*Journal of Chromatography*, 605 (1992) 269–275
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 228

# Micellar electrokinetic capillary chromatography of the enantiomers of amphetamine, methamphetamine and their hydroxyphenethylamine precursors

Ira S. Lurie

*Drug Enforcement Administration, Special Testing and Research Laboratory, 7704 Old Springhouse Road, McLean, VA 22102-3494 (USA)*

(First received January 15th, 1992; revised manuscript received March 31st, 1992)

ABSTRACT

The separation of the enantiomers of amphetamine, methamphetamine, ephedrine, pseudoephedrine, norephedrine and norpseudoephedrine in a single run via micellar electrokinetic capillary chromatography (MECC) is described. The procedure, which involves preliminary derivatization with 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) followed by MECC analysis, is far superior with respect to both resolution and speed of analysis versus similar efforts utilizing high-performance liquid chromatography. The MECC separation was obtained at 20 kV on a 48 cm × 50 μm I.D. (26 cm length to detector) capillary at 30°C using a run buffer consisting of 20% methanol and 80% sodium dodecyl sulfate (SDS) solution [100 m*M* SDS, 10 m*M* phosphate, 10 m*M* borate (pH 9.0)]. The effects of organic modifier type, organic modifier concentration, voltage, temperature and SDS concentration on the resolution of the GITC derivatives are described. The application of the above methodology to forensic samples is presented.

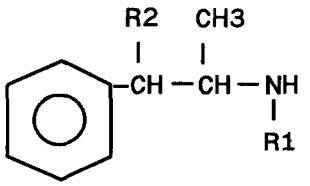
## INTRODUCTION

The separation of optical isomers such as phenethylamines (amphetamine, methamphetamine, norephedrine, norpseudoephedrine, ephedrine and pseudoephedrine) is important for forensic analysis. Isomer determination can help identify synthetic methodolologies. For example, amphetamine or methamphetamine synthesized via the Leuckart reaction (*i.e.*, from phenylacetone) will exist as the racemate, whereas that synthesized via direct stereospecific reduction of one of the enantiomeric α-hydroxyphenethylamines (*i.e.*, from norephedrine, norpseudoephedrine, ephedrine or pseudoephedrine) will exist as an optically pure *d* or *l* isomer [1]. In addition, since these compounds are often encountered in combinations, the determination of their isomers could provide additional intelligence information other than synthetic route.

Gas chromatography (GC) and high-performance liquid chromatography (HPLC) have both been utilized previously for the separation of the enantiomers of phenethylamines [2–5]. Resolution of the optically active compounds was obtained either by derivatization with an optically active reagent with subsequent separation of the resulting diastereomers on a non-chiral stationary phase [2,4], or by direct analysis using a chiral stationary phase [3,5]. None of the reported procedures are capable of resolving the enantiomers of norephedrine, norpseudoephedrine, ephedrine, pseudoephedrine, amphetamine and methamphetamine (Fig. 1) in a single run.

One approach for the capillary electrophoretic (CE) separation of enantiomers involves the use of buffer additives such as cyclodextrin derivatives [6–8], chiral surfactants [9–12] or cyclodextrin plus

*Correspondence to:* I. S. Lurie, Drug Enforcement Administration, Special Testing and Research Laboratory, 7704 Old Springhouse Road, McLean, VA 22102-3494, USA.




| R1 | R2 | COMPOUND | ABSOLUTE CONFIGURATION |
|---|---|---|---|
| H | H | Amphetamine | 2S - (+)<br>2R - (-) |
| $CH_3$ | H | Methamphetamine | 2S - (+)<br>2R - (-) |
| $CH_3$ | OH | Ephedrine | 1R, 2S - (-)<br>1S, 2R - (+) |
| $CH_3$ | OH | Pseudoephedrine | 1R, 2R - (-)<br>1S, 2S - (+) |
| H | OH | Norephedrine | 1R, 2S - (-)<br>1S, 2R - (+) |
| H | OH | Norpseudoephedrine | 1R, 2R - (-)<br>1S, 2S - (+) |

Fig. 1. Structures, absolute configurations and optical rotations of phenethylamines examined.

chiral surfactant [13]. The use of cyclodextrin derivatives as mobile phase additives have been previously reported for the separation of the enantiomers of norephedrine, pseudoephedrine and ephedrine [6,7]. Another approach for the CE analysis of optical isomers involves the micellar electrokinetic capillary chromatographic (MECC) separation of diastereomers resulting from the reaction of enantiomers with chiral derivatizing reagents [14,15]. MECC, a form of CE developed by Terabe *et al.* [16], is capable of providing high resolution for both neutral and ionic compounds [15–18]. In this chromatographic technique, compounds may have different abilities to partition into a micelle which is retarded by electrophoretic migration. 2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) has been previously shown to be an excellent chiral derivatization reagent for the analysis of primary and secondary amines (such as phenethylamines) via HPLC [4] and the analysis of amino acids via MECC [15]. Reactions proceed fairly quickly under mild conditions and give products with high UV extinction coefficients. This increased sensitivity is particularly advantageous for MECC in view of the small optical path lengths used for the on-column UV detection.

This paper describes the MECC separation of the enantiomers of amphetamine, methamphetamine, ephedrine, pseudoephedrine, norephedrine and norpseudoephedrine. In addition the effect of various chromatographic parameters on the separation is detailed.

## EXPERIMENTAL

### *Equipment*

A Model 270A-HT capillary electrophoresis unit (Applied Biosystems, San Jose, CA, USA) interfaced with a Turbochrom 3 chromatographic data handling system (PE Nelson, Cupertino, CA, USA) was used for all CE studies.

The fused-silica capillaries (Polymicro Technologies, Scottsdale, AZ, USA) used in this study were conditioned by successively aspirating with 1 *M* sodium hydroxide 10 min, water 10 min and the run buffer 10 min. For separations employing heptakis (di-O-methyl)-β-cyclodextrin, a 57 cm × 50 μm I.D. (35 cm length to detector) capillary was used. For separations using cyclodextrin plus sodium dodecyl sulfate (SDS) and for those runs using sodium taurocholate 72 cm × 50 mm I.D. (50 cm length to detector) capillaries were used. Finally, for separations using SDS, a 48 cm × 50 μm I.D. (26 cm length to detector) capillary was used. All separations were carried out with UV detection at 210 nm and with the autosampler cooled to 4°C.

For flow injection analysis a Series 4 liquid chromatograph (Perkin-Elmer, Norwalk, CT, USA) fitted with an ISS 100 autosampler (Perkin-Elmer) and a 1040 M diode array detection system (Hewlett Packard, Waldbronn, Germany) were used.

### *Materials*

Heptakis(di-O-methyl)-β-cyclodextrin (Sigma, St. Louis, MO, USA), sodium taurocholate (Sigma) and SDS (Aldrich, Milwaukee, WI, USA) were used as received. Sodium borate, sodium phosphate (monobasic), tris(hydroxymethyl)aminomethane hydrochloride (trizma) buffer, phosphoric acid and sodium hydroxide were reagent grade. Deionized water from a Milli-Q system (Millipore, Bedford, MA, USA) was used to prepare all buffers. Methanol, acetonitrile and tetrahydrofuran were HPLC grade. GITC was obtained from Polysciences (Warrington, PA, USA). The drug standards used were

part of the reference collection of the Drug Enforcement Administration's Special Testing and Research Laboratory.

One run buffer consisted of a 0.01 *M* phosphate–borate buffer (pH 9.0) containing 0.015 *M* heptakis (di-O-methyl)-β-cyclodextrin and 100 m*M* SDS. Other run buffers consisted of a 0.01 *M* phosphate–borate buffer (pH 9.0) containing 0.05 *M* sodium taurocholate and this same solution adjusted to pH 11.7 with 1 *M* sodium hydroxide. For MECC analysis using GITC derivatives, a stock solution containing 10 m*M* borate and 10 m*M* phosphate (pH 9.0) was used for the preparation of all run buffers.

*GITC derivatization procedure*

A standard solution containing all six racemic phenethylamines was prepared by dissolving 4 mg of each substance in 1.0 ml of 50% (v/v) aqueous acetonitrile containing 0.2% triethylamine; complete solution was achieved by vortexing for 30 s. A 100-μl volume of the resulting solution was then reacted with 100 μl of (1.28%, w/v). GITC–acetonitrile solution; complete solution was achieved by vortexing for 60 s. After standing for 15 min (to complete the reaction), the solution was diluted to 1.0 ml with 0.01 *M* phosphate–borate buffer (pH 9.0) containing 100 m*M* SDS. After final vortexing for 20 s, the solution was filtered through a UniPrep filter (Whatman, Clifton, NJ, USA) and finally injected onto the capillary electrophoresis instrument using a vacuum-assisted injection of 0.5 s. This procedure is a modification of a previously described derivatization protocol [15]. The structure of GITC-derivatized amphetamine is shown in Fig. 2.

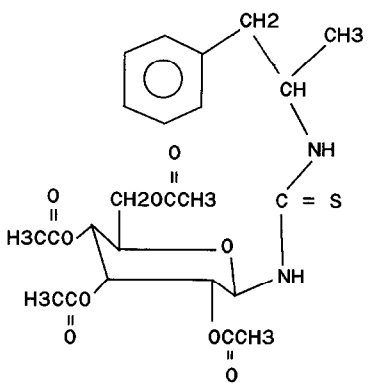



Fig. 2. Structure of GITC derivative of amphetamine.

## RESULTS AND DISCUSSION

*Use of chiral additives*

The first approach investigated for the CE separation of the enantiomers of phenethylamines involved the use of a chiral cyclodextrin derivative as a additive. Using similar conditions as Swartz [7], attempts to separate the enantiomers of amphetamine and methamphetamine using cyclodextrins proved unsuccessful. It has been reported for HPLC that three points of interaction are required between the enantiomer and the β-cyclodextrin derivative for a separation to occur [19]. It appears that a benzylic hydroxyl can provide one of these points, since the enantiomers of ephedrine, pseudoephedrine, norephedrine and norpseudoephedrine all resolve using the above mobile phase conditions. In addition, use of the chiral surfactant taurocholate in buffers of pH values 9.0 and 11.7 failed to resolve the enantiomers of ephedrine or methamphetamine (separation of other phenethylamines were not attempted). At pH 9.0 the predominantly positively charged ephedrine and methamphetamine had little affinity for the micelle and eluted before $t_0$ (neutral marker; methanol peak), while at pH 11.7 these compounds existed predominantly as the free base and had affinity for the micelle and therefore eluted after $t_0$. Finally, the use of the non-chiral micelle dodecyl sulfate with cyclodextrin at pH 9.0 failed to resolve the enantiomers of ephedrine or methamphetamine.

*MECC analysis of GITC derivatives*

*Effect of organic modifiers.* Pre-column derivatization with optically pure GITC was investigated. As shown in Fig. 3, extensive overlap existed between the compounds of interest when MECC was employed with a run buffer containing SDS and no organic modifier. The addition of an organic modifier to the buffer (Fig. 4) greatly improved the separation by increasing the peak capacity and by decreasing the micelle–water partition coefficient. Organic modifiers have been previously shown to reduce electroosmotic flow in MECC systems, thus extending elution range, and also to reduce solute capacity factors [20]. Using the MECC conditions shown in Fig. 4 methanol appears to be the modifier of choice. Using 20% methanol in the run buffer results in at least partial resolution of all

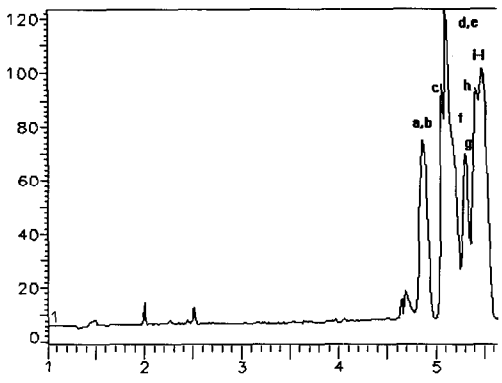



Fig. 3. MECC separation at 210 nm of phenethylamine-GITC derivatives with 100 m*M* SDS in 10 m*M* phosphate–10 m*M* borate buffer (pH 9.0) in a 48 cm (26 cm length to detector) × 50 μm capillary at 40°C with a voltage of 20 kV; current 86 μA. Peaks: a = 1*S*,2*S*-(+)-norpseudoephedrine; b = 1*R*,2*S*-(−)-ephedrine; c = 1*R*,2*S*-(−)-norephedrine; d = 1*R*,2*R*-(−)-norpseudoephedrine; e = 1*R*,2*R*-(+)-ephedrine; f = 1*S*,2*S*-(+)-pseudoephedrine; g = 1*S*,2*R*-(+)-norephedrine; h = 1*R*,2*R*-(−)-pseudoephedrine; i = 2R-(−)-methamphetamine; j = 2R-(−)-amphetamine; k = 2*S*-(+)-methamphetamine; l = 2*S*-(+)-amphetamine. Abscissa: time in min; ordinate: mAU.

enantiomers. In addition, for reasons that are presently unclear, amphetamine and methamphetamine exhibit reduced response in the presence of acetonitrile.

Since MECC is partially based on a liquid–liquid partition mechanism, it exhibits some similarity to reversed-phase HPLC. Therefore the percent organic modifier present for methanol, acetonitrile and tetrahydrofuran in the MECC separation were based on the relative percentages which gave equal solvent strength in a previously reported reversed-phase HPLC separation [21]. As shown in Fig. 4, this was a reasonable assumption for acetonitrile and tetrahydrofuran.

*Effect of percent methanol*

A study of the effect of % methanol indicates that when the run buffer contains 20% methanol, as shown in Fig. 5, all enantiomers are at least partially resolved. Amphetamine and methamphetamine again exhibit reduced response when 25% methanol is used. Changing the organic modifier concentration alters the partition coefficient as well as possibly changing the micellar size [22]. It is apparent from Figs. 4 and 5, and as was also observed by Gorse *et al.* [23], that both the type and amount of organic modifier used can alter selectivity. Interestingly, the elution order changes with different organic modifier types because partition coefficients are altered [20].

*Effect of temperature*

The effect of temperature on the separation is shown in Fig. 6. For these MECC conditions the best separation is obtained at 30°C where, except for 1*R*,2*S*-(−)-norephedrine and 1S,2*S*-(+)-pseudoephedrine, all compounds are well resolved. For reasons that are unclear it is apparent that lower

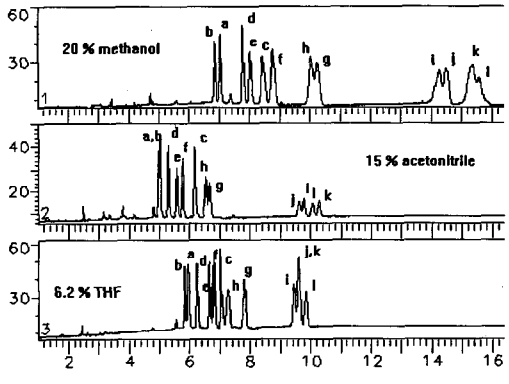



Fig. 4. Effect of organic modifier on MECC separation of phenethylamine-GITC derivatives. Conditions as in Fig. 3 except for organic modifier; methanol, current 54 μA; acetonitrile, current 88 μA; and tetrahydrofuran (THF), current 93 μA. Peaks and axes as in Fig. 3.

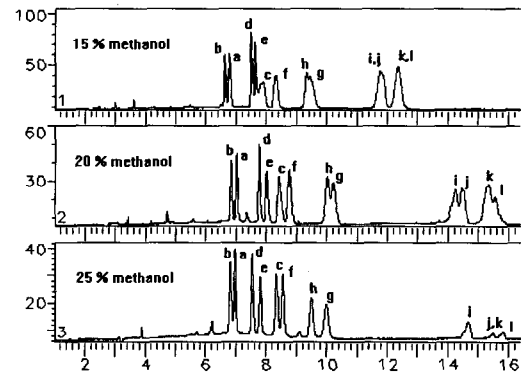



Fig. 5. Effect of methanol concentration on MECC separation of phenethylamine-GITC derivatives. Conditions as in Fig. 3 except for methanol concentration; 15% methanol, current 58 μA; 20% methanol, current 54 μA; and 25% methanol, current 45 μA. Peaks and axes as in Fig. 3.

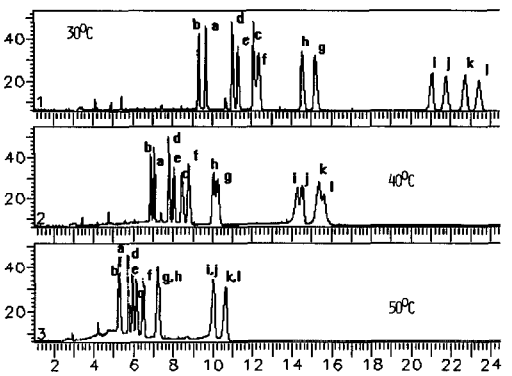



Fig. 6. Effect of temperature on MECC separation of phenethylamine-GITC derivatives. Conditions as in Fig. 3 except for temperature and the presence of 20% methanol in run buffer; 30°C, current 48 μA; 40°C, current 54 μA; and 50°C current 58 μA. Peaks and axes as in Fig. 3.

temperatures result in higher efficiencies. In fact, the efficiency (number of plates, $N$) for 2$R$-(−)-methamphetamine is 44 000, 27 000 and 22 000 at temperatures of 30, 40 and 50°C, respectively. In addition to its effect on column efficiency, temperature can also change selectivity in MECC by its effect on micelle partitioning [24]. A combination of these two factors could contribute to the overall increase in resolution at lower temperatures.

*Effect of voltage*

As shown in Fig. 7, lowering the voltage from 20 to 15 kV increases the overall resolution. Although the efficiency for 2$R$-(−)-methamphetamine decreases from 27 000 to 20 000, resolution increases for several pairs of compounds (including the late eluters) due to an apparent increase in selectivity. In describing column efficiency in MECC as related to voltage, Sepaniak and Cole [25] showed at low to moderate voltages the major contribution to plate height to be axial diffusion (which decreases with increasing voltage). The change in selectivity with voltage can be described in terms of joule heating effects inside the capillary which effect the solute distribution coefficient $K$ [24]. At 25 kV, which represents a relatively high field strength of 521 V/cm, no peaks were detected presumably due to a degradation of separation due to joule heating effects.

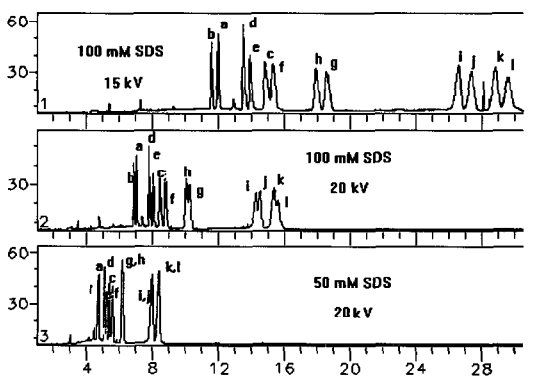



Fig. 7. Effect of voltage and SDS concentration on MECC separation of phenethylamine-GITC derivatives. Conditions as in Fig. 3 except for voltage, SDS concentration and the presence of 20% methanol in run buffer; 15 kV, 100 m$M$ SDS, current 34 μA; 20 kV, 100 m$M$ SDS, current 54 μA; and 20 kV, 50 m$M$ SDS, current 34 μA. Peaks and axes as in Fig. 3.

*Effect of SDS concentration*

In addition, as shown in Fig. 7, lowering the SDS concentration results in decreased resolution. A similar result was obtained for diastereomers of GITC-derivatized amino acids due to changes in selectivity and migration times [15]. The efficiency ($N$) of 2R-(−)-methamphetamine decreased from 27 000 to 19 600 when the SDS concentration was lowered from 100 m$M$ to 50 m$M$. Since resolution depends on the square root of $N$, it is apparent from Fig. 7 that a decrease in selectivity as well as migration times is playing a major role. The change in selectivity is due to differences in interactions of solutes with micelles, as detailed by Armstrong and Stine [26] in describing micellar pseudophase in HPLC. When an SDS concentration of 200 m$M$ was used, no peaks were detected. This was presumably due to a degradation of separation as a result of joule heating effects caused by the increased current.

*Resolution of twelve phenethylamine isomers*

Using an organic modifier concentration of 20% methanol, a temperature of 30°C, a voltage of 20 kV and a 100 m$M$ SDS concentration, all compounds are baseline resolved except for 1$R$,2$S$-(−)-norephedrine and 1$S$,2$S$-(+)-pseudoephedrine, which have a resolution of approximately 1 (see Fig. 6). This may not represent the best possible separation; however such determination is beyond the scope of this manuscript.

The use of a relatively short 48 cm capillary (26

cm length to detector) resulted in faster separations at the expense of plate number. A plate count of 44 000 for 2*R*-(−)-methamphetamine is significantly below what was reported for GITC derivatives of amino acids analyzed via MECC [15]. For these compounds, Nishi *et al.* [15] reported plate numbers in excess of 100 000 using capillary lengths of 65 cm (50 cm length to detector). Use of shorter columns resulted in a higher field strength where joule heating effects, *i.e.* temperature gradients within the column, might be expected to be responsible for the decreased plate count. However, several authors have shown the temperature gradient in the capillary to have a neglible effect on plate height [27–29].

*MECC versus HPLC*

In spite of the relatively low plate numbers obtained using the shorter capillaries, the MECC separations are still far superior to what is obtained via HPLC [4]. As shown in Fig. 8, the separation of GITC-derivatized amines via HPLC results in far less resolution and longer analysis time than obtained via MECC. For this same HPLC system, amphetamine–GITC derivatives failed to resolve in spite of a 60-min elution time. It is also of interest to compare the elution order of the individual optical isomers using HPLC *versus* MECC. For the optical isomers separated in Fig. 8, only ephedrine–GITC derivatives have the same elution order using both techniques. Since both separation techniques depend to a large extent on the hydrophobicity of the solutes, identical elution orders would not have been unexpected. Nishi *et al.* [15] reported identical elution orders via HPLC and MECC for the GITC derivatives of the optical isomers of five out of the six compounds. It would appear that mobile phase interactions such as hydrogen bonding and dipole interactions play a major role in both separation techniques. For the HPLC separation of GITC-derivatized phenethylamines, the mobile phase contains tetrahydrofuran for MECC, methanol is used as the organic modifier.

*Application to forensic exhibit*

The applicability of the MECC technique to a forensic sample is shown in Fig. 9. By comparison with the standard run shown in Fig. 6, the sample was found to contain 1*R*,2*S*-(−)-ephedrine and 2*S*-(+)-methamphetamine. This result was consistent

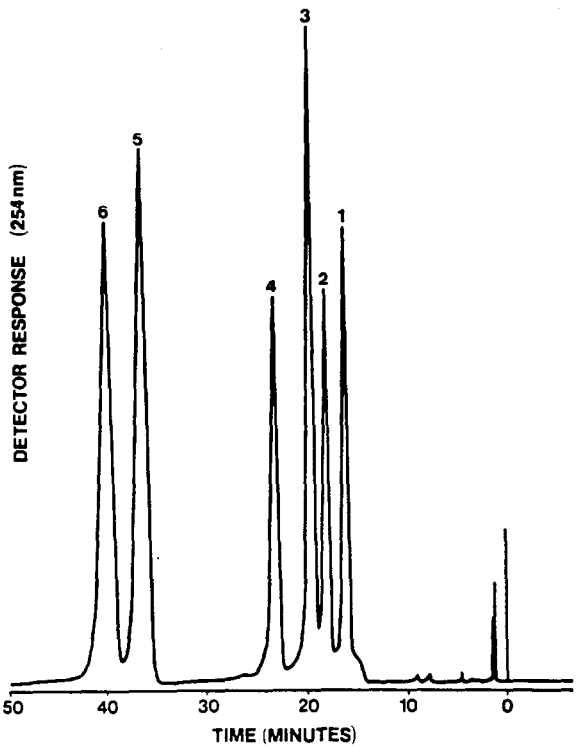



Fig. 8. HPLC separation of phenethylamine-GITC derivatized amines using tetrahydrofuran–water (3:7) mobile phase at a flow-rate of 1.5 ml/min. Peaks: 1 = 1*R*,2*R*-(−)-pseudoephedrine; 2 = 1*S*,2*S*-(+)-pseudoephedrine; 3 = 1*R*,2*S*-(−)-ephedrine; 4 = 1*S*,2*R*-(+)-ephedrine; 5 = 2*S*-(+)-methamphetamine; 6 = 2*R*-(−)-methamphetamine. From ref. 4.

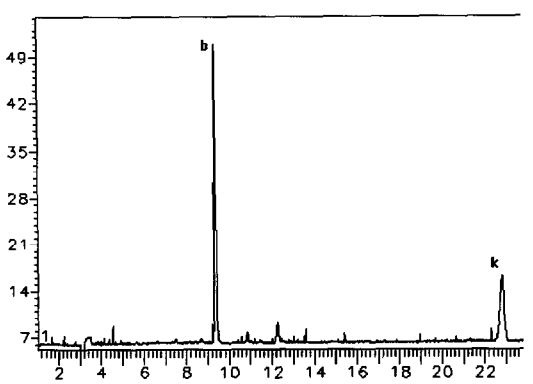



Fig. 9. MECC separation of GITC-derivatized exhibit. Conditions as in Fig. 3 except for a temperature of 30°C and the presence of 20% methanol in run buffer. Peaks and axes as in Fig. 3.

with what had been reported for this exhibit using a combination of GC-mass spectrometry and microscopic techniques.

Flow injection analysis using a diode array detector reveals that the extinction coefficient at 210 nm for the GITC derivative of methamphetamine is approximately twice that of both underivatized methamphetamine and that obtained at the UV maximum wavelength (254 nm) of the GITC derivative. For more selective detection the higher wavelength of 254 nm would be recommended since the extinction coefficient of GITC derivatized methamphetamine is approximately fifty times that of the underivatized methamphetamine. It has been previously shown that at 250 nm the molar extinction coefficient of GITC is 1000 whereas those of GITC-amino acids are approximately 12 000 [30]. Apparently the N,N′-disubstituted thiourea linkage where lone pair electrons on two amide groups can contribute to UV conjugation (see Fig. 2) is reponsible for the higher extinction coefficients of the GITC derivatives.

ACKNOWLEDGEMENTS

The author thanks Dr. Robert Weinberger (CE Technologies, Chappaqua, NY, USA) and Dr. Michael Albin (Applied Biosystems, Foster City, CA, USA) for helpful discussions. The author would also like to thank Charles Harper for the preparation of those figures containing chemical structures.

REFERENCES

1 H. F. Skinner, *Forensic Sci. Int.*, 48 (1990) 123.
2 A. H. Beckett and B. