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Journal of Chromatography B, 825 (2005) 79–87
JOURNAL OF CHROMATOGRAPHY B
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Enantiomeric determination of amphetamines: Exploring a novel one-step solid-phase microextraction-based approach

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Received 16 October 2004; accepted 17 January 2005
Available online 5 February 2005

Abstract

The recent advances in fiber manufacturing technology, solid-phase microextraction (SPME) is now widely studied for its effectiveness for the pretreatment of various categories of samples. This study explores a novel SPME approach for enantiomeric analysis of amphetamines, in which absorption/derivatization are accomplished in one step. Specifically, (*S*)-(−)-*N*-(Trifluoroacetyl)-prolyl chloride was adopted as the chiral derivatizing reagent and added directly into the sample matrix. Analytical parameters, such as temperature, absorption/desorption duration, and the amount of derivatizing reagent, were studied to determine their effects on the yield of analytes. The derivatization products resulting from this study show excellent desorption characteristics on the polydimethylsiloxane-coated fiber adopted in this study. Optimal operational parameters (absorption: 70 °C for 10 min; injection: 250 °C for 5 min) cause minimal negative impact on the fiber, allowing repeated use of the fiber for more than 30 times. For quantitation, data were collected under selected ion monitoring (SIM) mode using *m*/*z* 237 and 251 to designate derivatized amphetamine and methamphetamine. This method was evaluated and proved to be effective in (a) quantitative determination of the enantiomeric pairs of amphetamine and methamphetamine – in terms of repeatability, linearity, and limits of detection and quantitation; and (b) generating case-specimen data comparable to those derived from a conventional Liquid–liquid extraction approach. Good linearity for the calibration curves were established in the range of 1000–50 ng/mL for both analytes. The limits of detection for these analytes were 30 ng/mL.

Keywords: Amphetamine; Methamphetamine; Enantiomer separation; Derivatization; GC–MS

1. Introduction

Solid-phase microextraction (SPME) presents a great potential in many areas of the analytical science, where sample pretreatment is used to purify and concentrate the analytes. With recent advances in the fiber manufacturing technology, SPME is now widely studied for its applications in pretreating various categories of specimens [1].

Previous studies on SPME as sample pretreatment method for the analysis of amphetamines nicely illustrated the evolution of this technology's application [2–9]. At first, SPME was applied to water samples and later to biological samples such as urine or blood. Chemical derivatization of the analytes, often required for chromatographic analysis, was carried out through the addition of the derivatizing reagents into the sample matrix [5] or into the chromatographic injection port [3]. A two-step approach was adopted by Jurado et al. [8] in which a polydimethylsiloxane fiber was placed in the headspace of the sample vial for the absorption of 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethamphetamine. The fiber was then removed from the sample vial and placed into the headspace of a trifluoroacetic anhydride containing vial for derivatization. A recent report [9] simplified this procedure by placing the fiber in the headspace of the derivatizing reagent-containing vial, which was placed in the headspace of the sample vial. Analytes in the sample were allowed to reach the fiber through the holes on the upper sides of the insert vial.

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doi:10.1016/j.jchromb.2005.01.017

The present study represents a further progress in the application of the SPME technology. A one-step process was adopted to complete the absorption/derivatization process for the analysis of the enantiomeric compositions of amphetamines by adding the derivatizing reagent directly into the sample matrix in a regular sample vial.

2. Experimental

2.1. Drug standards and case specimens

Drug standards (D,L-amphetamine, D-amphetamine, D,L-methamphetamine, and D-methamphetamine, 1.0 mg/mL) and internal standards (D,L-amphetamine-d_8 and D,L-methamphetamine-d_8, 0.1 mg/mL) in methanol were purchased from Radian/Cerilliant Co. (Austin, TX, USA). Derivatization reagent, (*S*)-(−)-*N*-(trifluoroacetyl)-prolyl (L-TPC) was purchased from Aldrich (St. Louis, MS, USA). Supelco^{TM} solid phase microextraction fiber holder and fibers coated with 100 μm of polydimethylsiloxane were purchases from Supelco (Bellefonte, PA, USA).

Twelve case specimens analyzed in this study came from a workplace drug-testing laboratory. These urine specimens had been tested positive for amphetamine and methamphetamine by both immunoassay and GC–MS methods and were scheduled for disposal. No information on the history or the collection time of these specimens was available.

