ELSEVIER

Journal of Chromatography A, 824 (1998) 241–245

JOURNAL OF CHROMATOGRAPHY A

Short communication

Determination of nicotine in water by gradient ion chromatography

G.P. Ayers*, P.W. Selleck, R.W. Gillett, M.D. Keywood

Division of Atmospheric Research, CSIRO, PMB 1, Aspendale 3195, Australia

Received 31 March 1998; received in revised form 28 July 1998; accepted 3 August 1998

Abstract

A sensitive gradient ion chromatographic method has been demonstrated for determination of nicotine in aqueous solution. The method provides an improvement in detection limit, plus a reduction in analysis time, compared with a previously published ion chromatographic method.

Keywords: Nicotine

1. Introduction

A case for development of a sensitive, aqueous-phase method for determination of nicotine was articulated by Lewis et al. [1] on the basis that ''where nicotine is used as a tracer for environmental tobacco smoke (ETS), the samples are sometime water extracts of acid-coated filters or denuders that have been used to sample indoor air''. An invitation to assist another Australian agency in a pilot study on indoor air quality, with nicotine to be collected on acid-impregnated filters and quantified as a surrogate for ETS, led us to the same conclusion concerning the need for a simple method for determination of aqueous phase nicotine. Like Lewis et al. [1] our approach was based on a form of ion chromatography (IC) analysis, which can be seen as a specific variant of the more common high-performance liquid chromatography (HPLC) analytical method [2].

In the pilot study two methods of gaseous nicotine sampling were to be trialed, along the lines adopted by Eatough et al. [3] and Hammond et al. [4]. One was a low-volume active sampling method in which a small pump drew air through an aerosol impactor, followed by a triple filter pack containing a PTFE aerosol filter followed by a pair of acid-impregnated glass-fibre filters for collection of gaseous nicotine. The second method to be trialed was a passive sampling method, in which the nicotine was also to be collected on an acid- impregnated filter, but in this case solely by molecular diffusion. The much lower sampling rate achieved by the passive sampling method ensured the need for a sensitive method of nicotine determination. Description of the analytical method developed for determination of aqueous nicotine is the focus of this note. Full details of other aspects of the pilot study and its findings will be presented elsewhere.

2. Experimental

2.1. Instruments

The IC system employed in this work was based around a Dionex GP40 gradient pump, AS3500

*Corresponding author.

0021-9673/98/$ – see front matter
PII: S0021-9673(98)00632-3

autosampler and UI20 detector interface all under computer control (via PeakNet software). An LDC/Milton Roy Spectromonitor D variable-wavelength UV–visible detector was employed, with the chromatographic separations achieved on a Dionex OmniPac PCX-500 column preceded by a Dionex OmniPac Guard PCX-500 column.

The nicotine passive air-sampling system employed was based on the well characterised passive sampler design of Ferm [5].

2.2. Consumables

All chemicals employed were analytical-reagent grade or better. Deionised water was obtained from a Milli-Q system with organics removal and filtration through a 0.22-μm filter. Standard solutions were prepared from 40% (w/v) aqueous nicotine hemisulfate solution (Sigma, stock No. N-1019), accurately standardised by determination of sulfate using IC (Dionex DX500) with suppressed conductivity detection. The environmental nicotine sampling substrates were 47 mm diameter Gelman A/E glass fibre filters for the active sampler, and 25 mm diameter Whatman GF/A filters for the passive samplers.

2.3. Procedures

Chromatographic conditions employed were a flow-rate of 1.0 ml min^{-1}, injection volume of 50 μl, and detector wavelength of 260 nm. Mobile phase composition was varied linearly between the gradient set-points shown in Table 1.

The coating solution used to collect nicotine on both the active and passive samplers was 4% $NaHSO_4$ by mass dissolved in water–5% methanol. The filters were soaked in coating solution and dried under high purity nitrogen prior to use. After exposure, the collected nicotine was obtained as an aqueous solution by extracting each filter in a small polyethylene bags with an aliquot of deionised water, 40.0 ml or 5.0 ml for the active filters and 2.0 ml for the passive filters. Extraction was for 1 h, with periodic shaking. For calculation of nicotine levels from the passive samplers, a diffusion coefficient of 0.063 cm^2 s^{-1} was employed [3].

3. Results and discussion

3.1. Chromatography

The procedure outlined above returned a well-separated nicotine peak at a retention time of 7.6 min. Peak area response was linear over the range of standard solutions tried: for seven higher concentration standards from 8 to 512 μ*M*, an r^2 of 0.9999 was achieved with intercept of 3.2 ± 2.2 μ*M*; for five low concentration standards from 0.5 to 8 μ*M*, an r^2 of 0.9992 and intercept of -0.06 ± 0.10 μ*M* were achieved.

