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Enantiomeric determination of ephedrines and norephedrines by chiral derivatization gas chromatography–mass spectrometry approaches

Sheng-Meng Wang [a, b], Russell J. Lewis [b], Dennis Canfield [b], Tien-Lai Li [a], Chang-Yu Chen [c], Ray H. Liu [b, c, d, *]

[a] *Central Police University, Taoyuan, Taiwan*
[b] *Bioaeronautical Sciences Research Laboratory, FAA Civil Aerospace Medical Institute, Oklahoma City, OK, USA*
[c] *Department of Medical Technology, Fooyin University, Kaohsiung Hsien, Taiwan*
[d] *Department of Justice Sciences, University of Alabama at Birmingham, Birmingham, AL, USA*

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Abstract

Concerned with variations in abuse potential and control status among various isomers of ephedrines and norephedrines, this study was conducted to develop an effective method for the simultaneous analysis of eight ephedrine-related compounds along with structurally similar cathinones. Among various approaches studied, a 60-m HP-5MS (0.25 mm i.d., 0.25 μm film thickness) was successfully used to characterize the following compounds that were derivatized with (−)-α-methoxy-α-trifloromethylphenylacetic acid (MTPA): (+)-cathinone, (−)-cathinone, (+)-norephedrine, (−)-norephedrine, (+)-norpseudoephedrine, (+)-ephedrine, (−)-ephedrine, (−)-pseudoephedrine, (+)-pseudoephedrine. (−)-Cathine standard was not available, but should also be resolvable under this analytical procedure. This method was successfully applied to the analysis of selected cold remedies for characterizing the enantiomeric compositions of the compounds present in these samples.

Keywords: Enantiomer separation; Ephedrine; Norephedrine; Derivatization; GC–MS

1. Introduction—significance in enantiomeric analysis of ephedrines

"Chirality" is currently a topic at the forefront of academic research as evidented by the award of the 2001 Noble Prize in Chemistry to "three scientists who devised techniques for catalytic asymmetric synthesis — the use of chiral catalysts to accelerate the production of single-enantiomer compounds for pharmaceutical use and a wide range of other applications." [1] In the pharmaceutical industry, drug firms are actively involved in developing new drugs as single enantiomers and in carrying out "racemic switches" — redeveloping racemic mixture drugs as single enantiomers — resulting in a significant increase in the percentage of drugs marketed as single enantiomers [2].

Enantiomeric analysis of abused drugs is also an important issue in forensic laboratories. Data resulting from enantiomeric analysis can (a) provide information for sentencing guidance for certain drug-related offenses; (b) assist in drug-related investigations; and (c) determine whether the drug of concern is derived from a controlled substance. For example, ephedrine and pseudoephedrine (ψ-ephedrine) are common over-the-counter (OTC) pharmaceuticals. They are also frequently used as adulterants in packaging drugs of abuse [3]. (−)-Ephedrine has been a popular precursor for illicit manufacturing of (+)-methamphetamine [4,5]. Investigation of clandestine laboratory activities reported [6,7] the use of ephedra plant (Ma Huang) material for methamphetamine manufacturing; (−)-ephedrine and (+)-ψ-ephedrine in this plant are extracted for conversion to methamphetamine in these illicit manufacturing processes. Thus, the identification of ephedrine and ψ-ephedrine and their enantiomeric composition in methamphetamine samples may help identify the

* Corresponding author. Tel.: +886 7 781 1151x244; fax: +886 7 782 7162.
E-mail addresses: mt124@fy.edu.tw, rayliu@uab.edu (R.H. Liu).

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drug's precursor material and provide valuable information to the investigation process. Also of significant analytical concern is the reported false methamphetamine identification in urine specimens due to excessive consumption of ephedrine and ψ-ephedrine [8].

Enantiomeric analysis of abused drugs in the authors' laboratories date back to 1981, mainly involving gas chromatographic and nuclear magnetic resonance spectrometric approaches [9–13]. More recent studies utilized liquid chromatography and capillary electrophoresis [14,15]. Concerned with the presence of ephedrine-related compounds in OTC cold remedies and its implications in sport drug testing [16], this study was conducted to develop a method that can be effectively used to determine the enantiomeric compositions of the following structurally related compounds: ephedrines, Ψ-ephedrine, norephedrines (phenylpropanolamine, or PPA), norpseudoephedrine (nor-Ψ-ephedrine, or cathine), and cathinones. Methods thereby developed were then applied to selected OTC cold remedies to detect the presence and enantiomeric compositions of these compounds.

