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Analytical determination of nicotine in tobacco by supercritical fluid chromatography–ion mobility detection

Ching Wu[a], William F. Siems[a], Herbert H. Hill, Jr.[a,*], Rich M. Hannan[b]

[a]*Department of Chemistry, Washington State University, Pullman, WA 99164-4630, USA*

[b]*USDA-ARS, Regional Plant Introduction Station, 59 Johnson Hall, Washington State University, Pullman, WA 99164, USA*

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Abstract

A preliminary investigation using supercritical fluid chromatography–ion mobility detector (SFC–IMD) to analyze the nicotine contained in tobacco was carried out. Nicotine in commercially available cigarettes and tobacco leaves was extracted into chloroform and determined by an IMD after SFC separation. The detection limit was at the picogram level for nonselective monitoring and the subnanogram level for nicotine selective detection. This system provided a simple analytical method for the analysis of nicotine and other alkaloids in tobacco. Compared to currently utilized methods, the SFC–IMD provided advantages of high sensitivity, nicotine specific detection and lower instrumentation cost. The potential of combining online supercritical fluid extraction with SFC–IMD for analysis of alkaloids in tobacco was demonstrated.

Keywords: Tobacco; Ion mobility spectrometry; Nicotine

1. Introduction

Ion mobility spectrometry (IMS) has been known as a gas phase separation and chromatographic detection technique for several decades [1]. It can be used either as a selective chromatographic detector (IMD) to determine specific target compounds or as a universal detector similar to a flame ionization detector (FID). The selectivity of an IMD relies on the identity and mobility of gas-phase ions that are produced from the analyte. The ion mobility of these gas-phase ions is related to charge, collision cross section, and mass of the ion. Ions with different mobilities will require different times to drift through the IMD tube and reach the ion collector. Selective detection by IMD is achieved, then, by monitoring specific gas-phase ions produced from the target analyte at specific drift time.

During the last decade, IMD has been developed as a unique supercritical fluid chromatography (SFC) detector [2–6]. As a ambient pressure detection technique, IMD has several advantages as an SFC detector. Previous studies have demonstrated that the IMD is compatible with most ionizable mobile phases used with conventional SFC [2]. One of the most important advantages of the IMD is its sensitive detection of nonvolatile and nonchromophore containing compounds. For example, the SFC separation and selective detection of Triton X-100 surfactant oligomers (1000 a.m.u.) demonstrated the potential

*Corresponding author.

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utility of SFC–IMD for high-molecular-mass compounds analysis [2]. The SFC–IMD method for selective detection of underivatized 2,4-dichlorophenoxyacetic acid in a soil sample has also been accomplished [7].

The advantages of IMS over other detection techniques are that IMS provides a variety of operation modes similar to mass spectrometry (MS). In addition, previous work has shown that IMS is a very sensitive detection method for amines and other nitrogen-containing compounds [6]. For this series of compounds, the detection limit was as low as subpicograms. This selectivity and high sensitivity provide the potential of utilizing IMS as a nicotine detection method. Under the selective detection mode of IMS, it would be possible to selectively determine nicotine from incompletely separated chromatographic eluents.

Many chromatographic methods have been developed to qualitatively and quantitatively determine nicotine contained in tobacco leaves or biological fluids. GC–thermal energy analyzer (TEA), GC–nitrogen–phosphorus detector (NPD), GC–MS, ion pair reversed-phase chromatography–UV absorption, and thermospray liquid chromatography (LC)–MS [8–12] offered good choices. The detection limit of these methods were in the range 10–1200 pg. However, with the exception of MS, most of these analytical methods relied on chromatographic separation alone to distinguish nicotine from other alkaloids. A simpler and less expensive alternative is desired for selective detection of nicotine and its metabolites in either tobacco products or biological fluids. This study demonstrates the possibility of utilizing SFC–IMS to selectively detect nicotine in tobacco extracts.