2.2. SPME procedure

The absorption/derivatization basic protocol involved placing 1-mL drug-free urine in a 4.5-mL sample vial, followed by the addition of amphetamine and methamphetamine and respective internal standards (250 ng each), L-TPC (50 μL), K_2CO_3-saturated solution (100 μL), and NaCl (0.3 g). The vial was capped and vortexed for 10 min, followed by the insertion of the fiber into the headspace of the vial. The absorption/derivatization process was then changed to study the effects of the following parameters: adsorption duration, temperature, amount of the derivatization reagent, analyte concentrations, age of the fiber.

2.3. Liquid–liquid extraction (LLE) procedure

Established procedures [10] were adopted for the comparative LLE study. Briefly, K_2CO_3-saturated solution (0.5 mL) and hexane (4 mL) were added into the 16 mm × 100 mm glass tube containing the analytes, internal standards, and the derivatization reagent. The mixture was vortexed for 10 min,

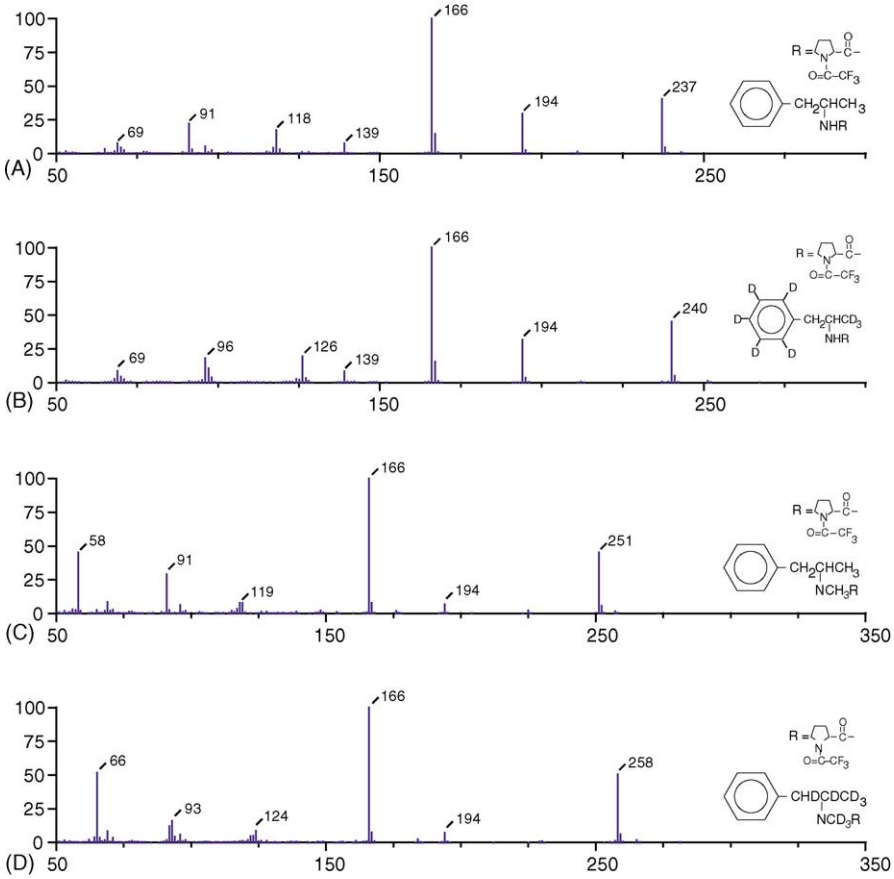

Fig. 1. Mass spectra of L-TPC-derivatized amphetamine (A), amphetamine-d_8 (B), methamphetamine (C), and methamphetamine-d_8 (D).

Table 1
Relative intensity and cross-contribution data[a] of ions with potential for designating the analyte and the adapted internal standard

	Methamphetamine-d_0			Methamphetamine-d_8		
	Ion (*m*/z)	Relative intensity	Analog's contribution	Ion (*m*/z)	Relative intensity	Analog's contribution
L-form	58	46.8	0.27	65	53.6	4.98
	91	28.5	5.53	93	16.0	0.85
	251[b]	45.8	0.04	258[b]	50.6	0.61
D-form	58	45.1	0.28	65	51.7	5.03
	91	28.8	5.41	93	16.1	0.83
	251[b]	45.3	0.03	258[b]	50.5	0.65
	Amphetamine-d_0			Amphetamine-d_8		
	Ion (*m*/z)	Relative intensity	Analog's contribution	Ion (*m*/z)	Relative intensity	Analog's contribution
L-form	118	16.4	0.90	126	19.1	6.14
	237[b]	39.4	1.70	240[b]	43.0	0.11
D-form	118	17.3	0.88	126	19.6	5.94
	237[b]	40.3	1.70	240[b]	45.2	0.10

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

centrifuged, and the upper organic layer was quantitatively transferred to a clean tube. The extract was dried under nitrogen at 45 °C, followed by the addition of ethyl acetate (200 μL) for reconstitution. Typically, 1 μL was used for GC–MS analysis.

2.4. Desorption–injection

A typical protocol adopted for the desorption–injection of the analytes for GC–MS analysis involved removing the processed fiber from the sample vial and placing it onto the GC injector. Injection was carried out by desorption at the injector's temperature (250 °C). Desorption time was varied to study the fiber's carry-over phenomenon.

2.5. 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, USA). A 25-m × 0.20-mm (0.25-μm film thickness) HP-5MS capillary column (Agilent: Wilmington, DE, USA) was used for this study. Helium carrier gas flow rate was 1.0 mL/min. The GC column was operated at an initial temperature of 60 °C for 5 min, raised to 250 °C at 25 °C/min, and a 2-min hold at the final temperature (total time = 20 min). The injector and GC–MS interface temperatures were maintained at 250 and 280 °C, respectively.

Full-scan mass spectra of L-TPC-derivatized amphetamine and methamphetamine and their deuterated analogs are shown in Fig. 1. These spectra were used for the selection of ions suitable for designating each analyte and internal standard in selected ion monitoring (SIM) mode. These ions and their "cross contribution" characteristics [11] are shown in Table 1. A typical total ion chromatogram of a standard solution derived from SIM mode is shown as Fig. 2.

3. Results and discussion

3.1. 3.1 Absorption/derivatization

Adsorption duration, temperature, and the amount of derivatizing reagent all contributed to the yields of the derivatized analytes on the fiber. The effects of these parameters were empirically studied using a standard solution (concentrations of amphetamine and methamphetamine: 250 ng/mL). Resulting data shown in Fig. 3(A)–(C) indicate the optimal duration, temperature, and derivatization reagent are 20 min, 70 °C, and 50 μL, respectively. To shorten analytical time, 10 min (instead of the optimal 20 min) along with 70 °C and 50 μL of derivatization reagent were adopted to carry out the experiments described hereafter.

3.2. Desorption

Complete desorption of the absorbed analytes would allow for re-use of the costly fiber and probably would also improve the limit of detection. Ion chromatograms shown in Fig. 4(A)–(E) indicate that three 1-min desorption at 250 °C completely removed the adsorbed analytes. As shown in Fig. 4(F) and (G), one 5-min desorption also removed the adsorbed analytes completely.

3.3. Evaluation of analytical parameters

The operational parameters (absorption time: 10 min, temperature: 70 °C, derivatization reagent: 50 μL; desorption duration: 5 min at 250 °C) established above were used to further study the effectiveness of this method for quantitative determinations of the enantiomeric pairs of amphetamine and methamphetamine.