2. Experimental

2.1. Standards and reagents

Standards (*R*(+)-cathinone, *S*(−)-cathinone, *S*,*R*(+)-ephedrine, *R*,*S*(−)-ephedrine, *S*,*S*(+)-Ψ-ephedrine, *R*,*R*(−)-Ψ-ephedrine, and *S*,*R*/*R*,*S*(±)-norephedrine, all in 1000 μg/mL in methanol) and internal standard (*S*,*R*(+)-ephedrine-d_3, 100 μg/mL in methanol) were purchased from Cerilliant Int. Co. (Austin, TX). S,S(+)-Nor-Ψ-ephedrine standard (1000 μg/mL) was purchased from Sigma Co. (St. Louis, MO). The structures of these compounds are shown in Fig. 1.

Chiral derivatization reagents and their sources are as follows: (−)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA), (*S*)-(−)-*N*-(trifluoroacetyl)prolyl chloride (L-TPC) (Aldrich: St. Louis, MO); 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate, *R*-(+)-α-phenylethyl isocyanate, 2,3,4-triacetyl-α-D-arabinopyranosyl isothiocyanate (Fluka Chemie Gmbh: Buchs, Switzerland). Achiral derivatization reagents and their sources are: 9-fluorenylmethyl chloromate, *N*-(phenylseleno)-phthalimide, *N*,*O*-bis(trimethylsilyl)-acetamide (BSA), pentafluoropropionic anhydride (PFPA), helptafluorobutyric anhydride (HFBA) (Aldrich: St. Louis, MO); 4-carboethoxyhexafluorobutyryl chloride (4-CB) (Lancaster: Windham, NH).

Nineteen readily available OTC cold remedies (13 syrup, 6 capsule) were purchased from local drug stores in the greater Taipei area.

2.2. Sample preparation

Typical extraction, derivatization, and GC–MS analysis studies utilized 2 mL of standard mixtures or specimens.

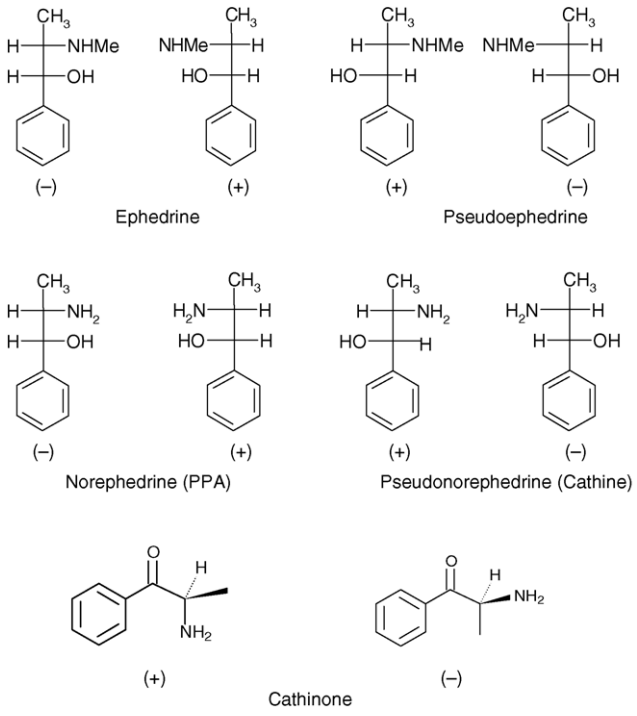

Fig. 1. Structures of ephedrine and structurally closely related compounds.

Standard mixtures were prepared to contain 1 μg of each analyte following the general procedure described below. Standards obtained from the suppliers (typically 1000 μg/mL in methanol) were first diluted to 10 μg/mL (in methanol). 100 μL of each standard was then taken and mixed into 2 mL of drug-free syrup.

The preparation of OTC samples was as follows. Those in syrup forms were diluted (typically diluting 100 μL to 2 mL), while those in capsule forms were emptied and dissolved into 10 mL of blank syrup with further dilution (typically diluting 20 μL to 2 mL).

To determine the extraction efficiency, the amounts of the analyte found at the conclusion of the analytical process (without and with the extraction step) were compared. Specifically, triplicates containing the analyte of interest were prepared by mixing 100 μL of the diluted standard (10 μg/mL in methanol) in clean tubes, then dried under nitrogen. These tubes were then processed in parallel with another set (triplicates) of standards that contain the same amount of the analyte (in 2 mL solution) and have been proceeded through the extraction step.

2.3. Derivatization procedure

Standard mixtures and OTC specimens in aqueous solutions were extracted and derivatized following either a one-step or two-step procedure as described below. Using L-TPC as example, the one-step procedure involved mixing 2-mL sample, 100-μL internal standard ((+)-ephedrine-d_3, 10 μg/mL), 0.5-mL saturated K_2CO_3 solution, 50-μL L-TPC, and 6-mL ethyl acetate for 10 min. The mixture was then

Table 1
Gas chromatograph oven temperature programming parameters for the analysis of analytes resulting from three derivatization reagents

Derivatization reagent	Starting (°C)	Hold (Min)	Rate (°C/Min)	End (°C)	Hold (Min)	Rate (°C/Min)	End (°C)	Hold (Min)
HFBA	60	0	5	200	0	25	250	–
L-TPC	160	5	5	250	–	–	–	–
MTPA	160	0	5	220	1	25	250	–

centrifuged (5 min), followed by removing the upper layer to a clean tube which was dried under a nitrogen stream. The residue was typically reconstituted with 200-μL ethyl acetate of which 1 μL was used for each GC–MS analysis.

