, 100 ng each; E_{APP} = (a) +1200, (b) +1150 and (c) +1250 mV; columns, (a) LiChrosorb RP-18 (5 μ m), 25 \times 0.40 cm id, (b and c), Spherisorb RP-6 (5 μ m), 25 \times 0.46 cm id; flow rate, 1 ml min⁻¹.

Linear range and sensitivity

Calibration curves for butan-2-one peroxide, *tert*-butyl hydroperoxide and cumene hydroperoxide were linear over the

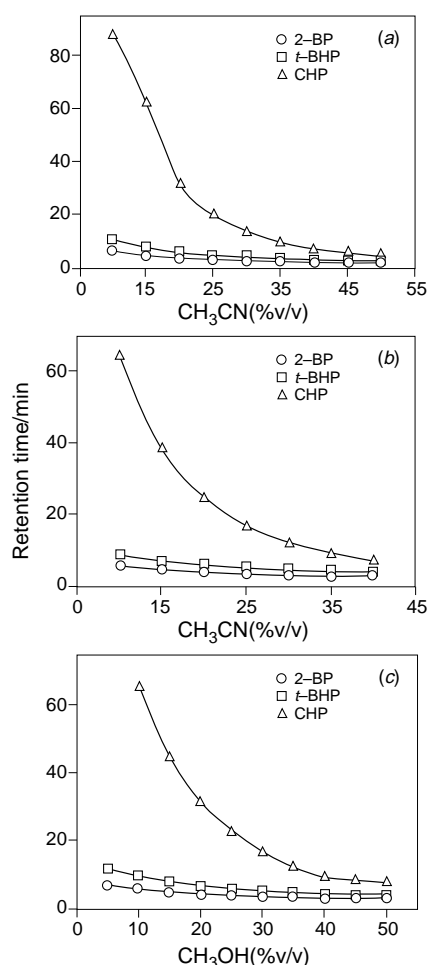


Fig. 4 Effect of organic modifier concentration on the retention time of the organic peroxides. Analytes, butan-2-one peroxide, *tert*-butyl hydroperoxide and cumene hydroperoxide, 100 ng each; E_{APP} = (a) +1200, (b) +1250 and (c) +1150 mV; columns, (a) LiChrosorb RP-18 (5 μ m), 25 \times 0.40 cm id, (b and c), Spherisorb RP-6 (5 μ m), 25 \times 0.46 cm id; flow rate, 1 ml min⁻¹.

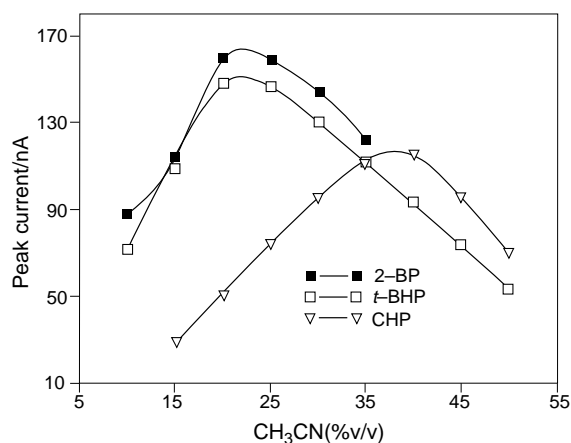


Fig. 5 Effect of organic modifier on the chromatographic response (peak height). Analytes, butan-2-one peroxide, *tert*-butyl hydroperoxide and cumene hydroperoxide, 100 ng each; E_{APP} = +1200 mV; column, LiChrosorb RP-18 (5 μ m), 25 \times 0.40 cm id; flow rate, 1 ml min⁻¹.

selected range of 0.2–200 ng (Table 1). The calibration curves (peak height *versus* amount injected) were linear ($r > 0.999$) and essentially passed through the origin. The deviation of the calibration curve from the origin, as measured by the y-intercept value, was about 2.03%₁ (2.06%₂), 0.26%₁ (0.19%₂) and 0.01%₁ (0.07%₂) for the above three analytes, respectively. The calibration sensitivity, based on measurement of the peak height, was also obtained for each analyte from routine calibration curves.

Applications

The three compounds were determined with two isocratic procedures. Known amounts of the analytes were added to laboratory distilled water and Cincinnati tap water. The results for the sample solutions presented in Table 2 show good agreement between the amount of analyte added and the recovered values. The data presented represent the average of at least three injections of each mixture and/or single analyte. The recoveries of butan-2-one peroxide, *tert*-butyl hydroperoxide

Table 1 Calibration data for the glassy carbon electrode

Analyte	Linear range/ng	Sensitivity/ units ng ⁻¹ ‡	Intercept (units)‡	r^2
2-BP*	2–200	604.0	–2432	0.9995§
	2–200	630.7	–2570	0.9995¶
<i>t</i> -BHP*	2–200	555.5	–283.7	0.9999§
	2–200	578.5	–223.0	0.9999¶
CHP†	0.2–200	338.8	8.70	0.9999§
	0.2–200	340.2	46.3	0.9999¶

Table 2 HPLC–amperometric determinations of butan-2-one peroxide (2-BP), *tert*-butyl hydroperoxide (*t*-BHP) and cumene hydroperoxide (CHP). At least three determinations were performed on each Cincinnati tap water solution and each laboratory de-ionized, distilled reagent water solution.

Matrix	Sample	Peroxide	Peroxide added/ng	Peroxide determined/ng (mean \pm s)	Recovery (%) (mean \pm s)
Reagent Water	A	2-BP*	3	3.0 \pm 0.1	100.5 \pm 3.7
		<i>t</i> -BHP*	3	2.9 \pm 0.0	98.3 \pm 0.6
	B	2-BP*	7	7.1 \pm 0.2	100.7 \pm 2.1
		<i>t</i> -BHP*	7	6.9 \pm 0.0	99.3 \pm 0.6
Tap Water	C	2-BP*	12	11.8 \pm 0.2	98.7 \pm 1.7
		<i>t</i> -BHP*	12	11.9 \pm 0.1	99.3 \pm 0.7
	D	2-BP*	5	5.5 \pm 0.1	109.7 \pm 0.9
		<i>t</i> -BHP*	5	4.7 \pm 0.0	94.8 \pm 0.9
Reagent Water	A	CHP†	5	5.3 \pm 0.05	105.1 \pm 1.1
		CHP†	10	9.7 \pm 0.10	97.7 \pm 1.0
	C	CHP†	20	20.4 \pm 0.06	101.9 \pm 0.3
		CHP†	5	5.1 \pm 0.12	102.9 \pm 2.4
Tap Water	E	CHP†	8	8.1 \pm 0.03	100.7 \pm 0.5
		CHP†	17	17.1 \pm 0.26	100.9 \pm 1.5
	G	CHP†	20	20.1 \pm 0.13	100.5 \pm 0.7

* Analysis performed in 5% v/v CH₃OH and 0.05 M phosphate buffer, flow rate 1 ml min⁻¹; working electrode, unmodified glassy carbon; E_{APP} = +1150 mV *versus* Ag/AgCl; sample loop, 20 μ l; column, Spherisorb RP-6 (5 μ m), 25 \times 0.46 cm id; temperature, ambient. † Analysis performed in 35% v/v CH₃OH and 0.05 M phosphate buffer. All other conditions as for 2-BP and *t*-BHP.

and cumene hydroperoxide from laboratory distilled water and Cincinnati tap water averaged 102.4 ± 4.9 , 98.0 ± 2.1 and $101.4 \pm 2.3\%$, respectively.

Conclusion

This study demonstrates that organic peroxides and hydroperoxides, of differing organic character and structure, can be effectively separated and determined by HPLC with oxidative amperometric detection at an unmodified glassy carbon electrode. We investigated various experimental parameters in order to be able to optimize the chromatographic procedure, and subsequently observed their effect on the magnitude of the analytical signal, retention time, degree of separation, *etc.* Under isocratic elution conditions acetonitrile appears to be the preferred organic modifier because of its greater solvent strength, lower viscosity and, as a consequence, lower system operating pressure and the apparent enhanced amperometric oxidative responses of the analytes, *etc.*

The optimum electrode potential for the simultaneous determination of the organic peroxides and hydroperoxides was approximately +1.150 V *versus* the Ag/AgCl. Additionally, alkyl and aromatic peroxides and hydroperoxides can be simultaneously determined in a single isocratic experiment using organic modifier concentrations between 25 and 35% v/v. The type of reversed-phase column, whether C₁₈, C₈ or C₆, appeared to have little effect on the analyte retention times or degree of separation.

Our research on these and other organic peroxides and hydroperoxides and other potential ozonation disinfection by-products (*e.g.*, hydrogen peroxide, chloramines, aldehydes, ketones, acids, bromate) is continuing in the following areas: (a) reductive amperometric detection in a thin-layer cell, (b) oxidative and reductive coulometric detection and determination, including isocratic and gradient elution, (c) development of electrocatalytic and biocatalytic sensors, (d) off-line electrochemical characterization, (e) developing and evaluating corroborative spectroscopic measurement procedures, (f) the identification and elimination of interferences and (g) determining the effect of pH on analyte behavior.

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The mention of commercial products does not constitute endorsement or recommendation for use by the US Environmental Protection Agency.

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