ELSEVIER

Journal of Chromatography A, 688 (1994) 55–65

JOURNAL OF CHROMATOGRAPHY A

Application of the particle beam interface to high-performance liquid chromatography–thermal energy analysis and electron impact mass spectrometry for detection of non-volatile N-nitrosamines

Stanley M. Billedeau*, Thomas M. Heinze, Jon G. Wilkes, Harold C. Thompson, Jr.

Food and Drug Administration, National Center for Toxicological Research, HFT-230, 3900 NCTR Road, Jefferson, AR 72079, USA

First received 15 June 1994; revised manuscript received 12 September 1994

Abstract

Interest in the analysis of non-volatile N-nitrosamines has recently been renewed due to the development of several new reversed-phase HPLC interfaces to thermal energy analysis (TEA) or chemiluminescence detection. A new application of a counter flow gas diffusion cell (CFGDC)-based particle beam LC interface (Universal Interface, Vestec) is described for the HPLC–TEA analysis of the non-volatile N-nitrosamines, N-Nitrosodiethanolamine (NDELA) and N-nitrosomethyl-*p*-amino-2-ethylhexylbenzoate (NMPABAO). The interface incorporates a thermospray vaporizer, desolvation chamber, and CFGDC to reduce the LC effluent to a dry aerosol and a single-stage momentum separator to form a particle beam of the non-volatile analyte. Using this system, the LC–TEA response to NDELA was linear in the range 6–200 ng total amount injected. Several experiments are reported indicating the effect of thermospray tip temperature, He carrier flow-rate, and mobile phase composition on TEA response. Minimum detection limits (5 ng NDELA injected on column) are comparable to other LC–TEA interfacing methods. Several advantages over existing methodology which include ease of use, ruggedness and MS compatibility are discussed. Additional LC–particle beam MS data are reported indicating that full scan electron impact MS identification of the N-nitrosamine contaminants in cosmetics is possible for confirming TEA detection data.

1. Introduction

In recent years, the emphasis on analysis of non-volatile N-nitrosamines in foods, beverages, cosmetics and various other consumer products [1–4] has increased due to GC–thermal energy analysis (TEA) data confirming the presence of potentially carcinogenic volatile N-nitrosamines in such products. The lack of technology for interfacing reversed-phase HPLC with TEA, a detection method primarily used for GC analysis of N-nitroso compounds, has resulted in the development of two such interfaces, a KI/HOAc postcolumn reaction interface [5] and a UV photolysis-based interface [6]. Both of these interfaces are based on reactions which involve

* Corresponding author.

SSDI 0021-9673(94)00894-9

liberation of nitric oxide (NO gas) from the N-nitroso moiety rather than from pyrolysis as performed in the typical GC–TEA mode [7]. The liberated NO(g) and LC solvent is then swept by a flow of carrier gas into a series of cold traps which remove the LC mobile phase and residual vapors. The NO(g) survives the cold traps, enters the TEA detection cell, is mixed with ozone gas, and the resulting chemiluminescence detected using a sensitive photomultiplier tube. These two TEA interfacing techniques, although highly sensitive (1–10 ng total compound injected) and selective for a variety of N-nitroso compounds, are not suited to coupling with mass spectrometry (MS) due to the destructive reactions employed which alters the eluting compounds.

Recently, an US Food and Drug Administration (FDA) intra-agency collaboration was developed between the National Center for Toxicological Research (NCTR) and the Center for Food Safety and Applied Nutrition (CFSAN), Office of Cosmetics and Colors, Cosmetic Technology Branch, involving detection and identification of non-volatile N-nitroso compounds in cosmetics and their raw materials. The major objective of this project is to discover possible N-nitroso carcinogens in cosmetics for skin care, creams, lotions, or other long-term skin contact products, previously unreported in analytical tests. For identification of unknown non-volatile N-nitroso contaminants in these products, it was advantageous to perform HPLC–TEA analysis for flagging compounds of interest as possible N-nitroso carcinogens, as well as parallel HPLC–MS analysis on the intact compound to supply additional molecular mass, fragmentation, or functional group data for identifying TEA peaks of interest.

In this study, an HPLC–TEA interface is described utilizing a particle beam (PB) type of instrumentation developed initially for interfacing HPLC to MS [8]. The high solvent removal efficiency of this PB interface has made possible HPLC–TEA analysis with reversed-phase solvents (e.g. MeOH, water, CH_3CN,) without the need for solvent venting [9] or cryogenic trapping techniques [5,6] currently being used in alternative HPLC–TEA interfaces. The Vestec Model 702 B universal interface incorporates a thermospray vaporizer, desolvation chamber and counterflow gas diffusion cell (CFGDC) using a high counterflow (about 2 l/min) of helium to reduce the LC effluent to a dry aerosol of the intact non-volatile N-nitrosamine analyte. A single-stage momentum separator (specially designed for TEA) was used to form the particle beam which was directed into a 550°C pyrolysis tube of a Thermo Electron TEA Model 502 operated at 1.5 Torr vacuum (1 Torr = 133.322 Pa). To demonstrate the utility of the device, two non-volatile N-nitrosamines, N-nitrosodiethanolamine (NDELA) and N-nitrosomethyl-*p*-amino-2-ethylhexylbenzoate (NMPABA-O), were studied to test the sensitivity, reproducibility and linearity of response of the HPLC–PB-TEA system. An extraction method reported by Meyer and Powell [9] was modified for use in analysis of cosmetics by HPLC–PB-TEA. The application of this method for analysis of a skin care cosmetic product was successful in detecting NMPABAO contamination as well as several unknown TEA responding peaks.

2. Experimental

2.1. Standards and reagents

All solvents were distilled-in-glass HPLC grade or equivalent and all reagents were certified ACS grade or equivalent.

Non-volatile N-nitrosamine standards and cosmetic samples: the NDELA and NMPABAO standards and cosmetic samples in this study were obtained from the FDA, Center for Food Safety and Applied Nutrition, Cosmetics Section, Washington, DC, USA. The N-nitrosopyrrolidine (NPYR) and N-nitrosodibutylamine (NDBA) were obtained from Eastman Kodak (Rochester, NY, USA) The N-nitrosodiphenylamine (NDiPhA) was purchased from Sigma (St. Louis, MO, USA).

Nitrosation inhibitor: a solution containing 20 mg/ml hexyloxyaniline (HOA) in dichloromethane–hexane (1:9, v/v) was used to inhibit formation of N-nitrosamines during extraction. A 1-g quantity of HOA was dissolved in 5 ml

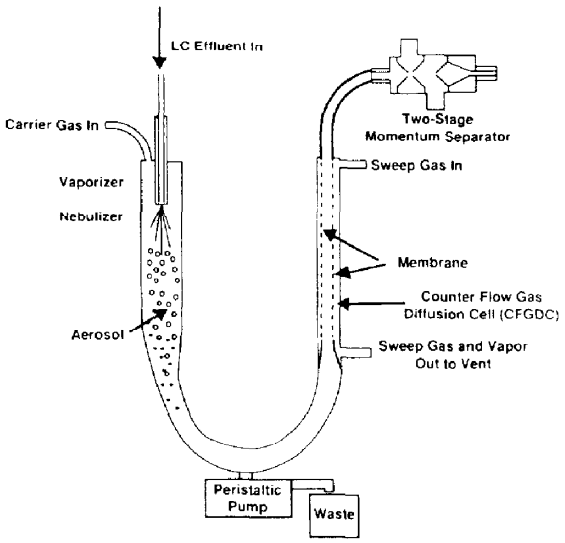

Fig. 1. Diagram of the Vestec separator, which includes the universal interface and a two-stage momentum separator.

dichloromethane and subsequently diluted to 50 ml total volume with hexane.

Ammonium acetate: the ammonium acetate used to make up HPLC mobile phase(s) was purchased from Fluka with a purity of >99%.

Safety note: N-nitrosamines are an extremely hazardous class of compounds and should be handled with the utmost caution.

2.2. PB-TEA

A diagram of the universal interface is shown in Fig. 1. Up to 1.5 ml/min flow of an HPLC mobile phase effluent is delivered to a heated capillary tube with a 0.75 mm thermospray vaporizer tip. The volatile mobile phase and non-volatile analyte(s) are nebulized into a heated desolvation chamber with a 2 l/min helium carrier. Larger droplets of solvent are removed to waste by a peristaltic pump connected to the bottom of a J-tube. The aerosol effluent from the J-tube is directed into the CFGDC where a PTFE membrane operated with a counterflow of He gas at 4.5 l/min removes most of the remaining solvent vapors to a waste receptacle. The resulting dry aerosol is passed through a 4 mm I.D. PTFE tubing into a stainless-steel single-stage momentum separator with a focusing nozzle (0.4 mm) impinging on a skimmer cone (0.6 mm). Residual solvent vapors are reduced further by diffusion of small molecules into the vacuum in the region between the nozzle and skimmer; the less volatile analyte, present as relatively large residual particles in the aerosol, is transmitted as a beam of high momentum particles past the skimmer orifice. As shown in Fig. 2, this particle beam of intact N-nitroso compound persisting through the skimmer is connected via a specially designed stainless-steel flange and delivery tube into the 550°C pyrolysis tube of a Thermo Electron Model 502 TEA operated at 1.5 Torr vacuum. A filter cartridge (CRT gas stream filter; Thermedics, Waltham, MA, USA) was used as a final filter to remove residual pyrolysis products. Following the filter, the pyrolysis product of the N-nitroso compound, nitric oxide (NO gas), is mixed with ozone electrically generated from an oxygen flow of 10 ml/min (on the flowmeter) in the TEA

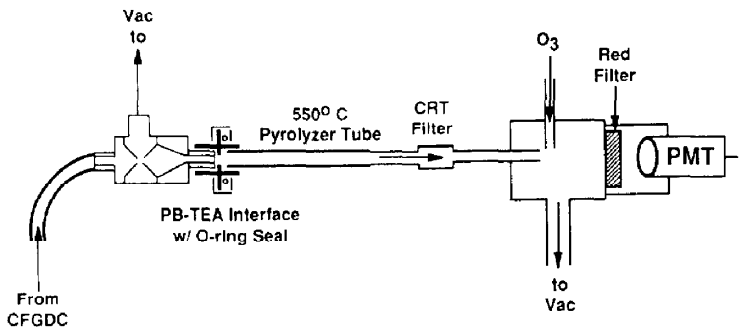

Fig. 2. Diagram of the PB-TEA interface with single-stage momentum separator and TEA detector. PMT = Photomultiplier tube; w/ = with; Vac = vacuum.

detector cell where the resulting chemiluminescence is detected by an extremely sensitive photomultiplier tube.

