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Journal of Chromatography A, 672 (1994) 135–140

JOURNAL OF CHROMATOGRAPHY A

Analysis of forensic samples using precolumn derivatization with (+)-1-(9-fluorenyl)ethyl chloroformate and liquid chromatography with fluorimetric detection

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(First received July 29th, 1993; revised manuscript received January 18th, 1994)

Abstract

An indirect chiral separation of forensic methamphetamine samples by liquid chromatography with fluorescence detection was developed. Carbamate derivatives of the methamphethamine enantiomers were formed by using (+)-1-(9-fluorenyl)ethyl chloroformate. The response appeared to be linear from 16.7 to 1674.0 ng/ml ($r = 0.9999$) for each enantiomer. The relative standard deviations in the within-day and between-day assays for (*S*)-(+)-methamphetamine and (*R*)-(−)-methamphetamine are reported. As the developed procedure can also be used to determine ephedrine and pseudoephedrine, it will provide valuable information concerning the method of synthesis and the purity of the product.

1. Introduction

In recent years, the number of methamphetamine forensic samples obtained from police courts has increased strikingly. Methamphetamine, which is known to cause addiction and to have intense psychostimulant actions, has two optical isomers, the (*S*)-(+)- and (*R*)-(−)-isomers. The latter isomer has greater sympathomimetic properties whereas the former has greater anorexic and stimulant properties [1]. The abuse of this drug is a serious social problem. Hence it is necessary to develop a method with good sensitivity and selectivity for investigating methamphetamine enantiomers.

The purity of methamphetamine can be attributed in part to its route of synthesis, based on the reduction of ephedrine or pseudoephedrine. The advantage of ephedrine as the starting material is that side-products are limited. Also, because ephedrine is a stereochemically pure natural product, the process generates the more potent dextrorotatory enantiomer of methamphetamine, not a racemic mixture (Fig. 1). The absolute configurations of the α-carbon of (1*R*,2*S*)-ephedrine and (1*S*,2*S*)-pseudoephedrine are the same, so that (*S*)-(+)-methamphetamine could be prepared from either compound. Another approach for methamphetamine synthesis has been reported involving reaction between phenylacetone and methylamine (Leuc-

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0021-9673/94/$07.00
SSDI 0021-9673(94)00078-N

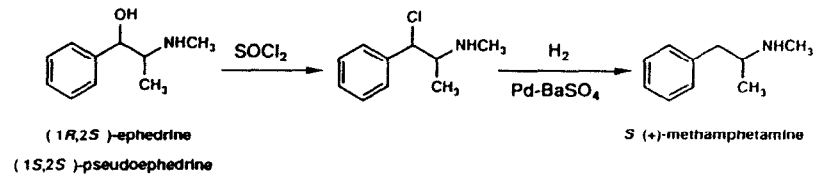

Fig. 1. Conversion of ephedrine and pseudoephedrine into (*S*)-(+)-methamphetamine.

kart procedure). This procedure generates the racemic mixture and, unless carefully purified, a higher proportion of contaminants. The purity of these illicit drugs is highly variable.

It has long been recognized that chromatographic methods can offer distinct advantages over classical techniques in the separation of stereoisomers. Several liquid chromatographic methods for the determination of amphetamine-related compounds have been reported [2–14]. Relatively fewer methods based on an indirect separation of enantiomers by precolumn derivatization with chiral reagents have been published [15–19]. Some of the commonly used reagents for amines give UV-sensitive derivatives for both primary and secondary amines. However, UV detection is hampered by the low molar absorptivities of these compounds in the UV region. We decided to use fluorescence detection, an intrinsically more sensitive method. This paper describes an approach for the derivatization of enantiomeric drugs using (+)-1-(9-fluorenyl)ethyl chloroformate reagent under optimized conditions using fluorimetric detection (Fig. 2). The detectability of enantiomers is enhanced and the chromatographic behaviour of basic compounds is improved as a result of such derivatization. This method was evaluated in terms of linearity, selectivity and detection limit. Further, we applied the method to the analysis of forensic samples that consisted of mixtures of methamphetamine product and its semi-products from clandestine laboratories. The results show that the method can simultaneously determine ephedrine, pseudoephedrine and methamphetamine enantiomers.

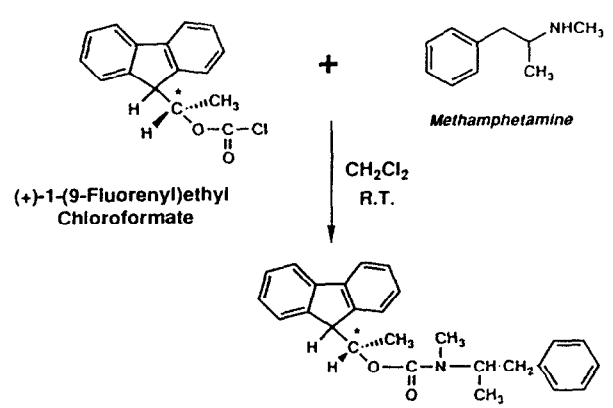

Fig. 2. Formation of carbamate derivatives from methamphetamine with (+)-FLEC.

2. Experimental

2.1. Reagents and chemicals

(1*R*,2*S*)-Ephedrine hydrochloride, (1*S*,2*S*)-pseudoephedrine hydrochloride, (*R*)-(−)-methamphetamine, (*S*)-(+)-methamphetamine hydrochloride and racemic methamphetamine hydrochloride were purchased from Sigma (St. Louis, MO, USA). (+)-1-(9-Fluorenyl)ethyl chloroformate [(+)-FLEC] ($[\alpha]_D^{25} = +70.0°$; $c = 1$ in $CHCl_3$; optical purity >99.5%) was obtained from Fluka (Buchs, Switzerland). All organic solvents and reagents were of either LC or analytical-reagent grade and used as received. Triply distilled water with an electric conductance 18.3 MΩ was used (obtained with a Millipore Milli-Q reagent water system).

2.2. Apparatus

The high-performance liquid chromatographic (HPLC) system consisted of a Model 600E system controller (Waters Chromatography Division, Millipore, Milford, MA, USA), a Waters Model 510 pump, a Waters Model 745B data module and a Shimadzu Model RF-535 spectrofluorimeter operated with excitation at 295 nm and emission at 315 nm. The mobile phase was

pumped through a $5C_{18}$-AR (5 μm) (Waters) reversed-phase column (25 cm × 3.9 mm I.D.) (Nacalai Tesque) with an isocratic flow-rate of 1.0 ml/min. Chromatography was performed at room temperature. Samples were injected automatically with a WISP-712 injector (Waters). Injections of 25 μl of all solutions were made.

2.3. Mobile phase

The mobile phase was acetonitrile–0.05 *M* phosphate buffer (65:35, v/v). It was filtered (0.45-μm Millipore filter) and degassed with an ultrasonic bath prior to use.

2.4. Standard solutions

An accurately weighed amount of (*S*)-(+)-methamphetamine or (*R*)-(−)-methamphetamine was dissolved in distilled water to give a concentration of 1674 ng/ml. For derivatization, the amine solution to be studied was placed in a 10-ml screw-capped glass tube, 0.5 ml of distilled water was added and the pH was made alkaline (about pH 12) with 1 *M* NaOH. The solution was reacted with 0.2 ml of (+)-FLEC–dichloromethane (0.1:10, v/v), 0.5 ml of dichloromethane was added and the solution was shaken for 30 min. The aqueous phase was discarded and the organic phase was washed twice with 0.5-ml portions of distilled water. The organic phase was evaporated to dryness under nitrogen and the residue was dissolved in 3 ml of methanol and mixed. This solution was passed through a 0.45-μm filter. The filtrate was then ready for injection into the chromatographic system.

2.5. Sample solution

A weighed aliquot (about 1 mg) of an illicit methamphetamine sample was dissolved in 1 ml of distilled water and the solution was derivatized in the same manner as the reference material.

2.6. Solution for linearity response

Calibration graphs were constructed. Solutions of seven concentrations of (*S*)-(+)- or (*R*)-(−)-methamphetamine, 16.74, 33.48, 100.44, 167.4, 334.8, 1004.4 and 1674.0 ng/ml, were prepared and each solution was derivatized through the whole procedure as described in Section 2.4. Each concentration was chromatographed six times.

3. Results and discussion

The effect of the (+)-FLEC concentration on derivatization was examined by varying the molar ratio of methamphetamine to (+)-FLEC from 1:1 to 1:7. The results indicated that ratios greater than 1:2 gave the maximum fluorescence intensity for these amines. In order to minimize chromatographic difficulties associated with excess of reagent, a molar ratio of 1:2 was adopted. The effect of reaction time was examined by allowing the derivatization to proceed for times ranging from 1 to 60 min. The results showed that maximum fluorescence intensity was observed at reaction times over 20 min, hence 20 min were used in all subsequent work. In order to establish the stabilities of the derivatized amines, derivatizations with concentrations of 167.4 ng/ml of (*S*)-(+) and (*R*)-(−)-methamphetamine were studied. The results showed that more than 99.5% of the amine in the original solution remained for 1 month.

Mobile phase conditions such as acetonitrile content, pH and salt concentration were investigated to determine their effects on the separation of the methamphetamine enantiomers and related compounds. Of these, the most critical factor was found to be pH. A pH of 6.0 was chosen because it produced the best combinations of separation and speed of analysis of the combined product. A buffer molarity of 0.05 was necessary to maintain the optimum pH of 6.0. A mobile phase concentration of acetonitrile of 65% was found to be the optimum for separating the enantiomers of methamphetamine (Table 1). Good selectivity with $\alpha = 1.04$ and capacity factors (k') of 17.0 and 17.7 for the (*S*)-(+)- and (*R*)-(−)-isomers, respectively, were found. The sensitivity of fluorescence detection was *ca.* 200 times higher than that of UV absorbance detection at 254 nm.

Table 1
Effect of organic solvent content in the mobile phase

Acetonitrile–0.05 *M* phosphate buffer (pH 6.0)	$k'_{(S)-(+)}$	$k'_{(R)-(-)}$	α
70:30	13.1	13.5	1.033
65:35	17.0	17.7	1.040
60:40	39.3	41.1	1.047

The calibration graphs passed through or near the origin. The linearity of the relationships between peak area (y) and each enantiomer concentration (x, ng/ml) was verified by injection of seven concentrations ranging from 16.7 to 1674.0 ng/ml. For typical calibration graphs, the regression equations and their correlation coefficients were calculated as follows: (*S*)-(+)-methamphetamine, $y = 1.0126x - 0.0392$ ($r = 0.9999$); (*R*)-(−)-methamphetamine, $y = 0.9974x - 0.0537$ ($r = 0.9999$). The detection limit for each enantiomer was down to 145.2 pg at signal-to-noise ratios higher than 3 (Fig. 3).

Reproducibilities (R.S.D.s) for both within-day and between-day assays were evaluated. A new calibration graph was prepared each day with a new series of samples. Each enantiomer was assayed in triplicate on five separate days. The within-day and between-day variabilities are shown in Table 2. The R.S.D.s in the within-day assay were between 0.11 and 0.80% for (*S*)-(+)-methamphetamine and between 0.22 and 1.50% for (*R*)-(−)-methamphetamine and those in the between-day assay were 1.00% for (*S*)-(+)-methamphetamine and 0.68% for (*R*)-(−)-methamphetamine.

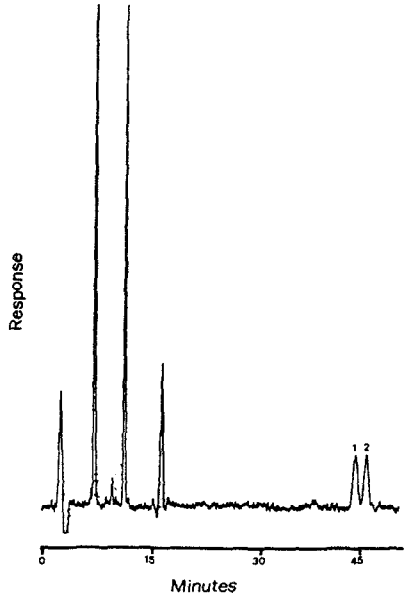

Fig. 3. Chromatogram showing detection limit of racemic methamphetamine derivative (290.4 pg). Peaks: 1 = 145.2 pg; 2 = 145.2 pg.

Typical chromatograms of carbamate derivatives of the (*S*)-(+)- and (*R*)-(−)-methamphetamine are shown in Fig. 4. The method can also separate carbamate derivatives of compounds related to methamphetamine, *e.g.*, ephedrine and pseudoephedrine, which are eluted prior to methamphetamine (Fig. 4). The first two peaks (retention times *ca.* 7 and 11 min) in each chromatogram are solvent peaks. The third peak (retention time *ca.* 16.7 min) in each chromatogram is from the (+)-FLEC reagent. The separation parameters of these enantiomeric compounds are given in Table 3.

The utility of enantiomeric composition determinations using the (+)-FLEC derivatization method is best illustrated with the analysis of some forensic samples. We analysed 50 illicit methamphetamine powder samples and two liquid samples seized in Taiwan. Only (*S*)-(+)-methamphetamine was detected in the powder samples (Fig. 4). (1*R*,2*S*)-Ephedrine, (1*S*,2*S*)-pseudoephedrine and (*S*)-(+)-methamphetamine were detected in the liquid samples. These observations showed that these forensic samples of (*S*)-(+)-methamphetamine had been synthesized from (1*R*,2*S*)-ephedrine and/or (1*S*,2*S*)-pseudoephedrine.

4. Acknowledgements

The authors thank Miss Hsiou-Chuan Chung for her assistance with the preparation of the manuscript. This work was supported by the Division of Drug Chemistry, National Laboratories of Foods and Drugs, Department of Health.

Table 2
Accuracy and reproducibility data

Isomer	Initial concentration (ng/ml)	Within-day variability			Between-day variability		
		Mean measured concentration (ng/ml)[a]	S.D. (ng/ml)	R.S.D. (%)	Mean measured concentration (ng/ml)[b]	S.D. (ng/ml)	R.S.D. (%)
(*S*)-(+)-Methamphetamine	1674.0	1674.0	5.00	0.29	–	–	–
	502.2	490.0	1.04	0.21	500.2	0.50	1.00
	50.2	54.0	0.08	0.15	–	–	–
(*R*)-(−)-Methamphetamine	1674.0	1673.0	3.61	0.22	–	–	–
	502.2	488.7	1.13	0.23	497.9	0.34	0.68
	50.2	54.7	0.41	0.70	–	–	–

[a] $n = 3$.
[b] $n = 5$. The between-day studies included 15 samples at each concentration level.

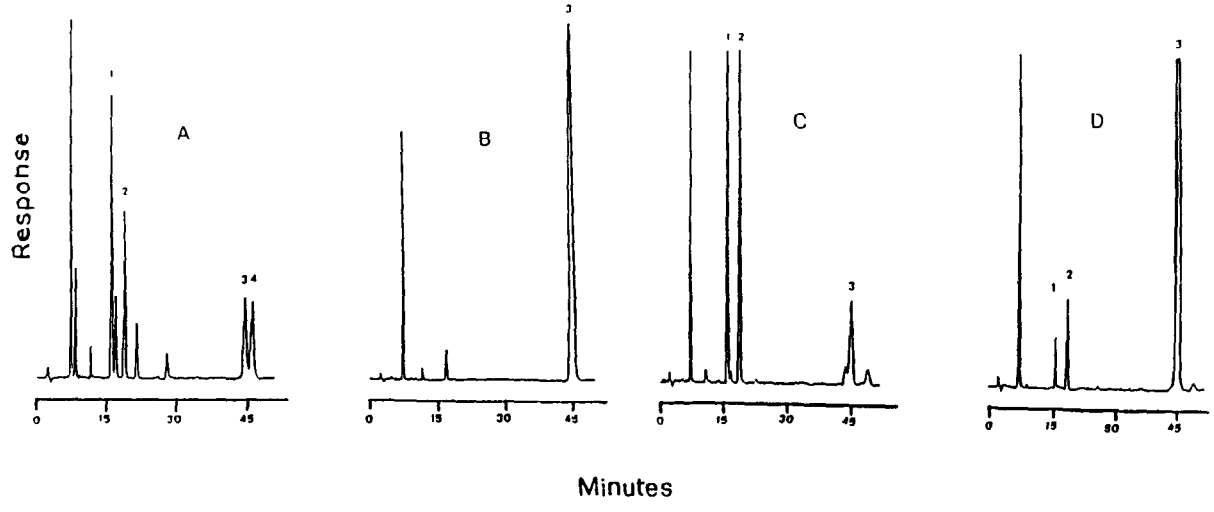

Fig. 4. Chromatograms of (A) standard amine compounds, (B) powder forensic sample, (C) liquid forensic sample 1 and (D) liquid forensic sample 2. Peaks: 1 = (1*R*,2*S*)-ephedrine–(+)-FLEC; 2 = (1*S*,2*S*)-pseudoephedrine–(+)-FLEC; 3 = (*S*)-(+)-methamphetamine–(+)-FLEC; 4 = (*R*)-(−)methamphetamine–(+)-FLEC.

Table 3
Separation parameters of enantiomeric drugs

Drug	$k'_{(S)-(+)}$	$k'_{(R)-(-)}$	α
Methamphetamine	17.00	17.70	1.04
Ephedrine	6.11	5.53	1.10
Pseudoephedrine	7.82	7.23	1.08

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