Fig. 1 contains a plot showing a chromatogram from a high-level standard. The positive and negative detector responses at times prior to about 6 min were present in all chromatograms including blanks, indicating that these baseline excursions resulted from in mobile phase extinction due to mixing. This was of no consequence for the nicotine determination, since the baseline was flat past 6 min, by which time a constant mobile phase composition (and thus constant extinction) had been achieved (Table 1). The gradient was necessary as isocratic separation of nicotine had not proved possible in a range of experiments prior to the adoption of the method described here.

Based on standard chromatograms, produced using

Table 1
Mobile phase composition: linear gradient between composition shown (as % of each component)

Component	Time (min)					
	0.0	0.2	0.50	0.75	1.75	10
(A) CH_3CN–water (90:10)	15	15	40	53	60	60
(B) 1.0 *M* KCl	10	10	10	10	30	30
(C) 0.5 *M* HCl	5	5	10	10	10	10
(D) Water	70	70	40	27	0	0

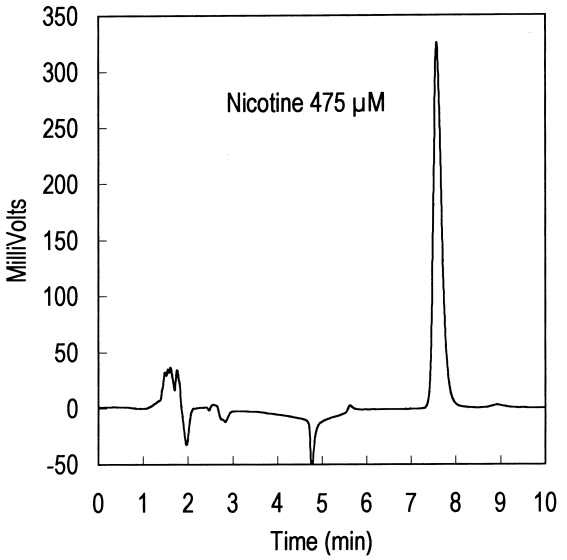

Fig. 1. Standard chromatogram with a 50-µl injection for a nicotine concentration of 475 µ*M*. All peaks prior to 6 min derive from solvent mixing.

a 50-µl injection volume, the limit of detection determined as a peak height three-times the baseline peak-to-peak noise level, was ~0.01 µ*M* (~2 ng ml^{-1}). Since injection volumes up to 10-times that tested here are feasible with the column used, a detection limit of order 1 nmol l^{-1} (i.e., <1 ng ml^{-1}) appears possible.

Practical application of the method requires two additional checks: (1) that other alkaloids or related compounds typical of cigarette smoke are well separated chromatographically from nicotine, and (2) that extraction of nicotine from the sampler filters was efficient and reproducible. Regarding the first point, the adoption of a gradient method recommended itself not only because it enabled elution of nicotine rapidly while maintaining excellent peak shape, but also because it enabled very clear separation of nicotine from other similar or related compounds. Application of the method to standard solutions of cotinine, pyridine and 2-ethyenylpyridine produced elution in that order, as reported previously by Lewis et al. [1], but with retention times of 2.2, 2.8 and 3.1 min, well separated from nicotine at 7.5 min.

Regarding the second point, recovery was checked by pipetting varying, known amounts of nicotine standard solution on to five bisulfate-impregnated collection filters, which were then air dried. The filters were extracted for 1 h with shaking, as noted above, and analysed as for the ambient samples. At expected extract nicotine concentrations of 500, 50, 20, 10 and 5 µ*M* recovery was determined to be 95, 95, 95, 96 and 94% respectively.

3.2. Test measurements

Two simple experiments were carried out to check that the dynamic range of the method and the sensitivity achieved under ideal laboratory conditions (i.e., using standard solutions) were also achieved in more complex sample matrices of actual environmental samples. The test of dynamic range was carried out using the active filter-pack sampler that employed a pair of acid-sulfate impregnated backup filters to collect gas-phase nicotine at ~1 l min^{-1} flow-rate. A strong source of gaseous nicotine was provided by connecting the system to a cigarette smoking test rig. Smoke from two cigarettes of differing brands was sampled, each from a single burn of the whole cigarette, with all smoke drawn from the cigarette passed through the active sampling system. Aqueous extracts of the front and back filters using de- ionised water were subjected to the nicotine analysis. The results presented in Table 2 show a wide dynamic range for the technique, evident in the <1% breakthrough recorded for the filter pairs. This

Table 2
Nicotine levels in extracts of cigarette smoke collected using an active dual filterpack sampler

Cigarette	Filter	Extract (ml)	Nicotine (µ*M*)	Nicotine (mg)
1	Front	40	549	3.6
1	Back	5	3.1	0.0
2	Front	40	440	2.9
2	Back	5	0.83	0.0

The total nicotine level per cigarette is given in the final column.

