Flow injection Fourier transform infrared determination of nicotine in tobacco

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A fully automated procedure is proposed for the Fourier transform infrared (FTIR) determination of nicotine in tobacco. The method is based on the on-line extraction of nicotine with $CHCl_3$. Samples, weighed inside empty extraction cartridges, were humidified with NH_3 and the cartridges were installed in a flow manifold in which they were extracted with 2 ml $CHCl_3$ for 2 min, then 400 μl of the extract were introduced into a micro-flow cell using a carrier of $CHCl_3$ and the IR spectrum was registered continuously. The absorbance, in the wavenumber range 1334–1300 cm^{-1}, was measured, obtaining a peak as a function of time. The area of this peak was interpolated on a calibration line established from standard solutions of nicotine in chloroform treated in the same way as samples. The method provided a limit of detection of 0.1 mg ml^{-1} nicotine, an RSD lower than 2% and a sampling frequency of the whole procedure of 6 h^{-1}. Results obtained for natural samples of cut tobacco and cigar compared well with those obtained by a batch FTIR procedure, involving an off-line extraction with a total time of 16 min. However, for yellow tobacco cigarette, an on-line extraction time of 10 min was required to obtain a good recovery of nicotine.

Introduction

The large number of samples processed by the tobacco industry and the importance of some tobacco parameters, such as alkaloids, for the information and health of consumers require the development of automated procedures.

The first automated method reported for total alkaloids in steam distillates of tobacco leaf was based on its spectrophotometric determination using cyanogen bromide[1] and modular instruments for tobacco analysis have integrated this reaction for the fast automated determination of nicotine alkaloids[2] and alkaloids together with other parameters such as NO_3^-, NH_4^+ and PO_4^{3-}[3] or reducing sugars.[4,5]

However, cyanogen bromide is a toxic reagent and storage problems, volatility and apparent deterioration of this reagent have been reported, hence additional efforts have been made in order to replace it and to effect the on-line generation of cyanogen chloride from potassium thiocyanate and NaOCl[6] or from chloramine-T and potassium cyanide.[7]

The single alternative to the spectrophotometric automated determination of alkaloids, based on infrared spectrometry, is that developed by Long[8] using an automated pyrolysis technique.

In a previous study,[9] we developed a simple Fourier transform infrared (FTIR) procedure for the inbatch determination of nicotine in tobacco samples, based on the extraction with chloroform, which is suitable to be automated by carrying out the extraction process on-line. In fact, the main idea of the present study was to consider tobacco leaves as a solid support in which alkaloids were naturally concentrated and, taking into consideration the exciting possibilities reported previously for on-line elution of analytes preconcentrated by solid phase extraction,[10–12] to develop a fully automated procedure by carrying out the on-line extraction of nicotine with $CHCl_3$ from solid samples weighed inside empty extraction cartridges and humidified with NH_3.

Experimental

Apparatus and reagents

A Nicolet (Madison, WI, USA), Magna 750 FTIR spectrometer equipped with a temperature-stabilized DGTS detector, a long-lasting Ever-Glo source and a KBr beamsplitter, was employed for spectral measurements with a nominal resolution of 4 cm^{-1} using a Spectra Tech (Warrington, UK) micro-flow-through cell with ZnSe windows and a pathlength of 0.457 mm. The on-line nicotine extraction and FTIR measurements were carried out using the manifold shown in Fig. 1. Samples, weighed inside extraction cartridges of 10 mm id and 3.6 ml volume, were placed in an ultrasonic water-bath (Selecta, Barcelona, Spain)

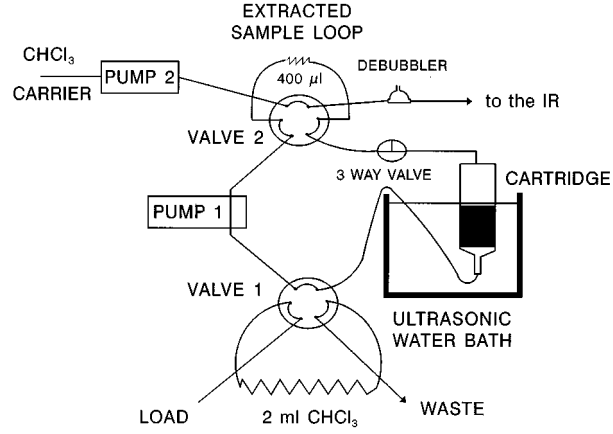

Fig. 1 Manifold employed for FI-FTIR determination of nicotine in tobacco.

installed between two interconnected six-way Rheodyne (Cotati, CA, USA) Type 50 valves. Two Gilson (Villiers-le-Bel, France) Minipuls 2 peristaltic pumps were employed to create a closed-flow system (pump 1) and to transport the extracted samples to the detector (pump 2). A laboratory-made glass debubbler was installed before the micro-flow-through IR cell and a three-way directional valve was used for cleaning the closed-flow extraction system. Viton (isoversinic) flexible tubes of 3 mm od and 1 mm id were employed as pump tubing and 0.8 mm id PTFE connecting tubes were used to construct the manifold.

(−)-Nicotine from Fluka (Buchs, Switzerland), $CHCl_3$ containing 150 mg l^{-1} of amylene as stabilizer from Scharlau (Barcelona, Spain) and ammonia solution (30%) from Panreac (Barcelona, Spain), all of analytical-reagent grade, were employed as reagents.

Various types of cigarettes and cut and shag tobacco samples were obtained from the Spanish market and were analysed without any previous treatment. Data reported in this work correspond to nicotine concentrations in wet samples, the average humidity of cigarette samples being 4.5% m/m.

Recommended procedure

A 0.2 g tobacco sample was accurately weighed inside an empty extraction cartridge of 10 mm id and 3.6 ml volume and humidified with 1 ml of 0.1 M NH_3 solution. The cartridge was inserted in the manifold shown in Fig. 1 and partially immersed in an ultrasonic water-bath until the position indicated in Fig. 1, which shows that the part containing the sample was completely submerged. The loop of valve 1 was filled with 2 ml of $CHCl_3$ in the load position and then the valve was moved to the inject position and $CHCl_3$ introduced into the cartridge from the bottom switching on pump 1. After $CHCl_3$ introduction into the cartridge, the direction of the flow was reversed to work with the minimum dead volume, and the extraction of nicotine from tobacco was carried out for 2 min in a closed-flow system which was previously full of air. After this time, 400 μl of the extract were sampled with valve 2 and injected into the carrier flow to be transported to the detector.

FTIR spectra were continuously recorded between 4000 and 600 cm^{-1}, using a nominal resolution of 4 cm^{-1} and by accumulating two scans per spectrum. From these spectra, peak area absorbance values at 1316 cm^{-1}, measured between 1334 and 1300 cm^{-1}, were obtained as a function of time and the area of the corresponding FI recording was employed as the quantitative analytical paremeter, making a baseline correction between consecutive injections after an 11 point smoothing of the recordings. Values for samples were interpolated on a calibration curve obtained from the injection of 400 μl of nicotine standards dissolved in $CHCl_3$ and measured in the same way as samples.

Results and discussion

FTIR spectra of nicotine solutions and tobacco extracts

The mid-infrared spectra of nicotine solutions in chloroform present a series of characteristic bands in the wavenumber range 1500–900 cm^{-1}, as can be seen in Fig. 2. When tobacco samples were humidified with aqueous 0.1 M NH_3 and extracted in chloroform, stabilized with amylene, the FTIR spectra show the same bands as nicotine standards but with a baseline shift due to the presence of water, hence the band at 1316 cm^{-1}, with a baseline established between 1300 and 1334 cm^{-1}, was selected to be employed for the determination of nicotine.

Effect of the experimental conditions for flow injection measurement and on-line extraction

The carrier flow is a critical parameter in flow injection analysis, affecting the analytical sensitivity, reproducibility and sampling frequency. To evaluate the effect of carrier flow on the FI determination of nicotine, 400 μl aliquots of a solution containing 2.0 mg ml^{-1} nicotine were measured at different carrier flow rates from 0.5 to 1.33 ml min^{-1}. As can be seen in Table 1, for five injections of the same solution, an increase in the carrier flow rate causes a decrease in the analytical sensitivity and repeatability of measurements but increases the sampling frequency. Therefore, in order to achieve a compromise between analytical sensitivity and reproducibility and productivity, a carrier flow rate of 0.66 ml min^{-1} $CHCl_3$ was selected, obtaining under these conditions a sampling frequency of 36 h^{-1} and an RSD of 0.9%.

The effect of sample volume injected into the system after extraction was evaluated from 100 to 500 μl for a nicotine concentration of 1.9 mg ml^{-1} and using a carrier flow rate of 0.66 ml min^{-1}. The peak height of the FI recordings increased when the injection volume increased but a drastic decrease in the sampling frequency was observed, from 54 to 35 h^{-1} as a function of the peak shape (Fig. 3).

Using peak area values, a linear dependence between analytical signal and injection volume was found, the regression equation being $A = (0.001 \pm 0.005) + (0.000407 \pm 0.000015)V$ (V in μl) with a regression coefficient $r = 0.998$, thus opening up the possibility of using different injection volumes for the analysis of samples with different nicotine concentrations.

As a compromise between sensitivity and sampling frequency, an injection volume of 400 μl, which provide a sample frequency of 40 h^{-1} and an RSD for five different injections of the same solution of 1.2 %, was selected.

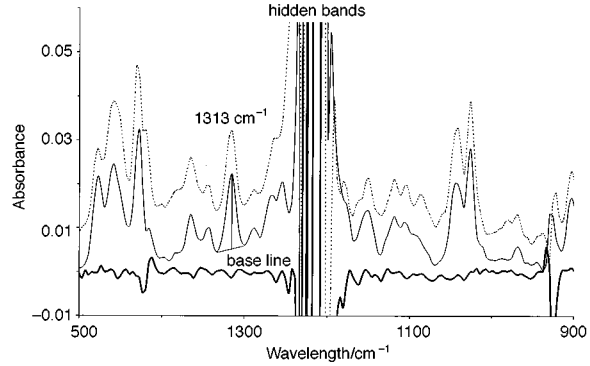

Fig. 2 FTIR spectra of a nicotine solution (——) and a nicotine extract of tobacco sample(.....). Experimental conditions $CHCl_3$ solutions containing 1.85 mg ml^{-1} and extract of tobacco sample of 2.12 mg ml^{-1} of nicotine, $CHCl_3$ being employed as a blank (——).

Table 1 Effect of $CHCl_3$ carrier flow rate on FI-FTIR determination of nicotine

Carrier flow rate/ ml min^{-1}	Area ± s_{n-1}	Sampling frequency/h^{-1}	RSD (%)
0.50	0.213 ± 0.003	30	1.5
0.56	0.192 ± 0.004	33	2.2
0.66	0.165 ± 0.001	36	0.9
0.73	0.142 ± 0.001	38	0.8
0.82	0.136 ± 0.003	44	2.4
0.98	0.110 ± 0.004	52	3.5
1.33	0.082 ± 0.002	59	2.5

Experimental conditions: nicotine solution in chloroform containing 2.0 mg ml^{-1}, sample volume 400 μl. Area values, expressed in arbitrary units, were measured from the absorbance spectra in the wavenumber range 1334–1300 cm^{-1} from two scans at a resolution of 4 cm^{-1}. The standard deviation (s_{n-1}) and RSD correspond to five independent injections.

In order to evaluate the effect of the $CHCl_3$ volume employed for the on-line extraction of nicotine, a fixed amount of 0.2 g of cut tobacco was treated in the manifold shown in Fig. 1 with different volumes of $CHCl_3$, from 1 to 3.4 ml, and using an injection loop of 400 μl for FI-FTIR measurements. As can be seen in Fig. 4, total recovery of nicotine was obtained in all cases. The continuous band in Fig. 4 corresponds to the average value ± the standard deviation of analyses carried out by off-line extraction. However, on using extraction volumes lower than 2.0 ml it is difficult to carry out various replicate measurements of the same extract and, for this reason, a volume of 2 ml was selected.

The effect of $CHCl_3$ recirculation time to extract nicotine was studied for 0.2 g of tobacco using a chloroform volume of 2 ml and a flow rate of 1.69 ml min^{-1}. The results obtained indicate that solvent recirculation times ⩾2 min provide a quantitative extraction of nicotine (Fig. 5) and, trying to improve the sampling frequency as much as possible, a 2 min extraction time was chosen.

The effect of flow rate of the solvent in the closed system for the on-line extraction of nicotine was studied from 0.66 to 2.01 ml min^{-1} using in all cases a fixed extraction time of 2 min, 2 ml of $CHCl_3$ and a tobacco mass of 0.2 g. The flow rate was not a critical parameter for the nicotine extraction, as it was observed to have no effect in the range studied (Fig. 6). However, in order to obtain a good sampling frequency a flow rate of 1.69 ml min^{-1} was chosen.