2. Experimental

2.1. Instrumentation

All experiments were performed on a Lee Scientific Model 501 SFC system (Lee Scientific, Salt Lake City, UT, USA) in which a SB-Biphenyl-30 fused-silica capillary column (Dionex Lee Scientific Division, Sunnyvale, CA, USA) was employed. The column was 10 m×50 μm I.D., 195 μm O.D., and was coated by 30% biphenyl and 70% methylpolysiloxane, 5-μm coating film thickness. A glass frit restrictor was recommended by the manufacturer connected at the end of the column with a fused-silica coupler (Dionex Lee Scientific Division, Sunny Vale, CA, USA). The restrictor was 30 cm× 50 μm I.D. The average linear velocity of the CO_2 mobile phase was 1.5 cm/s. A splitless injector with a 100-nl injection volume rotor was used. The column was tightly held on injector by a stainless steel screw with a double end ferrule, thus dead volume between injector and column was minimized. Both pressure–temperature program and density–temperature program were used in this study.

The IMD used in this study was a modification of the detector that has been reported previously [13]. In this study, all of the experiments were operated with the positive mode of the IMD. The compressed nitrogen gas that was filtered with a molecular sieve 13x (Alltech Associates, 2051 Waukegan Road, Deerfield, IL, USA) was used as drift gas at 1.5 l/min. If it was not specified, the IMD was operated at a tube temperature at 250°C, an interface heater temperature at 325°C and a total drift voltage of 4000 V. Fourier transform (FT) mode ion mobility spectrum was obtained with an initial frequency of 20 Hz and a final frequency of 10 020 Hz. The total sampling time was set at 8 s. Chromatographic conditions for each experiment are described individually.

2.2. Materials and reagents

Nicotine standard was made up by dissolving nicotine (Sigma) in chloroform. Tobacco samples were prepared by the following procedure: 0.6784 g of unprocessed Kentucky burley tobacco leaves (University of Kentucky, Tobacco and Health Research Institute, Cooper and University Drives, Lexington, KY, USA) was extracted by 10 ml chloroform in a sonicator for 2 h; the extracts were filtered with a 0.4-μm PTFE filter. Commercial cigarettes samples were prepared in the same manner. The samples were from the following cigarettes: 0.755 g Merit (Philip Morris, Richmond, VA, USA), 0.6813 g Winston Select Light (R.J. Reynolds Tobacco, Winston-Salem, NC, USA), 0.6427 g Hi-Lite (Japan Tobacco).

3. Results and discussion

The SFC–IMS system described above was used for selective detection of nicotine in tobacco leaves and commercial cigarettes. First of all, the behavior of nicotine in this SFC–IMD was investigated with a known concentration of nicotine standard. As shown in Fig. 1, when the nicotine sample was introduced into the IMS tube, a single peak corresponding to nicotine product ion was observed at 22.85 ms. The peak was well resolved from solvent peak (16.63 ms). The calculated reduced mobility constant [1] (K_o) of nicotine ion was 1.56 cm^2/V s while that of the reactant ion [$(H_2O)H^+$] was 2.47 cm^2/V s.

By setting the IMS window between 19.0 and 26.7 ms, solvent ions were eliminated and the nicotine product ion was completely collected. In this case, the IMS provides the highest response for nicotine sample. The response curve, $R=1.6\cdot10^{-3}Q+1.7\cdot10^{-2}$ (where R is response in pA and Q is the injection amount in pg), was obtained by least square root regression fitting of experimental data. The result demonstrated an r^2 value of 0.997. The relative standard deviation of different sampling points around the average response line was 3.8%, i.e. SFC–IMS system provided a detection method for nicotine with a response error of <3.8% for injection amounts ranging from 50 to 1000 pg. A dynamic response range over three orders of magnitude was observed. The calculated detection limit of nicotine was 20 pg when the signal-to-noise ratio was 3.

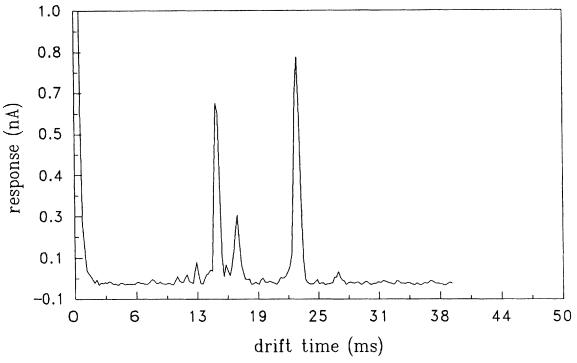

Fig. 1. Spectra of nicotine product ion; IMS conditions: tube temperature 250°C, heater temperature 325°C, drift voltage 4000 V, SSQ frequency range: 20–5010 Hz.

The extract of commercial cigarettes (Merit), was employed to demonstrate the possibility for selective detection of nicotine. Similarly, the selectivity was achieved by moving the IMS monitoring window to collect ions with selected drift time (Fig. 2). In these chromatograms, the solvent peak was eliminated by adjusting IMS scan windows. (A) shows the chromatogram of nonselective mode detection. In this case, IMS window was set from 20.0 to 39.6 ms. With the exception of solvent ions, most of the product ions of different compounds that were contained in the sample were monitored. One major peak and several minor peaks were observed. By narrowing down the window size around the drift time of nicotine product ion, only nicotine product ions were collected when the IMS window was set from 22.3 to 23.3 ms. A single nicotine peak is shown in chromatogram (B). On the other hand, (C) shows the chromatogram of the IMS windowing set from 28.5 to 48.2 ms. Under this condition, the other components in the extract were detected except nicotine. To prove there was no nicotine product ion contribution in this chromatogram, 0.1 ng/nl nicotine

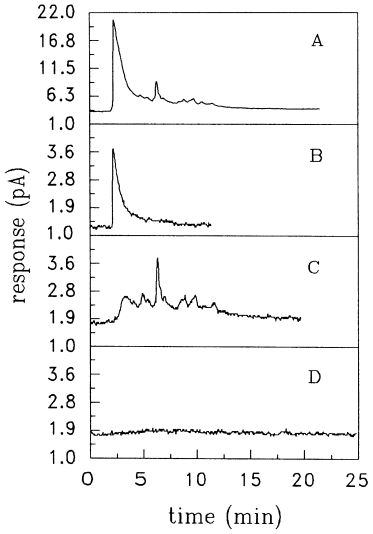

Fig. 2. Selective detection of nicotine; SFC conditions: asymptotic pressure program 150–400 atm., half rise time 1.5 min, total time 15 min, hold 30 min; linear temperature program 160–80°C, initial time 2 min, final time 25 min. IMS conditions: tube temperature 250°C, heater temperature 325°C, drift voltage 4000 V, monitoring window (A) 19.0–39.55 ms, (B) 22.3–23.3 ms, (C) 28.5–48.15, (D) 28.5–48.15.

standard was injected under the same IMS window setting. Its result is shown in (D). At this point, a selective detection method of nicotine was used. For this selective detection mode, detection limit was determined to be 350 pg nicotine.

This SFC–IMS system shows a large potential to compete with current detection methods for tobacco specific *n*-nitrosamines and their metabolites. Table 1 demonstrated the detection limit and selectivity of different detection methods of nicotine. With non-selective mode, IMS provided a lower detection limit than NPD and UV detectors; With selective mode, IMS provided a simple detection method with comparable detection limit with MS.

With this detection method, tobacco leaf and several commercial cigarettes samples were analyzed (Fig. 3). Similar to Fig. 2, solvent peaks were eliminated in these chromatograms. Compare the chromatograms obtained in both the selective and nonselective modes, only a single peak for nicotine was observed in selective mode. In addition, chromatograms (C) and (G) had similar responses in selective mode, but different response strength in nonselective mode (D) and (H). This result indicates that the chromatographic conditions used in this study can not separate the extract completely, but the IMS can be used to selectively detect the target compound, which can not be achieved by universal detectors, such as the FID or the NPD. Finally, with the assumption of 100% extraction, the nicotine contents were quantified as Leaf 0.78 mg/g, Merit 1.02 mg/g, Hi-lite 0.76 mg/g and Winston 1.20 mg/g. These results were very similar to the manufacturer's reported values of nicotine content.

As a nicotine detection method, IMS demonstrated high sensitivity and selectivity. However, other

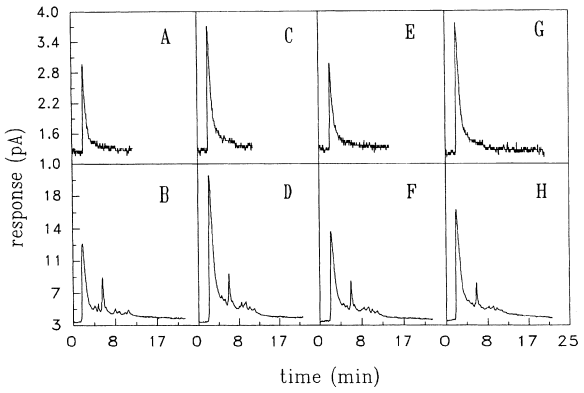

Fig. 3. Selective detection of nicotine in tobacco samples; SFC conditions: asymptotic pressure program 150–400 atm, half rise time 1.5 min, total time 15 min, hold 30 min; linear temperature program 160–80°C, initial time 2 min, final time 25 min. IMS conditions: tube temperature 250°C, heater temperature 325°C, drift voltage 4000 V, monitoring window: (A,C,E,G) 22.3–23.3 ms selective mode, (B,D,F,H) 19.0–39.55 ms nonselective mode; (A,B) tobacco leaves, (C,D) Merit, (E,F) Hi-lite, (G,H) Winston.

alkaloids in tobacco extract may have similar ion mobilities to nicotine. In this case, optimizing chromatographic separation could be one of the approaches to improve the entire system performance. With a better chromatographic separation, a wider IMS monitoring window can be used to decrease the detection limits. Compared with GC operating conditions, SFC requires a lower temperature for the separation of nicotine. This may prevent nicotine from decomposition during the separation process. Furthermore, supercritical fluid extraction (SFE) has already been used as an extraction technique for the analysis of low level nicotine content in tobacco extracts. With the SFE techniques, an on-line SFE–SFC–IMS system would have the additional advan-

Table 1
Comparison of various nicotine analytical methods

Separation method	Detection method	Detection limit S/N=3 (ng)	Selectivity	Reference
SFC	IMS	0.35	Nicotine selective	
		0.02	Nonselective	
GC	MS	0.01	Nicotine selective	[8]
GC	NPD	<1	Nitrogen selective	[9]
GC	Thermal energy analyzer		Nonselective	[12]
Ion pair LC	UV	1.2	Nonselective	[10]
LC	Thermospray MS	0.9	Nicotine selective	[11]

tage of fast on-line extraction, separation and selective detection of tobacco samples.

An IMD is a very simple instrument to operate. One merely selects the flow-rates, dials in the reduced mobility value and runs the chromatography. The instrument used in this study however, is a research grade instrument and is somewhat more difficult to operate since it does not have the advantage of commercial software. As with most analytical instruments these days, it is the user interface rather than the instrument, which determines the ease of operation. With respect to SFC, IMS is inherently more complicated to operate than simple flame ionization detectors but less complicated than mass spectrometers.

4. Conclusions

SFC–IMD offers a simple, selective and rapid alternative for the determination of the nicotine content of tobacco leaf. The extraction method used in this study provided a recovery efficiency of about 100% for tobacco. The linear response range of SFC–IMD was over three orders magnitude with a detection limit of 20 pg for nonselective detection and 350 pg for nicotine specific detection. This system provided a simple analytical method for the analysis of nicotine and other alkaloids in tobacco. Compared to current methods, the SFC–IMD provided advantages of a high sensitivity, compound specific detection, The potential exists to combine with on-line SFE for fast nicotine screening in tobacco products.

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