Using MTPA derivatization as example, the two-step process was carried out as follows. Typically, the internal standard, 2-mL standard mixture (or specimen), and 0.5-mL saturated K_2CO_3 solution were mixed for 30-s. The mixture was then extracted with 6-mL ethyl acetate by shaking (10 min), followed by centrifugation (5 min). The upper layer was transferred into a clean tube and dried under nitrogen. For the derivatization step, the residue was added 50-μL *N,N*-dicyclohexycarbodiimide and 100-μL MTPA. The reaction mixture was thoroughly mixed, then incubated at 70 °C for 20 min. This same two-step procedure was used when HFBA was used for derivatization, except that 1 mL of 2-N NaOH, instead of 0.5-mL saturated K_2CO_3 solution, was used prior to the addition of ethyl acetate for extraction.

2.4. GC–MS analysis

GC–MS analysis was performed on a HP 5890 Series II GC interfaced to an HP 5971 MS (Agilent: Palo Alto, CA). Two columns used in this study were: 25-m HP 5MS (0.20-mm i.d., 0.33-μm film thickness) and 60-m HP 5MS (0.25-mm i.d., 0.25-μm film thickness) from Agilent (Wilmington, DE). HP-5MS is bonded and cross-linked with 5% phenylmethylpolysiloxane. Helium carrier gas flow rate was 1.0 mL/min. The injector and GC–MS interface temperatures were maintained at 250 and 280 °C, respectively. Temperature of the GC oven was programmed using different parameters for the analysis of products derived from different derivatization reagents (Table 1). For the 60-m column, a typical GC–MS run took 30 min or less.

The MSD was initially operated under full-scan mode to derive the retention time and full-scan mass spectrum information for each analyte. These information were then used for the identification of each analyte in standard mixtures and OTC specimens. Full-scan mass spectra were further used for the selection of ions suitable for use in selected ion monitoring (SIM) mode.

3. Results and discussion

Enantiomeric analysis of amphetamine-related drug has recent been reviewed [18]. There have been a few studies specifically related to epherdines [15,19,20], norephedrines [21,22], and cathine [21,22].

3.1. Chromatographic resolution and quality of ion pairs used for designating the analyte and internal standard

As shown in Section 2.1, a total of 11 derivatization reagents (5 chiral and 6 achiral) were included in this study. MTPA was found to be the most effective chiral derivatization reagent, allowing complete base-line resolution of the 10 structurally closely related compounds of interest shown in Fig. 2. (−)-Norpseudoephedrine was not available for this study; however, it should have been resolved were it included in the mixture.

Shown in Fig. 3 are the mass spectra of MTPA-derivatized enantiomers of norephedrines, ephedrines, and the internal standard. Mass spectra of the corresponding (−)-isomers are practically indistinguishable and, therefore, are not shown.

Derivatization products resulting from the commonly used L-TPC also result in good resolution of the analytes, with the exception of (−)-ephedrine and (−)-Ψ-ephedrine (Fig. 4). Furthermore, the quality of ions that may be used to designate the analytes and their deuterated analogs are inferior compared to those derived from the MTPA derivatization. For example, data shown in Table 2 indicate that, with L-TPC derivatization, there is only one ion pair (*m/z* 251–254) with low cross-contribution [17] and can be used to designate ephedrine and ephedrine-d_3, respectively. Contrarily, three high quality ion pairs (*m/z* 275–278, 274–277, and 200–203) are available using MTPA derivatization. The most promising ion pair for quatitation derived from MTPA derivatization (*m/z* 275–278) is also superior to the corresponding one (*m/z* 251–254) derived from the L-TPC derivatization.

When the determination of analytes' enantiomeric compositions is not needed, HFBA-derivatization was found very effective (Fig. 5). Analytical time can be further reduced by increasing the column temperature following the elution of cathine and norephedrine (peaks A and B in Fig. 5). Mass spectra of representative compounds with HFBA derivatization are shown in Fig. 6.

3.2. Evaluation of analytical parameters

Common analytical parameters, including limits of detection and quantitation (LOD and LOQ) and extraction efficiency, have been studied. Evaluations were performed

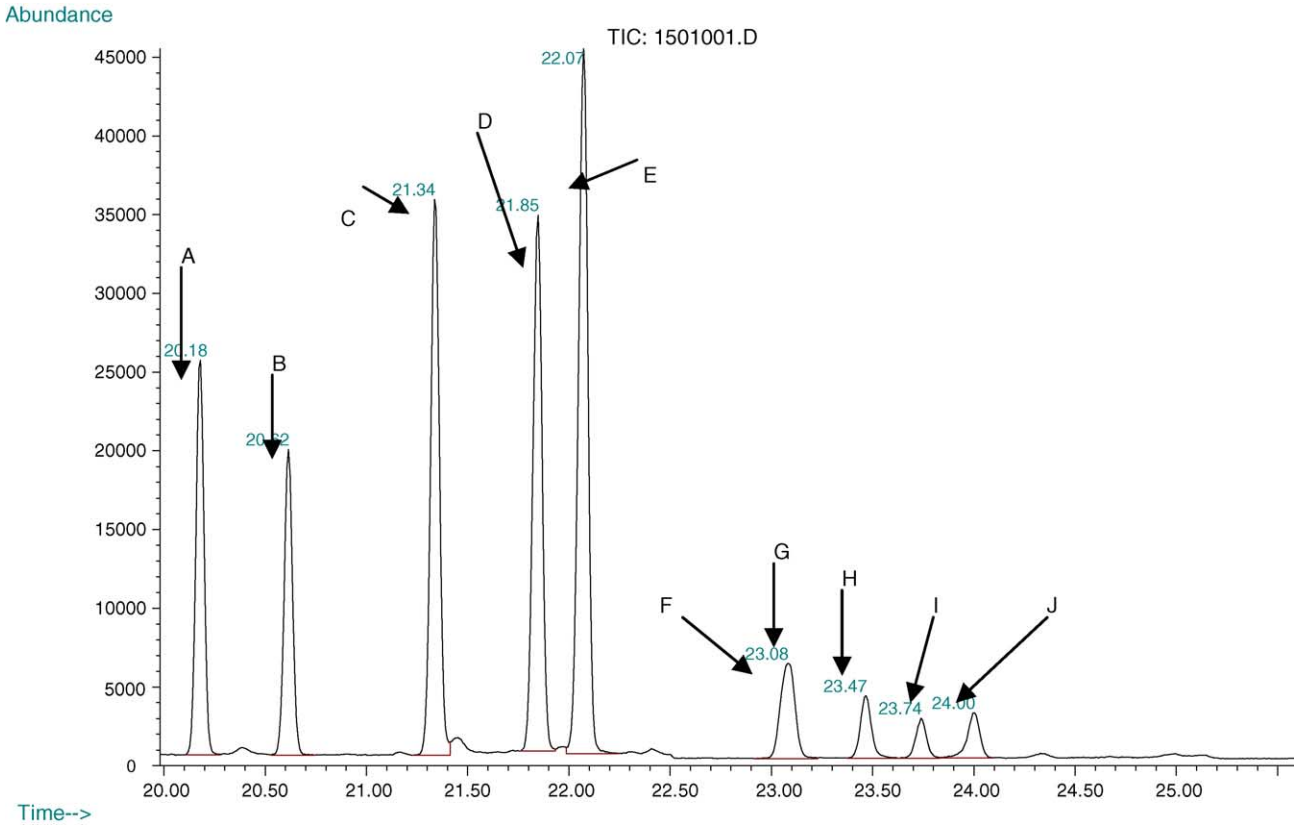

Fig. 2. Ion chromatogram of MTPA-derivatives: (+)-Cathinone (A), (−)-cathinone (B), (+)-norephedrine (C), (−)-norephedrine (D), (+)-nor-Ψ-ephedrine (E), (+)-ephedrine-d_3 (F), (+)-ephedrine (G), (−)-ephedrine (H), (−)-Ψ-ephedrine (I), and (+)-Ψ-ephedrine (J) (all as MTPA-derivatives).

Table 2
Relative intensity and cross-contribution data[a] of ions with potential for designating the analyte and the adapted internal standard

Derivatization group	(+)-Ephedrine			(+)-Ephedrine-d_3		
	Ion (*m/z*)	Relative intensity	Analog's contribution	Ion (*m/z*)	Relative intensity	Analog's contribution
L-TPC	58	20.1	3.14	61	31.4	15.4
	148	3.97	20.6	151	4.24	0.94
	251	13.9	2.28	254	22.6	2.05
MTPA	200	6.96	2.60	203	6.90	0.14
	274	52.9	1.54	277	52.7	0.20
	275	16.4	1.89	278	16.5	0.06

[a] Relative intensity are based on full-scan data and expressed in percentage, while analog's contribution (cross-contribution [17]) are derived from selected ion monitoring data and expressed in percentage.

on MTPA and HFBA derivatizations using ephedrine as the exemplar compound. Results listed in Table 3 were established using the criteria and procedure commonly adapted by the forensic toxicology community in the United States of America. Specifically, the presence of a specific analyte in a test sample is established if the ions monitored for a specific analyte are present at same and acceptable retention time with acceptable intensity ratios. It is considered acceptable if the retention time is within ±2% and the ion intensity ratios are within ±20% of that established by a standard. The LOD was defined as the lowest concentration of a standard solution meeting the above criteria, while LOQ was defined as the lowest concentration of a standard solution that met these criteria and with an observed analyte concentration that is within ±20% of the targeted value.

Table 3
Evaluation of common analytical parameters resulting from FHBA and MTPA derivatizations

Parameter	HFBA	MTPA
Recovery (%)[a]	72 ± 4[b]	90 ± 7[b]
LOD (μg/mL)	0.060	0.060
LOQ (μg/mL)	0.080	0.080

[a] Evaluated using triplicates of 2-mL standard solutions containing 0.500 μg/mL ephedrine.
[b] Mean ± standard deviation.

A series of standard solutions with the following concentrations of ephedrine were used for LOD and LOQ

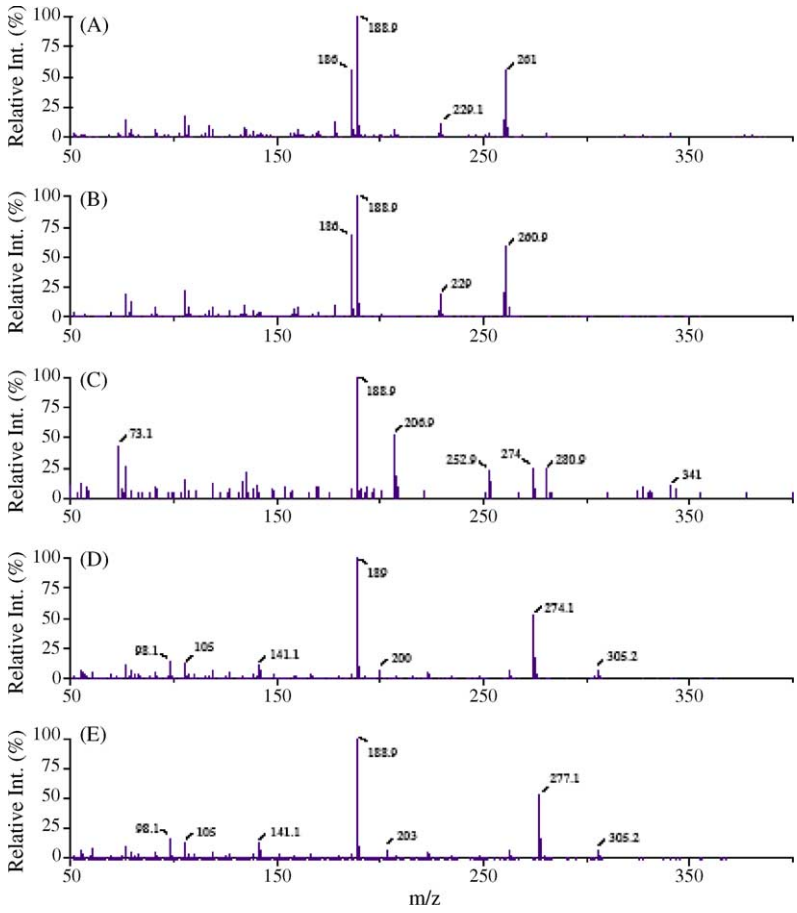

Fig. 3. Mass spectra of (+)-Ψ-norephedrine (A), (+)-norephedrine (B), (+)-Ψ-ephedrine (C), (+)-ephedrine (D), and (+)-ephedrine-d_3 (E) (all as MTPA-derivatives).

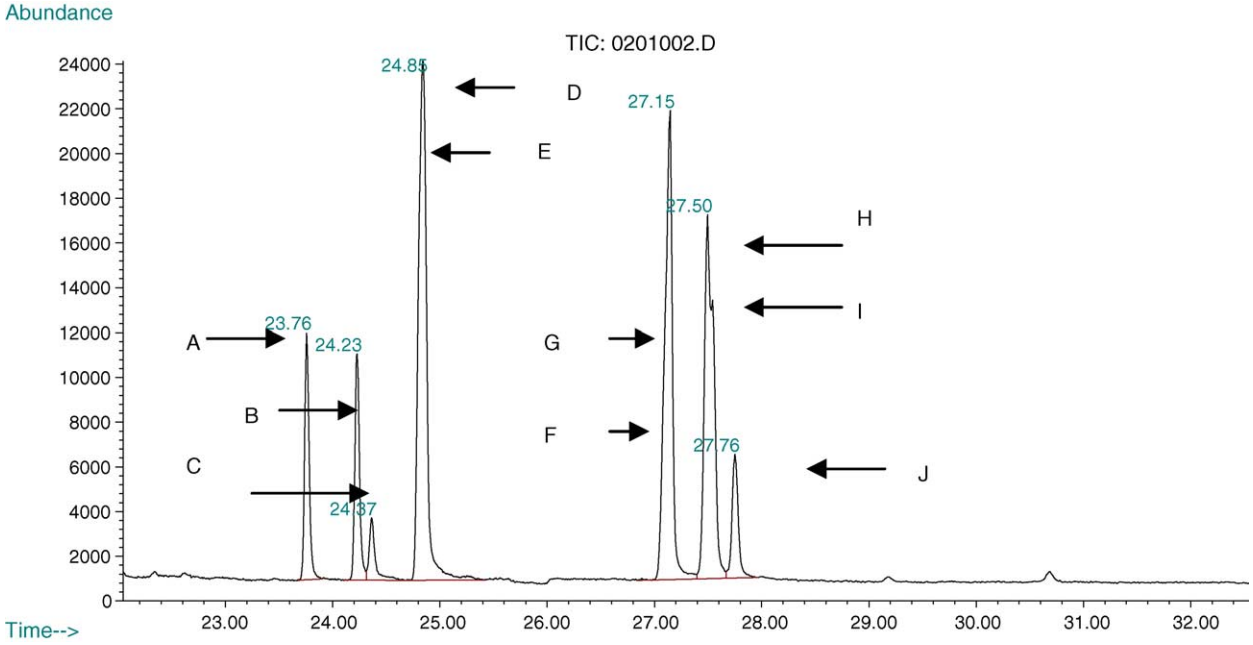

Fig. 4. Ion chromatogram of L-TPC-derivatives: (+)-Cathinone (A), (+)-Phenylpropanolamine (B), (−)-Cathinone (C), (−)-Phenylpropanolamine (D), (+)-cathine (E), (+)-Ephedrine (F), (+)-Ephedrine-d_3 (G), (−)-Ephedrine (H), (−)-Pseudoephedrine (I), (+)-Pseudoephedrine (J) (all as L-TPC-derivatives).

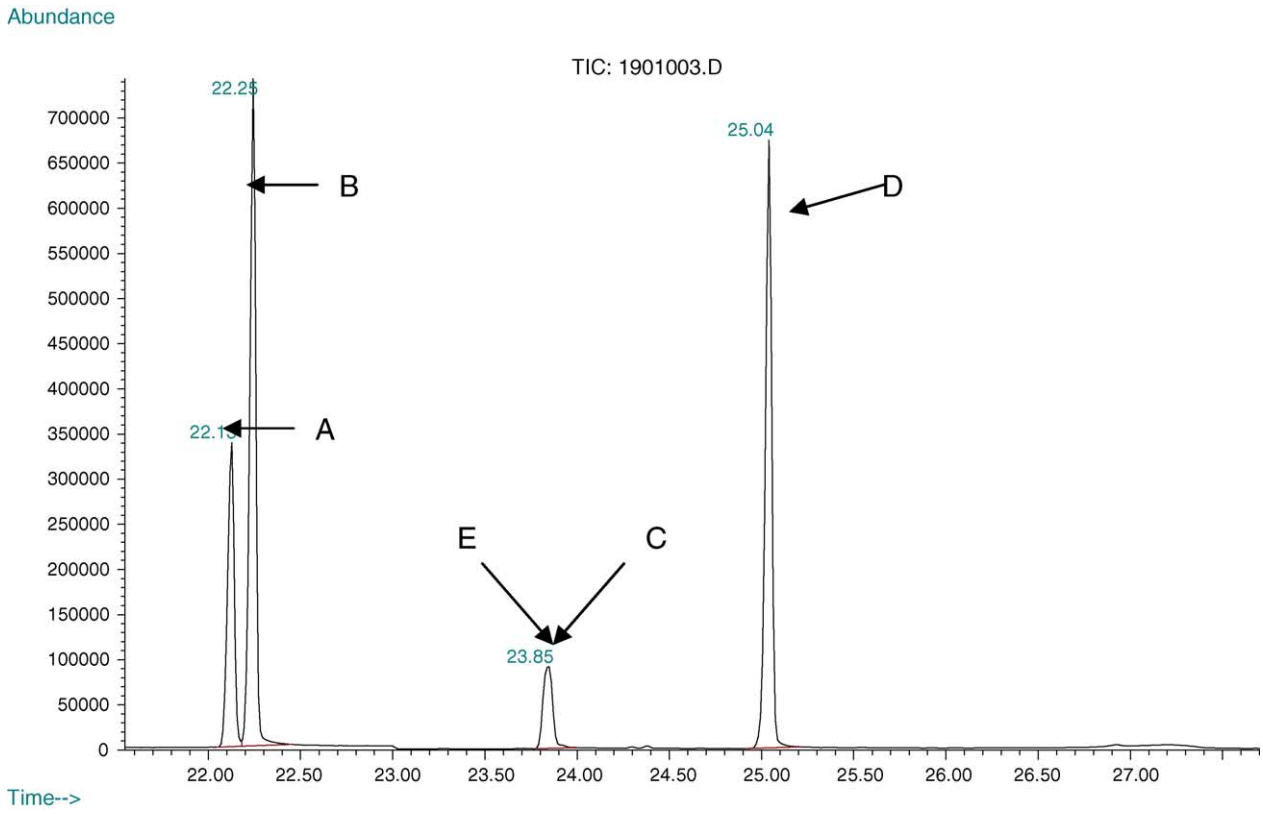

Fig. 5. Ion chromatogram of HFBA-derivatives: Cathine (A), (±)-norephedrine (B), (±)-ephedrine (C), (±)-Ψ-ephedrine (D), (+)-ephedrine-d_3 (E) (all as HFBA-derivatives).

Table 4
Enantiomeric composition (μg/mL) of the targeted 10 analytes found in various cold remedies

Sample	Derivative	Cathinone		Norephedrine		Nor-ψ-ephedrine		Ephedrine		ψ-Ephedrine	
		(+)	(−)	(+)	(−)	(+)	(−)[c]	(+)	(−)	(+)	(−)
2	MTPA	–[a]	–	–	–	0.039[b]	–	–	1.84	0.565	–
	HFBA	–		–		0.123		1.50		0.850	
9	MTPA	–	–	–	–	–	–	–	2.39	1.02	–
	HFBA	–		–		–		2.33		0.951	
10	MTPA	–	–	–	–	–	–	–	1.36	0.737	–
	HFBA	–		–		–		1.27		0.600	
15	MTPA	0.186	–	–	–	–	–	–	–	–	–
	HFBA	–		–		–		–		–	
16	MTPA	–	–	–	–	–	–	0.549	0.414	48.1	–
	HFBA	–		–		–		4.62		21.9	
17	MTPA	–	–	–	–	–	–	–	0.111	0.089	–
	HFBA	–		–		–		0.115		0.043[b]	
19	MTPA	1.35	26.8	1.19	2130	340	–	–	–	–	–
	HFBA	–		2860		0.940		–		–	

[a] Below LOD (0.060 μg/mL as established for ephedrine).
[b] Below LOD and LOQ as established for ephedrine. However, distinct chromatographic peaks and mass spectra were observed and the listed concentrations were estimated.
[c] No standard was available; thus, these analytical findings are tentative.

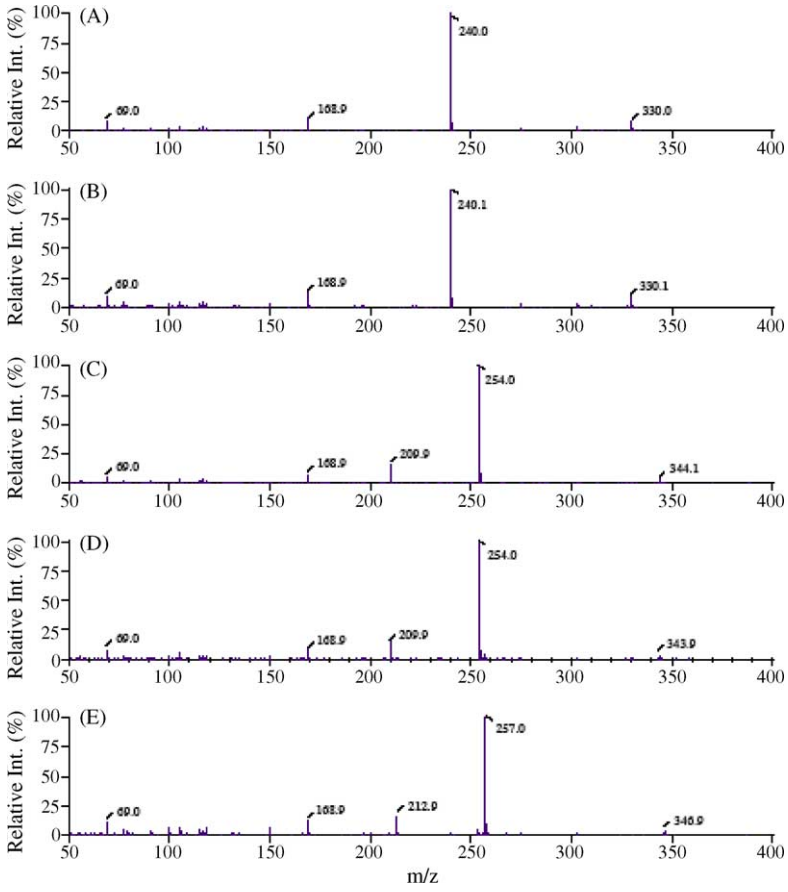

Fig. 6. Mass spectra of (+)-Ψ-norephedrine (A), (+)-norephedrine (B), (+)-Ψ-ephedrine (C), (+)-ephedrine (D), and (+)-ephedrine-d_3 (E) (all as HFBA-derivatives).

evaluations: 2.00, 1.00, 0.500, 0.250, 0.010, 0.080, 0.060, 0.040 μg/mL. Applying the criteria described above, the method's LOD and LOQ were determined to be 0.060 and 0.080 μg/mL for both HFBA and MTPA derivatives.

3.3. Application to the analysis of common OTC cold remedies

As reported in an earlier study [16], various ephedrine-related compounds were found in readily available OTC cold remedies. Attempts to correlate the occurrences and concentrations of these compounds in OTC remedies with the analytical findings derived from testing athletes during sport-competition events have not been conclusive. With this in mind, the authors thought an additional dimension of information (enantiomeric composition) may help studies of this nature. Thus, various chiral and achiral derivatization approaches were explored, of which the most effective ones were applied to the analysis of a limited number of OTC cold remedies (from 19 manufacturers). Preliminary data shown in Table 4 are promising and further studies will be pursued and applied to a comprehensive list of OTC remedies, selected prescription medicines, and relevant urine specimen sets.

4. Conclusion

An effective methodology has been established for the analysis of the following structurally related compounds and their enantiomers: cathinone, ephedrine, ψ-ephedrine, norephedrine, and nor-ψ-ephedrine. Using MTPA as the derivatization reagent, the resulting products can be baseline resolved by a 60-m HP 5MS capillary column. HFBA is effective when enantiomeric compositions is not needed. Preliminary application studies have also shown great potentials in providing an additional dimension of information (enantiomeric compositions) for source-tracing studies.

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References

[1] J. Long, A. Yarnell (Eds.), C & EN, October 15, 2001, p. 5.
[2] S.C. Stinson, C & EN, October 15, 2001, p. 79.
[3] F.T. Noggle, C.R. Clark, J. Assoc. Off. Anal. Chem. 66 (1983) 151.
[4] R.A. Ely, D.C. McGrath, J. Forensic Sci. 35 (1990) 720.
[5] F.T. Noggle, J. DeRuiter, C.N. Clark, Anal. Chem. 58 (1988) 1643.
[6] K.M. Andrews, J. Forensic Sci. 40 (1995) 551.
[7] K. Hutchinson, K.M. Andrews, Microgram 28 (1995) 256.
[8] Department of Health and Human Services, Notice to All DHHS/NIDA Certified Laboratories, February 4, 1991.
[9] J.R.H. Liu, W.W. Ku, Anal. Chem. 53 (1981) 2180.
[10] J.R.H. Liu, W.W. Ku, J.T. Tsay, M.P. Fitzgerald, S. Kim, J. Forensic Sci. 27 (1982) 39.
[11] J.R.H. Liu, W.W. Ku, M.P. Fitzgerald, J. Assoc. Off. Anal. Chem. 66 (1983) 1443.
[12] J.R.H. Liu, J.T. Tsay, Analyst 107 (1982) 544.
[13] J.R.H. Liu, S. Ramesh, J.T. Tsay, W.W. Ku, M.P. Fitzgerald, S. Angelos, C.L.K. Lins, J. Forensic Sci. 26 (1981) 656.
[14] J.K. Sellers, G.L. Duffitt, M.L. Gaines, R.H. Liu, Forensic Sci. Rev. 8 (1996) 91.
[15] R.M. Iwanicki, K. Maier, J.A. Zlotnick, R.H. Liu, T.-L. Kuo, F. Tagliaro, J. Forensic Sci. 44 (1999) 470.
[16] Y.-L. Tseng, H.-R. Hsu, F.-H. Kuo, M.-H. Shieh, C.-F. Chang, J. Anal. Toxicol. 27 (2003) 359.
[17] R.H. Liu, G.F. Foster, E.J. Cone, S.D. Kumar, J. Forensic Sci. 40 (1995) 983.
[18] J.-T. Liu, R.H. Liu, J. Biochem. Biophys. Methods 54 (2002) 115.
[19] K. Lemr, D. Jirovsky, J. Seveikéi, J. Liq. Chromatogr. 19 (1996) 3171.
[20] M.J. LeBelle, C. Savard, B.A. Dawson, D.B. Black, L.K. Katyal, F. Zreck, A.W. By, Forensic Sci. Int. 71 (1995) 215.
[21] K. Mathys, R. Brenneisen, J. Chromatogr. 583 (1992) 79.
[22] R. Brenneisen, S. Geisshusler, X. Schorno, J. Pharm. Pharmacol. 38 (1986) 298.