2.3. HPLC system

A Spectra-Physics Model 8800 ternary HPLC pump capable of operation in either isocratic or solvent programming mode was used to pump 1 ml/min of mobile phase into the appropriate HPLC column. An Altex Model 210 HPLC injector with a 50-μl loop was used for all standard and sample injections. A Supelcosil LC-18, 5 μm, 250 × 4.6 mm column operated isocratically at 1 ml/min of 5% MeOH–water (0.05 *M* ammonium acetate) was used to perform the initial NDELA experiments to test the feasibility of HPLC–PB-TEA analysis. A SynChropak SCD-100 (SynChrom, Lafayette, IN, USA), 250 × 4.6 mm column, solvent programmed at 1 ml/min of A–B (70:30) (start) to A–B (15:85) (final) in 8 min held for 5 min, was used for initial studies on TEA detection of NMPABAO. A MetaChem C_8, 5 μm, 250 × 4.6 mm column, with a solvent program of A–B (20:80) (start) to A–B (5:95) (final) in 2 min held for 13 min, was used for the analysis of cosmetic samples by electron impact (EI) MS. Mobile phase A = 100% aqueous 0.025 *M* ammonium acetate. Mobile phase B = 95% acetonitrile–water (saturated with ammonium acetate).

2.4. Mass spectrometers

A Vestec Model 201 quadrupole mass spectrometer was used to perform the initial PB-EI-MS experiments. The N-nitrosamine standards were flow injected into the PB-EI-MS system using MeOH–water (0.05 *M* ammonium acetate/1% acetic acid) (75:25) mobile phase flowing at 1 ml/min. The NDELA and NMPABAO were diluted in mobile phase to 100 ng/μl in separate flasks. A 70 eV EI mass spectrum, 150 μA emission, of 20-μl aliquots (2 μg total) of each compound was acquired by scanning the range from 46 to 400 u. The universal interface operating parameters were 60°C = T_1 (control temperature), 132°C = T_2 (thermospray tip temperature). 121°C = momentum separator temperature, 65°C = membrane separator temperature, and 2 l/min He carrier flow.

A Finnigan Model 4500 quadrupole mass spectrometer was used to perform the PB-EI-MS confirmational analysis on actual cosmetic extracts. A MetaChem C_8 250 × 4.6 mm column was used for cosmetic analysis. The MS conditions were 70 eV and 150 μA emission scanned from 50 to 350 u. The universal interface conditions were 112°C = T_2, 132°C = momentum separator temperature, 55°C = membrane separator temperature, and 2 l/min He carrier flow.

2.5. NDELA linearity

Standard solutions of NDELA containing 125, 250, 500, 1000, 2000 and 4000 ng/ml in MeOH–water (0.05 *M* ammonium acetate) (3.75:96.25) were prepared for HPLC–PB-TEA analysis for determining the minimum detection limit (MDL) and linearity. Triplicate 50-μl injections of these NDELA standards were analyzed on a Supelcosil C_{18} (previously described) using the diluent solution as an isocratic mobile phase at 1 ml/min. Interface parameters, T_2 and He carrier flow, were 140°C and 2 l/min, respectively. The retention for NDELA was 4.7 min on the HPLC–PB-TEA system.

2.6. Effect of variation of He carrier flow-rate upon response for NDELA using flow injection analysis–PB-TEA

The effect of varying the He carrier flow of the PB interface on the TEA response was determined by analyzing triplicate 50-μl injections of a 4 μg/ml NDELA standard solution (200 ng total injected) with He flow-rates of 1.0, 2.0, 3.0 and 4.0 l/min with all other variables remaining constant. A flow injection analysis method for HPLC–PB-TEA was used to reduce the time between assays and eliminate any variables which may be introduced by the HPLC column. The mobile phase and standard diluent were MeOH–water (0.05 *M* ammonium acetate) (3.75:96.25). The He counterflow on the

CFGDC was kept at twice the He carrier flow for each determination as recommended by the instrument manual for the interface.

2.7. Effect of ammonium acetate concentration in the mobile phase upon HPLC–PB-TEA response for NDELA

Duplicate injections of a 4 μg/ml NDELA standard were analyzed on the HPLC–PB-TEA system using concentrations of ammonium acetate 0, 0.01, 0.02 and 0.04 *M* in the mobile phase (MeOH–water, 3.75:96.25). A Supelcosil C_{18} column, 5 μm, 250 × 4.6 mm, was used with 1 ml/min flow of each mobile phase after equilibration with at least five column volumes of mobile phase prior to analysis.

2.8. Effect of thermospray tip temperature upon PB-TEA response for NMPABAO

A SynChropak SCD-100 column, 100 Å pore size, 250 × 4.6 mm, was used to perform HPLC separation of a NMPABAO standard (20 μl injected of 100 μg/ml). The mobile phase was acetonitrile–water (0.025 *M* ammonium acetate) (60:40) with 1 ml/min flow-rate. The tip temperature (T_2) of the thermospray was varied from 80 to 150°C in 10°C increments with the momentum and membrane separators kept constant at 130 and 60°C, respectively. Duplicate analyses were performed at each 10°C change in T_2.

2.9. Simplex optimization of TEA response for NMPABAO using flow injection–PB-TEA

The three interactive interface parameters, T_2, P_{ch} (desolvation chamber potentiometer setting), and He carrier flow-rate, were optimized using a Simplex optimization method [10,11]. Flow injection analysis was used to inject 50-μl aliquots of a 20 μg/ml NMPABAO standard for each experiment. Four different experiments were performed using different values for each of the interactive parameters listed. The TEA response (peak area) was used to rank the four experiments (BEST = highest TEA response, WORST = lowest TEA response). The average (P) of the top three values for each factor was the WORST (W) values to give $P - W$ values. A fifth experiment, R, was performed where $R = P + (P - W)$ and a sixth experiment, C_r, an example of variable-size Simplex optimization [11], where $C_r = P + (P - W)/2$. It should be pointed out that this is only an abbreviated example of Simplex optimization for PB-TEA and that several iterations of these experiments must be performed, discarding the W values and recalculating a new set of experiments to arrive at a comprehensive optimum.

2.10. Cosmetic extraction procedure

A 1.00-g sample of a skin cream, skin lotion or other cosmetic sample was weighed into a 15-ml tube with a PTFE-lined cap. A 50-μl aliquot of the HOA, nitrosation inhibitor, was added directly to the sample and vortexed thoroughly to mix. Sodium chloride (0.5 g) was added and vortexed again to reduce foaming. A 5-ml volume of hexane was added and vortexed and/or shaken for 5 min. The tube was centrifuged at 2000 *g* for 15 min at room temperature. The hexane extract (top layer) was transferred with a disposable pipet to a 15-ml graduated centrifuge tube with a PTFE-lined cap. The extract was concentrated by evaporating the hexane via nitrogen blowdown on a 60°C water bath. The oily residue was vortexed with exactly 1 ml of acetonitrile and centrifuged as before to separate the phases. A clear top layer with a slightly pink color due to the HOA was then analyzed by HPLC–PB-TEA and EI-MS.

2.11. Analysis of cosmetic extract using HPLC–PB-TEA

A 30-μl aliquot of the 1 ml acetonitrile extract was injected into a MetaChem C_8 HPLC column, 5 μm, 250 × 4.6 mm, using A–B (85:15) (6 min hold) to start and linear solvent programming to 100% A in 25 min. The HPLC flow-rate was 1 ml/min throughout the HPLC analysis. The interface conditions were $T_2 = 124$°C, momentum separator = 125°C and membrane

separator = 56°C. The TEA system was operated at 550°C pyrolyzer temperature, 1.5 Torr vacuum, and 10 ml/min ozone flow. Mobile phase A = acetonitrile–water (90:10), saturated ammonium acetate. Mobile phase B = 100% aqueous ammonium acetate (0.025 *M*).

3. Results and discussion

3.1. Initial PB experiments on N-nitrosamines using TEA and EI-MS

Standards of several N-nitrosamines, NDBA, NPYR, NDiPhA, NDELA, and NMPABAO, were subjected to EI-MS detection using flow injection analysis coupled with the universal interface. The volatile N-nitrosamines, NDBA and NPYR, showed no EI-MS response probably due to volatility losses during the desolvation steps prior to forming the aerosol particles. The NDiPhA, NDELA and NMPABAO were all successful in producing EI-MS spectra indicating good particle beam transport through the interface. The EI-MS spectra of NDELA and NMPABAO are shown in Fig. 3. The NDiPhA (M_r 198) spectra (not shown) identified the formation of some type of dimer ($M_r > 260$) apparently in the particle beam interface since an EI library 70 eV mass spectrum is available in our National Institute of Standards and Technology (NIST) database.

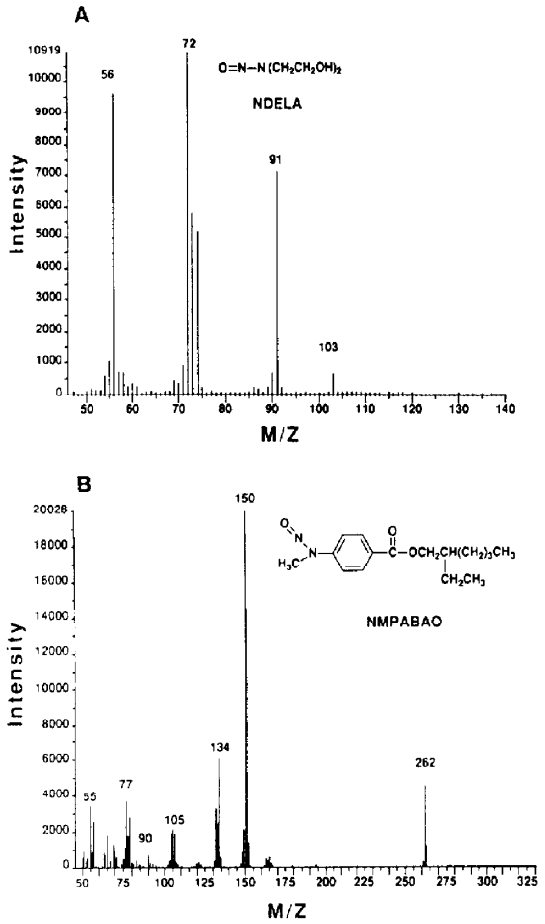

Fig. 3. Full-scan EI-MS of (A) NDELA and (B) NMPABAO using flow injection analysis coupled with PB-MS.

3.2. NDELA sensitivity and linearity study

After installation of the universal interface to the TEA system, flow injection analysis with 1 ml/min flow of MeOH–water (0.05 *M* ammonium acetate) (75:25) was used to test the response of the PB-TEA to NDELA standard injections.

The first HPLC column used to effect a separation of NDELA for HPLC–PB-TEA was a reversed-phase C_{18} which showed little retention of NDELA with the same flow injection mobile phase used previously. A MeOH–water (0.05 *M* ammonium acetate) (3.75:96.25) mixture eluted the NDELA in about 5 min at 1 ml/min from the C_{18} column (see Fig. 4). Using these HPLC conditions for PB-TEA analysis, the TEA response was shown to be linear for NDELA in the range 6–200 ng total compound injected with a correlation coefficient of 0.99901 and r^2 of 0.99802. The minimum detection limit for NDELA was 5 ng total injected on column.

3.3. Effects of thermospray tip temperature, He carrier flow, and ammonium acetate concentration on PB-TEA response to NDELA

Several experiments were performed to determine the effect of certain interface parameters on the sensitivity of response of the TEA. Data

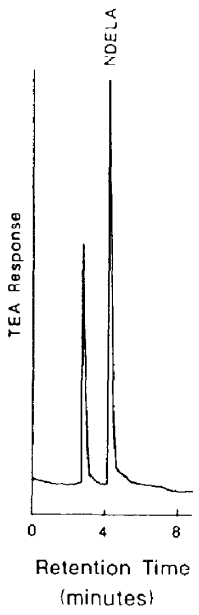

Fig. 4. HPLC–PB-TEA chromatogram of 750 ng NDELA standard injected on a Supelcosil LC-18, 5 μm, 250 × 4.6 mm column operated isocratically at 1 ml/min of MeOH–water (0.05 *M* ammonium acetate) (5:95).

presented in Fig. 5 and Table 1 has shown TEA sensitivity to be extremely dependent on thermospray tip temperature (T_2) and He flow with optimum response resulting at 110°C and 2.0

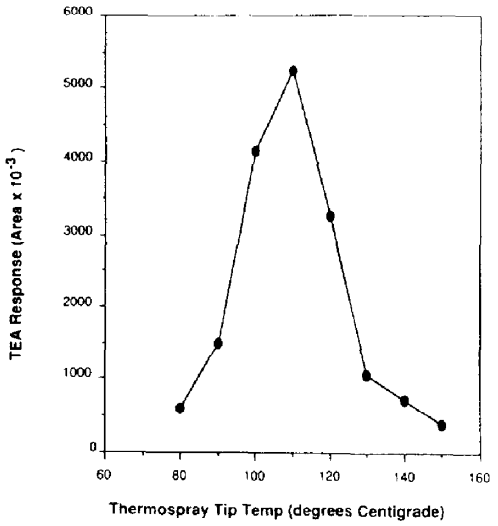

Fig. 5. Plot of TEA response versus thermospray tip temperature for HPLC–PB-TEA of NMPABAO.

Table 1
Effect of variation of flow-rate of helium carrier upon TEA response to NDELA (200 ng total amount injected) using flow injection analysis

He carrier flow (l/min)	NDELA response on TEA (mm peak height)			
	Inj 1	Inj 2	Inj 3	$\bar{x} \pm$ SD
1.0	84	83	87	85 ± 2
2.0	85	92	85	87 ± 4
3.0	74	74	71	73 ± 2
4.0	47	50	51	49 ± 2

Thermospray tip temperature (T_2) = 140°C; HPLC mobile phase = MeOH–water (0.05 *M* ammonium acetate) (3.75:96.25); spray chamber potentiometer setting (P_{ch}) = 2.80; momentum separator = 130°C; membrane separator = 60°C; He purge flow = 2 × carrier flow (l/min).

l/min, respectively, for NDELA at the prescribed HPLC conditions. The results shown in Table 2 indicated that a concentration in the range 0.02–0.04 *M* ammonium acetate must be present in the mobile phase for the best NDELA response on HPLC-PB-TEA. However, the smallest concentration of ammonium acetate giving optimum TEA response is recommended

Table 2
Relationship of ammonium acetate concentration in HPLC mobile phase to NDELA desolvation losses on universal interface as measured by TEA response using flow injection analysis

Ammonium acetate molarity (*M*)	TEA response (mm peak height)		
	Inj 1	Inj 2	$\bar{x} \pm$ S.D.
0	31	37	34 ± 4
0.01	64	64	64 ± 0
0.02	68	70	69 ± 1
0.04	71	70	71 ± 1
0.05	70	69	70 ± 1

Thermospray tip temperature (T_2) = 140°C; chamber (P_{ch}) = 2.80; momentum separator = 130°C; membrane separator = 60°C; He purge flow = 2 l/min. No TEA response was found for 50-μl injection of solvent blank (i.e. no NDELA present); all other injections were 50 μl of NDELA at 4 μg/ml in mobile phase (MeOH–water, 3.75:96.25) without ammonium acetate present.

to lessen the possibility of background response from low volatility impurities in the mobile phase. It should be noted that PB analysis of other analytes utilizing mobile phases containing higher percentages of organic solvents will exhibit very different optimum conditions for these interface parameters.

3.4. Simplex optimization of PB-TEA response to NMPABAO

Another N-nitrosamine contaminant, NMPABAO, found in some cosmetics containing the sunscreen ingredient, Padimate O, was studied. However, low sensitivity was first encountered in determining optimum PB parameters for NMPABAO. For different analytes and mobile phase ratios, these problems may be due to the interactive effects of T_2, P_{ch} and He carrier flow upon each other during aerosol transport. Simplex optimization [10] experiments were performed for these three interactive factors. The conditions from experiment R, $P + (P - W)$, which recorded the best TEA response, $5.19 \cdot 10^5$ area counts, for NMPABAO were 115°C tip temperature (T_2), 6.3 setting on the desolvation chamber potentiometer (P_{ch}) and 25 setting (1.2 l/min) on the He carrier flowmeter. However, these conditions will probably differ slightly for other analytes and substantially for other mobile phase mixtures. The TEA response using conditions for the sixth experiment, C_r, was $4.63 \cdot 10^5$ area counts or slightly less than the optimum response from the previous experiment R.

3.5. HPLC–PB-TEA analysis of cosmetic extract

For analysis of non-volatile N-nitrosamines in cosmetic samples, an extraction method previously reported by Meyer and Powell [9] was modified for applicability to HPLC–PB-TEA analysis. Several cosmetic samples could be extracted, centrifuged, evaporated and redissolved in 1 ml of acetonitrile and ready for HPLC–PB-TEA analysis within 1 h. A chromatogram of a 30-μl injection of a cosmetic sample (30 mg-equivalents of sample injected) is shown in Fig. 6A. NMPABAO was detected in the cosmetic at 8.5 ppm (w/w) (see Fig. 6A, at 8.0 min retention time) as compared to a NMPABAO standard (Fig. 7A). Upon UV irradiation of the sample at 365 nm for 16 h, the HPLC–PB-TEA chromatogram (Fig. 6B) indicated the loss of the NMPABAO peak due to photolysis as is typical for N-nitrosamines.

3.6. HPLC–PB-MS confirmation of NMPABAO in cosmetic extract

Full-scan EI-MS confirmation of NMPABAO in an actual cosmetic sample was performed by

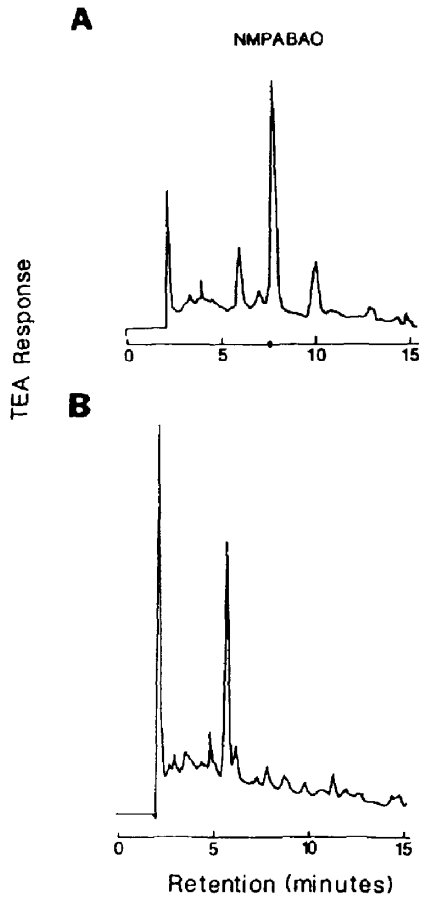

Fig. 6. HPLC–PB-TEA chromatograms of 30-μl injections of (A) a 1-g cosmetic skin cream sample in 1 ml extract and (B) the same extract after UV irradiation at 365 nm for 16 h.

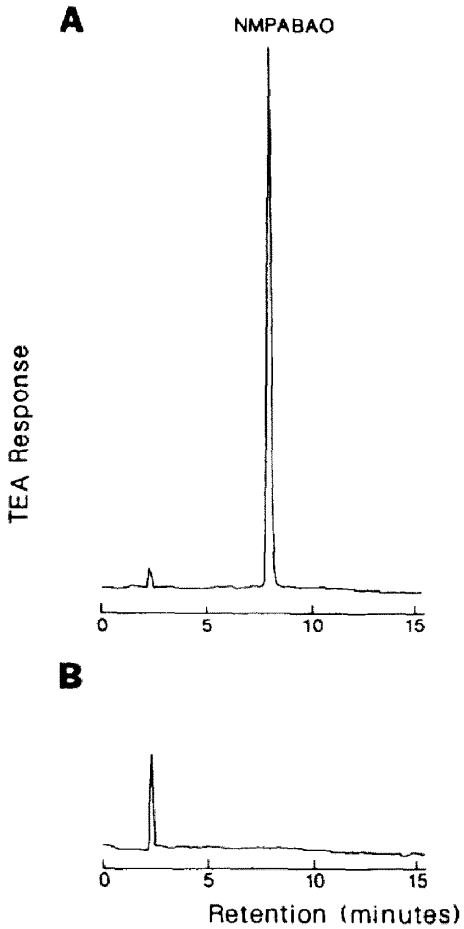

Fig. 7. HPLC–PB-TEA chromatogram of 30-μl injections of (A) 20 μg/ml of an NMPABAO standard solution and (B) reagent blank.

interfacing the HPLC–PB system to a mass spectrometer configured with a dual-stage momentum separator (as shown in Fig. 1). The reconstructed ion chromatogram (RIC) and ion scans at 262 and 263 m/z are shown in Fig. 8 using the same HPLC conditions (Fig. 6) for the cosmetic sample. Although numerous extraneous peaks appear in the RIC, a single-ion scan at 262 m/z and its EI-MS (Fig. 9, upper) has identified the peak at scan number 333 to be NMPABAO when compared to a NMPABAO standard. The peak in the 263 m/z ion scan (scan number 329, Fig. 8) was tentatively identified (no standard available) by EI-MS as MPABAO (Fig. 9, lower), the free amine of NMPABAO, since its spectra is consistent with the loss of NO (mass = 30) from NMPABAO (M_r 292) and addition of a hydrogen to give a molecular ion at 263 m/z. This further indicated that HPLC–PB-MS can be used to complement the HPLC–PB-TEA analysis of cosmetics by detecting the N-nitroso compound and the free amine, non-detectable by the TEA. However, most important for this application, HPLC–PB-MS has proven to be a very powerful, fast technique for identifying a N-nitrosamine contaminant found in a cosmetic product in a single HPLC separation without time consuming, peak collection procedures coupled with other MS introduction methods. Although for cosmetics containing lower levels of contaminants, a peak collection scheme can be used to increase detectability of this and other N-nitrosamine contaminants.

4. Conclusions

The first application of HPLC–PB-TEA for detection of non-volatile N-nitrosamines was successful in separating and detecting two compounds, NDELA and NMPABAO. Our modified cosmetic extraction method coupled with HPLC–PB-TEA was used to analyze for NMPABAO in a skin care cosmetic sample with confirmation by HPLC–PB-MS using EI ionization. Using the scenario of HPLC–PB-TEA analysis of cosmetics before and after UV photolysis, we hope to be able to screen several cosmetics for only TEA responding/UV degrading peaks. Other methods such as EI or CI-MS will then be applied to the identification of the most probable N-nitrosamine peaks.

The PB interface technology should be applicable to interfacing HPLC to TEA for detecting other low-volatility N-nitrosamines, N-nitroso derivatives, and TEA responding nitro-containing compounds in a number of different substrates. However, as with any new technique, additional research will be required to determine the scope and applicability of HPLC–PB-TEA to other analytical chemical problems.

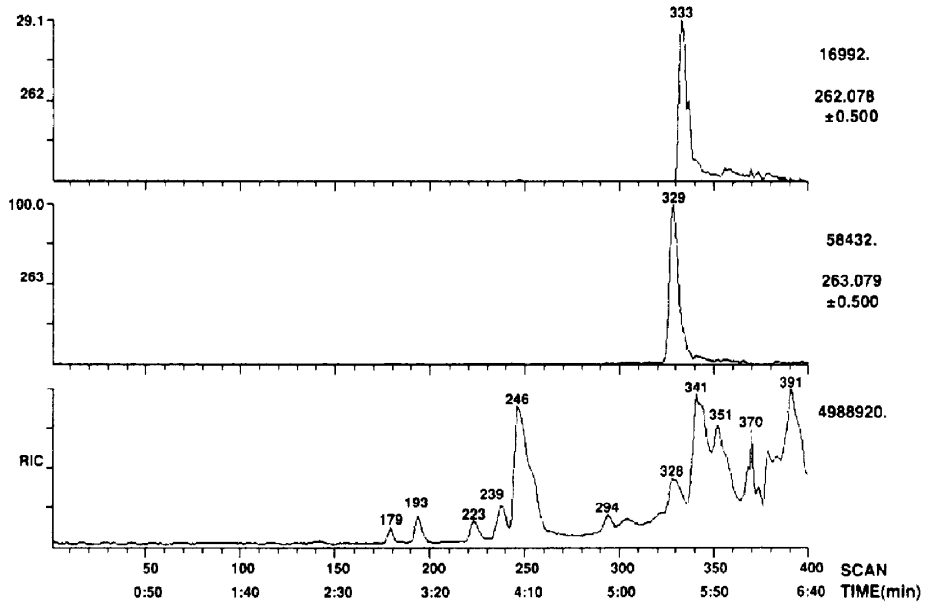

Fig. 8. RIC of HPLC–PB-MS analysis of a cosmetic skin cream extract with ion scans at 262 and 263 m/z.

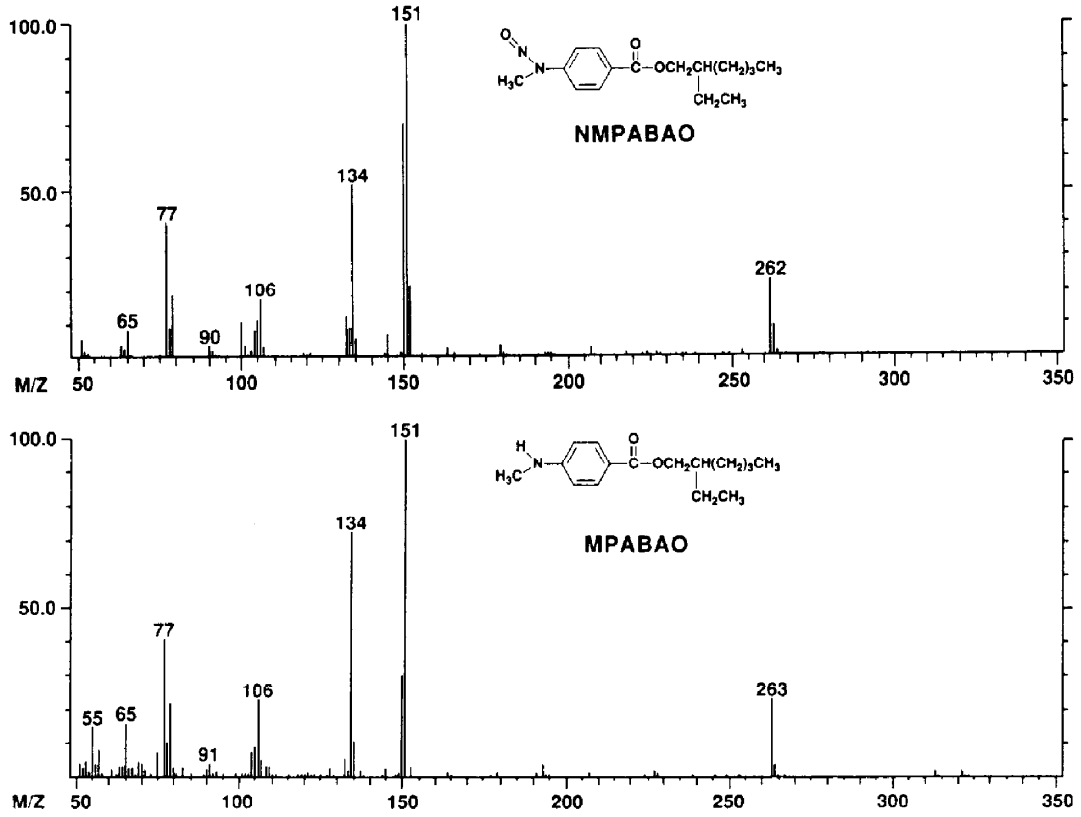

Fig. 9. EI-MS spectra of NMPABAO (upper, M_r 292) and MPABAO (lower, M_r 263) from scan numbers 333 and 329, respectively.

Acknowledgements

The authors would like to thank Don Havery and Hardy Chou of the Cosmetics Technology Section, Division of Colors and Cosmetics, CFSAN, Washington, DC, USA, for the NDELA and NMPABAO standards used in this study.

References

[1] B.J. Miller, S.M. Billedeau and D.W. Miller, *Food Chem. Toxicol.*, 27 (1989) 295–299.
[2] S.M. Billedeau, B.J. Miller and H.C. Thompson, Jr., *J. Food Sci.*, 53 (1988) 1696–1698.
[3] J.B. Morrison, S.S. Hecht and J.A. Wenninger, *Food Chem. Toxicol.*, 21 (1983) 69–73.
[4] H.C. Thompson, Jr., S.M. Billedeau, B.J. Miller, E.B. Hansen, Jr., J.P. Freeman and M.L. Wind, *J. Toxicol. Environ. Health*, 13 (1984) 615–632.
[5] D.C. Havery, *J. Anal. Toxicol.*, 14 (1990) 181–185.
[6] J.H. Hotchkiss and J.J. Conboy, *Analyst*, 114 (1989) 155–159.
[7] D.H. Fine, D. Lieb and R. Rufeh, *J. Chromatogr.*, 107 (1975) 351–357.
[8] M.L. Vestal, D. Winn, C.H. Vestal and J.G. Wilkes, in M.A. Brown (Editor), *LC/MS Applications in Agricultural Pharmaceutical and Environment Chemistry (ACS Symposium Series*, No. 421), American Chemical Society, Washington, DC, 1990, p. 215.
[9] T.A. Meyer and J.B. Powell, *J. Assoc. Off. Anal. Chem.*, 74 (1991) 766–771.
[10] J.G. Wilkes, *Dissertation*, University of Houston, Houston, TX, December 1991, pp. 127–131.
[11] A. Nelder and R. Mead, *Computer J.*, 7 (1964) 308.