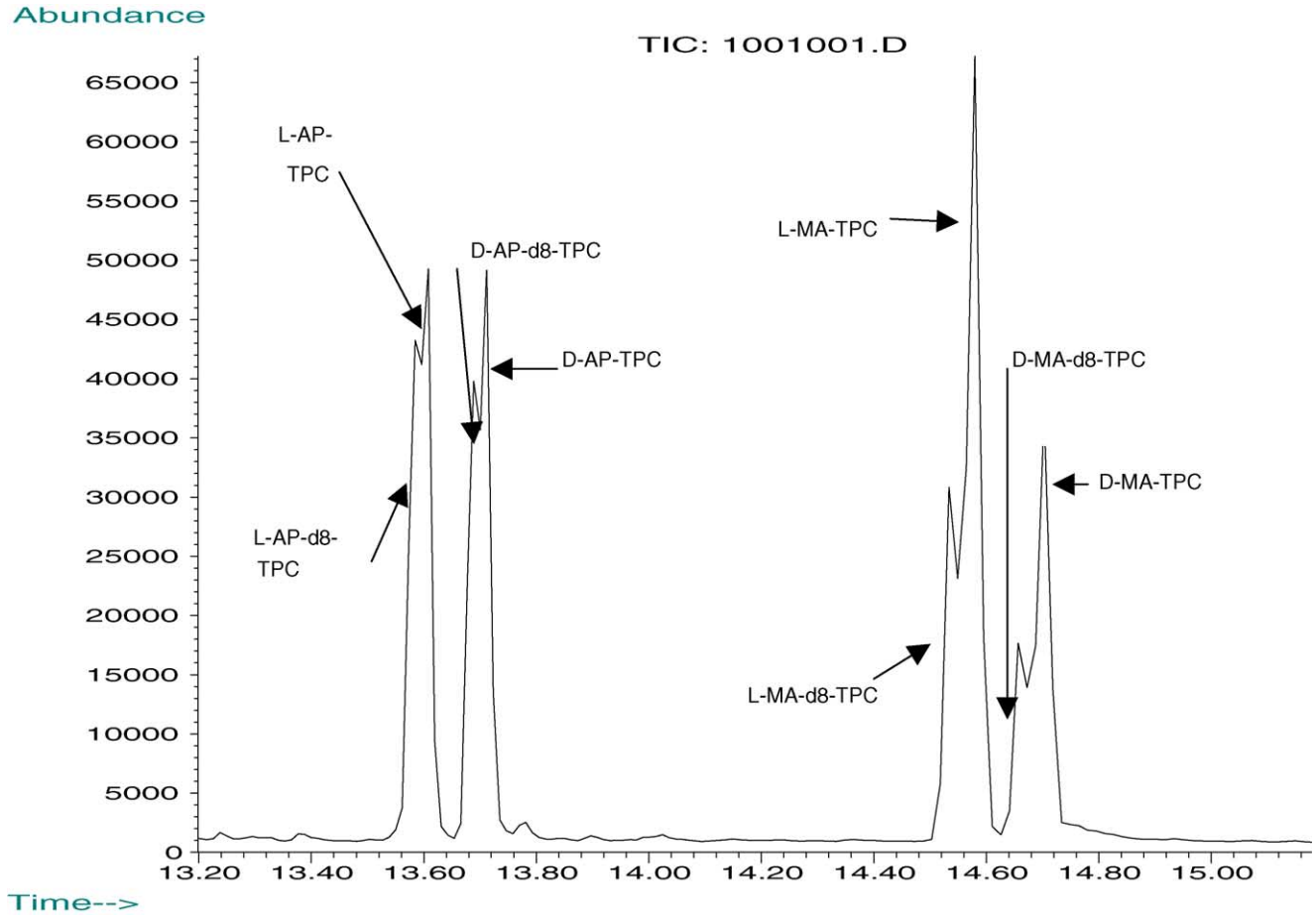

Fig. 2. Total ion chromatogram of a standard solution containing L-TPC-derivatized amphetamine, amphetamine-d_8, methamphetamine, and methamphetamine-d_8 (data collected under selected ion monitoring mode).

3.3.1. Limits of detection and quantitation (LOD and LOQ)

Commonly adopted criteria were used to confirm the presence of a specific analyte in a test sample, i.e., ions monitored for a specific analyte have to present at an acceptable retention time (±2%) with acceptable intensity ratios (±20%) as compared those established with a standard (250 ng/mL of amphetamine and methamphetamine and their respective internal standards). The LOD was defined as the lowest concentration of a standard solution meeting the above criteria, while the LOQ was defined as the lowest concentration of a standard solution that met these criteria and with an observed analyte concentration within ±20% of the targeted value.

A series of standard solutions with the following concentrations of both enantiomers of amphetamine and methamphetamine were used for LOD and LOQ evaluations: 1000, 500, 250, 100, 50, 40, 30, 20, 10, 5 ng/mL. Applying the criteria described above, the method's LOD and LOQ were determined as 30 and 50 ng/mL for both pairs of enantiomers.

3.3.2. Reproducibility and method linearity

Reproducibility was evaluated using triplicates of amphetamine/methamphetamine-containing standard solutions (250 ng/mL of each internal standard and each enantiomer of the analytes). Data shown in the upper portion of Table 2 were derived from the triplicates of the same standard solution, while the data shown in the lower portion of the

Table 2
Reproducibility of triplicates derived from one and three batches of standard solutions (250 ng/mL)

Replicate	Amphetamine		Methamphetamine	
	L-form	D-form	L-form	D-form
Standard solutions from the same batch				
1	261	290	269	262
2	233	270	258	246
3	260	287	260	256
Mean	251	283	263	255
S.D.	15.8	10.6	6.0	7.7
%CV	6.3	3.8	2.3	3.0
Standard solutions from three different batches				
2	261	290	269	262
3	248	255	284	247
Mean	253	272	267	249
S.D.	7.0	17.2	18.2	12.1
%CV	2.7	6.3	6.8	4.9