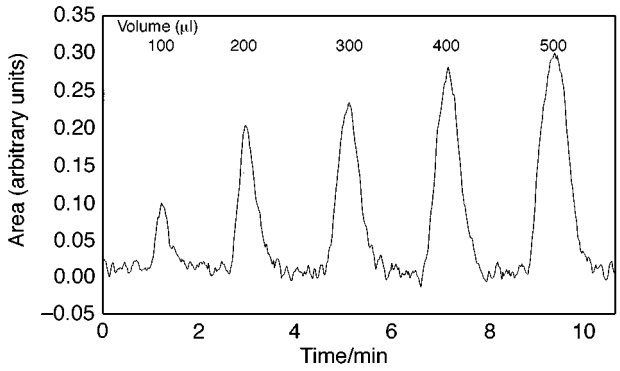

Fig. 3 Effect of extracted sample volume injected on the FI recordings obtained for nicotine. Peaks were obtained from the area of FTIR spectra measured between 1334 and 1300 cm^{-1}. Nicotine concentration, 1.9 mg ml^{-1}; carrier flow rate, 0.66 ml min^{-1}.

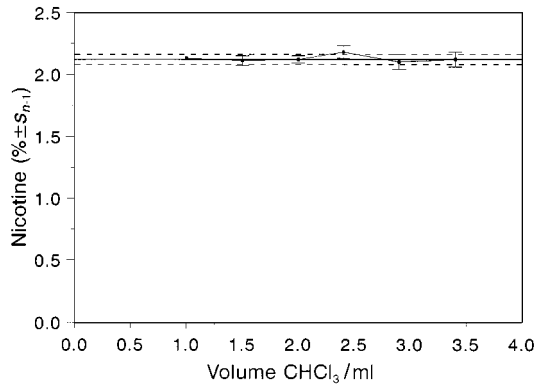

Fig. 4 Effect of $CHCl_3$ volume employed for the on-line extraction of nicotine from tobacco. Experimental conditions: sample mass, 0.2 g of cut tobacco containing 2.12 ± 0.04% nicotine; injection volume, 400 μl; carrier flow rate, 0.66 ml min^{-1}; recirculation flow rate 1.69 ml min^{-1}. Resolution 4 cm^{-1}; two accumulated scans. The error bands correspond to three independent measurements.

Analytical figures of merit of the FI-FTIR determination of nicotine

The calibration lines for the FI-FTIR determination of nicotine were obtained for the concentration range 0–3.06 mg ml^{-1} nicotine. A typical calibration line corresponds to $y = (0.005 \pm 0.002) + (0.086 \pm 0.001)\ C$ with $S_{(y/x)} = 0.003$ and a regression coefficient $r^2 = 0.9996$, y being the area of the FI peaks and C the nicotine concentration in mg ml^{-1}.

On the other hand, working with peak height values of the FIA recordings, established from the maxium spectrum, a calibration curve $y = (0.0003 \pm 0.0002) + (0.00896 \pm 0.0001)\ C$ with $r^2 = 0.996$ was obtained, thus indicating a loss of sensitivity compared with the use of peak area values.

The limit of detection, corresponding to 0.1 mg ml^{-1} nicotine, was established from the repeatability of blank measurements for a probability level of 99.6%. An RSD of 0.8% was determined from three independent analyses of a sample containing 2.12% m/m of nicotine. Hence it can be considered that samples with nicotine concentrations higher than 0.1% can be analyzed with an appropriate level of precision in only 10 minutes, including on-line all the different steps, from analyte extraction to FTIR determination, providing a sampling frequency of the whole method of 6 h^{-1}.

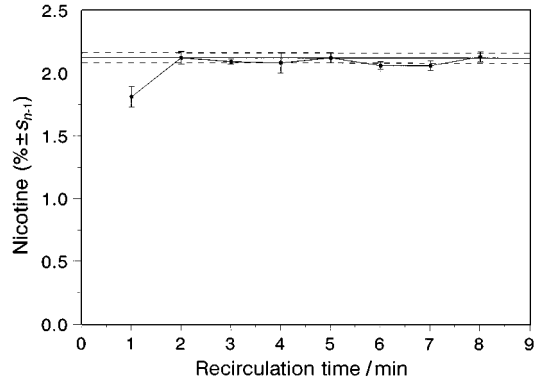

Fig. 5 Effect of $CHCl_3$ recirculation time on the on-line extraction and FI-FTIR determination of nicotine in tobacco. Experimental conditions: sample mass 0.2 g of cut tobacco containing 2.12 ± 0.04 % nicotine; solvent volume, 2 ml; recirculation flow rate 1.69 ml min^{-1}; sample injection volume, 400 μl; carrier flow rate 0.66 ml min^{-1}. Resolution 4 cm^{-1} and 2 accumulated scans. The error bands correspond to three independent measurements on the same sample.

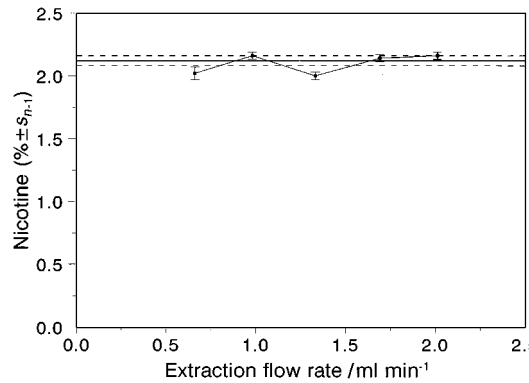

Fig. 6 Effect of $CHCl_3$ flow rate on the on-line extraction of nicotine and FI-FTIR determination. Experimental conditions: sample mass, 0.2 g of cut tobacco containing 2.12 ± 0.04% nicotine; solvent volume, 2 ml; time of extraction, 2 min; sample injection volume, 400 μl; carrier flow rate, 0.66 ml min^{-1}. Resolution 4 cm^{-1} and two accumulated scans. The error bands correspond to three independent measurements on the same sample.

Table 2 Results obtained by on-line extraction and FI-FTIR determination of nicotine in tobacco

Sample	Nicotine concentration ± s_{n-1} (%)	
	FI-FTIR	Reference method
Cut tobacco	2.10 ± 0.03	2.12 ± 0.04
Cigar	0.99 ± 0.02	0.96 ± 0.03

Results reported as are the average of three independent analyses. The reference method consisted of stopped-flow FTIR determination of nicotine by accumulating 25 scans, after off-line extraction using a total extraction time of 16 min.

Analysis of natural samples

The developed procedure for on-line extraction and FI-FTIR determination of nicotine was employed to analyse two natural samples of cut tobacco and cigar using nicotine solutions in $CHCl_3$ as standards. The results obtained are summarized in Table 2 and, as can be seen, the nicotine concentrations found are comparable to those obtained by a reference in-batch FTIR method involving off-line extraction and stopped-flow determination of nicotine using 25 cumulated scans with a total required time for the extraction of 16 min.[9]

Two yellow cigarette samples, analyzed in the same way as the previous ones, provided average nicotine concentrations of 1.29 ± 0.06 and 1.36 ± 0.02% by FI-FTIR, lower than those found after off-line extraction, which were 1.499 ± 0.005 and 1.56 ± 0.02%. However, by increasing the time of the on-line extraction from 2 to 10 min, the results were 1.37 ± 0.02 and 1.49 ± 0.03%, thus indicating that for some types of samples probably an increased extraction time would be required.

Conclusions

The procedure developed confirms the applicability of the on-line extraction and FI-FTIR measurement for the analysis of complex samples without needing tedious preliminary sample treatments. This methodology involves the use of a closed system and avoids the direct handling of toxic organic solvents, reduces the consumption of reagents and waste generation and opens up new possibilities for the automated determination of analytes in solid samples. However, the installation of extraction cartridges in the manifold must be carried out manually, hence this step is the major limitation of the methodology developed in order to provide a fully automated procedure. On comparing the developed procedure with the previous FTIR batch methodology,[9] it must be noted that the sensitivity is lower for FI than for off-line extraction and stopped-flow determination, the limit of detection of this latter method being half that found by FI. However, it is clear that, for the concentration level at which nicotine is present in tobacco, samples can be analysed correctly by FI-FTIR and that the reduction of toxic solvent handling and $CHCl_3$ consumed provides an environmentally friendly method which compensates for the lower sensitivity.

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