Table 3
Nicotine concentration (μM) in extracts from passive diffusion samplers placed at two locations in each of three bars and two homes, for a 48-h period.

Site	Extract (ml)	Nicotine (μM)	Nicotine ($\mu g\ m^{-3}$)
Bar 1	2	3.6	54
Bar 1	2	6.2	93
Bar 2	2	4.1	62
Bar 2	2	3.8	57
Bar 3	2	1.9	29
Bar 3	2	2.5	38
Smoker's home	2	0.57	8.2
Smoker's home	2	0.48	6.9
Non-smoker's home	2	<0.01	0.0
Non-smoker's home	2	<0.01	0.0

Corresponding atmospheric nicotine levels are given in the final column.

high collection efficiency for the impregnated filters is consistent with previous observations [4].

The second test involved deployment of passive gas samplers for a 48-h period in three bars, the home of a smoker, and the home of a non-smoker. The passive filters were extracted in deionised water, followed by analysis for nicotine.

Results are presented in Table 3, with a representative chromatogram shown in Fig. 2. The amounts measured accord well with previously published data for similar sampling situations [1–4,6]. Given a limit of detection of ~0.01 μM, the data in Table 2 confirm that the method presented here has adequate sensitivity, as well as dynamic range, to cope with both active and passive sampling of nicotine under ambient conditions.

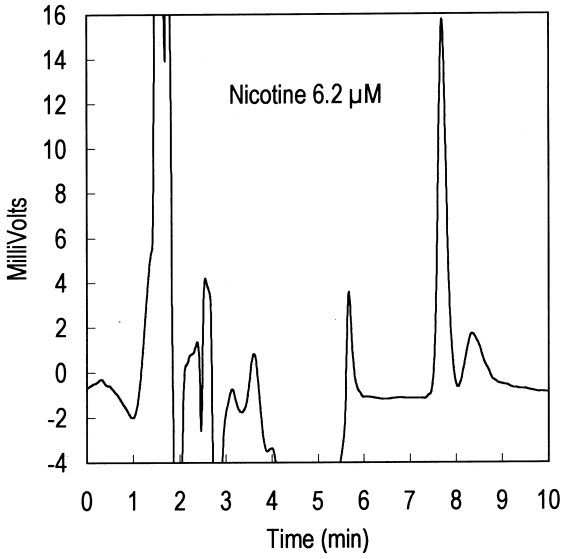

Fig. 2. Chromatogram from a passive air sampler extract yielding a nicotine concentration of 6.2 μM. The broad peak at ~8.5 min is an unidentified compound (perhaps an alkaloid) present with nicotine in all samples containing nicotine. All peaks prior to 6 min derive from solvent mixing.

4. Conclusions

A sensitive, rapid (10 min per analysis) method for determination of aqueous nicotine by gradient IC with UV detection has been described. The method was found to be linear in response over the concentration range investigated (0.5–512 μM), and has a limit of detection of ~0.01 μM (~2 ng ml^{-1}) for a 50-µl injection. Higher sensitivity could be achieved if desired, since the system can accommodate considerably larger injection volumes.

Acknowledgements

The authors gratefully acknowledge technical advice provided by T. Williams of Linbrook International.

References

[1] L.J. Lewis, J.D. Lamb, D.J. Eatough, L.D. Hansen, E.A. Lewis, J. Chromatogr. Sci. 28 (1990) 200.

[2] Z.S. Troje, Z. Frobe, D. Perovic, J. Chromatogr. A 775 (1997) 101.
[3] D.J. Eatough, C.L. Benner, J.M. Bayona, G. Richards, J.D. Lamb, M.L. Lee, E.A. Lewis, L.D. Harrison, Environ. Sci. Technol. 23 (1989) 697.
[4] S.K. Hammond, B.P. Leaderer, A.C. Roche, M. Schenker, Atmos. Environ. 21 (1987) 457.
[5] M. Ferm, A Sensitive Diffusional Sampler, Report L91-172, IVL (Swedish Environmental Research Institute), Gothenburg, 1991.
[6] NRC Committee on Passive Smoking, Environmental Tobacco Smoke: Measuring Exposure and Assessing Health Effects, National Academy Press, Washington, DC, 1986, ISBN 0309037301.