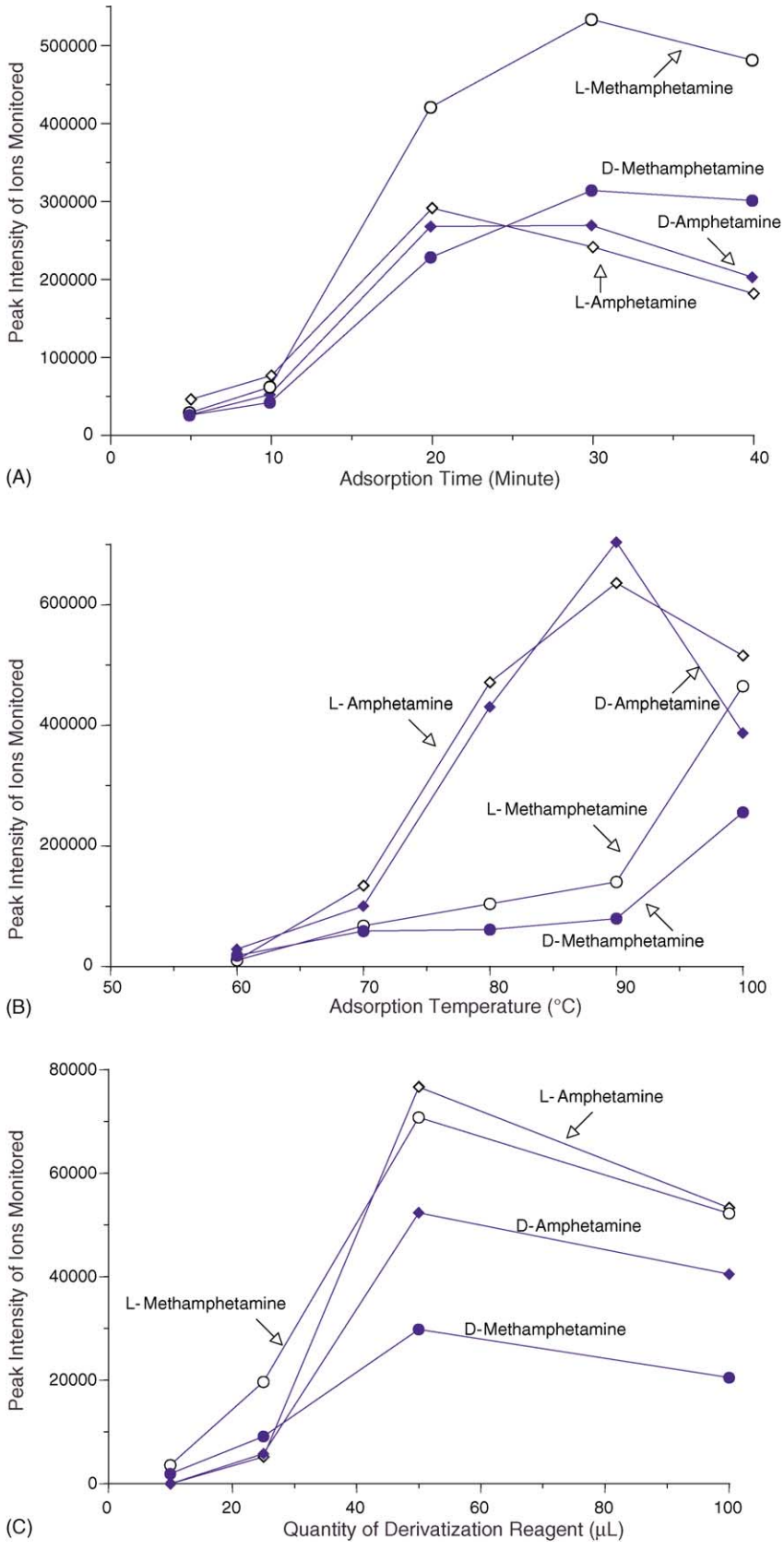

Fig. 3. Parameters affecting the yield of absorption/derivatization product: duration of absorption (A), temperature of absorption (B), quantity of derivatization reagent (C).

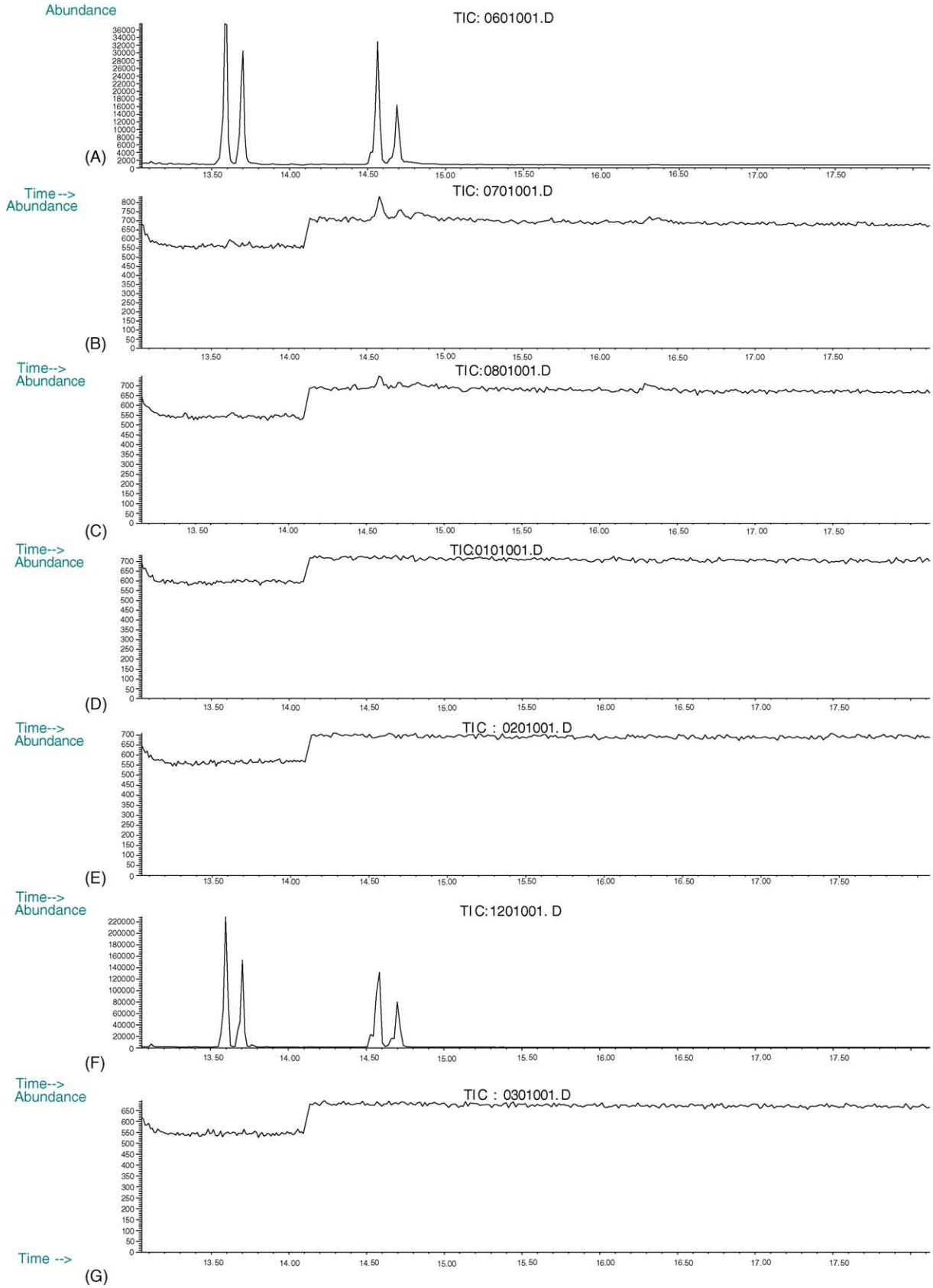

Fig. 4. Evaluation of desorption efficiency at 250 °C: consecutive 1-min desorption (A–E); consecutive 5-min desorption (F and G).

Table 3
Regression of calibration data (range 50–1000 ng/mL) derived from two different fibers, each in duplicates

Absorption		Amphetamine		Methamphetamine	
Fiber	Replicate	L-form	D-form	L-form	D-form
1	1	$y = -0.0612 + 0.0047x$ $r^2 = 1.000$	$y = -0.0888 + 0.0041x$ $r^2 = 0.997$	$y = -0.0399 + 0.0046x$ $r^2 = 1.000$	$y = -0.0367 + 0.0046x$ $r^2 = 1.000$
	2	$y = -0.0198 + 0.0054x$ $r^2 = 1.000$	$y = -0.0773 + 0.0054x$ $r^2 = 0.999$	$y = 0.0187 + 0.0047x$ $r^2 = 0.998$	$y = -0.0850 + 0.0054x$ $r^2 = 1.000$
2	1	$y = 0.0660 + 0.0043x$ $r^2 = 1.000$	$y = 0.0722 + 0.0048x$ $r^2 = 0.999$	$y = -0.0226 + 0.0050x$ $r^2 = 1.000$	$y = -0.0969 + 0.0051x$ $r^2 = 0.999$
	2	$y = 0.0324 + 0.0046x$ $r^2 = 0.999$	$y = 0.1612 + 0.0045x$ $r^2 = 0.998$	$y = -0.0152 + 0.0051x$ $r^2 = 0.998$	$y = -0.0078 + 0.0049x$ $r^2 = 0.999$

table were derived from three separately prepared standard solutions.

Method linearity was evaluated using two fibers and standard solutions containing the following concentrations of the enantiomeric pairs of amphetamine and methamphetamine: 50, 100, 250, 500, 1000 ng/mL (internal standard: 250 ng/mL). One fiber was repeatedly used to process these five standard solutions. This same fiber was used again to process another set of five standard solutions. Another fiber was used to repeat the same process. Regression data shown in Table 3 indicate excellent calibration characteristics.

3.4. Purity evaluation of the standards and the derivatization reagent

The derivatization reagent, L-TPC, and the analyte standards used in this study were not 100% enantiomerically pure. Furthermore, unlike a chiral column, the achiral column used in this study can only resolve the resulting four isomers into two chromatographic peaks [12,13]. It is thus very important to understand and to properly interpret the observed *apparent* enantiomeric composition data. To this aim, the SPME methodology established above was used to analyze the standard solutions (250 ng/mL of analytes without internal standard) prepared from standards (commercially labeled as 99% purity) of the following analytes: D-amphetamine, D-methamphetamine, racemic amphetamine, racemic methamphetamine.

This study was performed with triplicates and the resulting data are shown in Table 4. Data shown in the upper portion of the table indicate the presence of significant amounts of the L-enantiomers in the D-amphetamine and D-methamphetamine standards. Similarly, data shown in the lower portion of the table show more than 2% deviations from the expected value, 1.00, for the enantiomeric ratios.

It should be noted that these *apparent* values do not reflect the *exact* enantiomeric compositions of the standards used in this study. As demonstrated before [12,13], D-amphetamine-D-TPC and L-amphetamine-L-TPC are an enantiomeric pair and elute with the exact same retention time by an achiral column. The *apparent* peak area designated for L-amphetamine contains contribution due to the presence of D-TPC in the derivatization reagent (L-TPC). Correction factors can be applied to obtain data that are more representative of true values and interested readers are referred to literature references for further details [12,13]. In conclusion, enantiomeric purity of the chiral derivatization reagent should be fully considered when interpreting the observed *apparent* enantiomeric composition data derived from the analysis by an achiral column.

3.5. Case specimen

To fully validate the SPME methodology, the established protocol was further applied to the analysis of 12 case specimens. Results from this method are listed in Table 5 along with data from a conventional LLE extraction with a follow up derivatization step [10]. Differences in the quantitation

Table 4
Apparent enantiomeric compositions of commercial D- and D,L-amphetamine and methamphetamine resulting from the use of L-TPC containing a small amount of D-TPC

Analyte	Ion intensity and intensity ratio observed in replicates			
	1	2	3	Mean
D-amphetamine				
L-amphetamine[a]	4275	3921	3428	
D-amphetamine[a]	74091	84607	80150	
L/D ratio	0.058	0.046	0.043	0.049
D-methamphetamine				
L-methamphetamine[a]	4755	4552	4525	
D-methamphetamine[a]	57253	59349	55165	
L/D ratio	0.083	0.076	0.082	0.080
D,L-amphetamine				
L-amphetamine[a]	50346	55287	64374	
D-amphetamine[a]	50537	49281	67476	
L/D ratio	0.996	1.122	0.954	1.024
D,L-methamphetamine				
L-methamphetamine[a]	43137	49587	63206	
D-methamphetamine[a]	41449	48754	61893	
L/D ratio	1.041	1.017	1.021	1.026

[a] A small portion of the ion intensity observed for this compound was contributed by its enantiomer. See related text for details.

Table 5
Comparison of case-specimen enantiomeric composition data derived from SPME and liquid–liquid extraction (LLE) methods

Specimen	SPME			LLE			Mean and % deviation			
	L-form	D-form	L/D	L-form	D-form	L/D	L-form	Dev.	D-form	Dev.
Methamphetamine										
1	1280	12169	0.11	1546	10638	0.15	1422	±10	11404	±7
2	219	1821	0.12	146	1427	0.10	183	±20	1624	±12
3	593	7436	0.08	429	7768	0.06	511	±16	7602	±2
4	1960	13845	0.14	1233	11753	0.10	1597	±23	12799	±8
5	340	3766	0.09	252	3310	0.08	296	±15	3538	±6
6	1254	10745	0.12	1315	9931	0.13	1285	±2	10338	±4
7	704	3090	0.22	591	2564	0.23	648	±9	2827	±9
8	5836	2482	2.35	6317	2010	3.14	6077	±4	2246	±11
9	486	5398	0.09	435	4680	0.09	461	±6	5039	±7
10	608	6667	0.09	543	5649	0.10	576	±6	6158	±8
11	747	6761	0.11	449	5632	0.08	598	±25	6197	±9
12	972	8600	0.11	950	6276	0.15	961	±1	7438	±16
Amphetamine										
1	72	2477	0.03	63	2702	0.02	68	±7	2590	±4
2	42	1010	0.04	32	976	0.03	37	±14	993	±2
3	36	1329	0.03	37	1318	0.03	37	±1	1324	±1
4	94	2581	0.04	67	2545	0.03	81	±17	2563	±1
5	93	2675	0.03	72	2763	0.03	83	±13	2719	±2
6	92	3324	0.03	79	2973	0.03	86	±8	3149	±6
7	78	1466	0.05	79	1381	0.06	79	±1	1424	±3
8	403	580	0.69	538	482	1.12	471	±14	531	±9
9	50	1971	0.03	43	1928	0.02	47	±7	1950	±1
10	84	2376	0.04	66	2230	0.03	75	±12	2303	±3
11	78	2220	0.04	73	2100	0.03	76	±3	2160	±3
12	90	1308	0.07	58	1276	0.05	74	±22	1292	±1

and enantiomeric composition data resulting from these two methods are within experimental errors.

Since the metabolic fates of D- and L-methamphetamine proceed in the biological system with different rates [14], the enantiomeric compositions of methamphetamine and its metabolite, amphetamine, will not be the same. Again, as indicated earlier, the observed enantiomeric composition data are *apparent* values and should be interpreted with caution.

4. Conclusions

Data derived from this study have demonstrated the effectiveness of the proposed one-step absorption/derivatization SPME methodology for the analysis of amphetamine and methamphetamine from urine specimens. By adding the chiral derivatizing reagent, L-TPC, directly into the sample matrix, the derivatized analytes can be successfully absorbed and desorbed from the polydimethylsiloxane-coated fiber (100-μm film thickness). Quantitation and enantiomeric composition data derived from absorption at 70 °C for 10 min and a 5-min desorption (injection) at 250°C are comparable to those generated by a conventional 2-step approach involving LLE and a separate derivatization step.

This study also demonstrated that the *apparent* enantiomeric compositions resulting from the use of an *achiral column* and a *chiral derivatization reagent with significant optical impurity* should be interpreted with caution.

Acknowledgments

The author is thankful to helpful discussion with Dr. Ray H. Liu of Fooyin University (Kaohsiung Hsien, Taiwan) and the University of Alabama at Birmingham (Birmingham, AL, US). He is also grateful to the financial support provided by the (Taiwanese) National Bureau of Controlled Drugs, Department of Health (DOH92-NNB-1005) and the provision of a research environment by the US. Federal Aviation Administration's Civil Aerospace Medical Institute (Oklahoma City, OK), which greatly facilitate the completion of this manuscript.

References

[1] G. Shen, K. Lee, Anal. Chem. 75 (2003) 98.
[2] M. Yashiki, T. Kojima, T. Miyazaki, N. Nagasawa, Y. Iwasaki, K. Hara, Forensic Sci. Int. 76 (1995) 169.
[3] N. Nagasawa, M. Yashiki, Y. Iwasaki, K. Hara, T. Kojima, Forensic Sci. Int. 78 (1996) 95.
[4] F. Centini, A. Masti, I, Forensic Sci. Int. 83 (1996) 161.
[5] H.G. Ugland, M. Krogh, K.E, J. Chromatogr. B 701 (1997) 29.
[6] H.L. Lord, J. Pawliszyn, Anal. Chem. 69 (1977) 3899.

[7] C. Battu, P. Marquet, A.L. Fauconnet, E. Lacassie, G. Lachatre, J. Chromatogr. Sci. 36 (1988) 1.
[8] C. Jurado, M.P. Gimenez, T. Soriano, M. Menendez, M. Repetto, J. Anal. Toxicol. 24 (2000) 11.
[9] M.-K. Huang, C. Liu, S.-D. Huang, Analyst 127 (2002) 1203.
[10] S.-M. Wang, T.-C. Wang, Y.-S. Giang, J. Chromatogr. B 816 (2005) 131.
[11] R.H. Liu, T.-L. Lin, W.-T. Chang, C. Liu, W.-I. Tsay, J.-H. Li, T.-L. Kuo, Anal. Chem. 74 (2002) 618A.
[12] R.H. Liu, W.K. Ku, Anal. Chem. 53 (1981) 2180.
[13] R.H. Liu, W.W. Ku, J.T. Tsay, M.P. Fitzgerald, S. Kim, J. Forensic Sci. 27 (1982) 39.
[14] A.H. Beckett, M. Rowland, Nature 206 (1965) 1260.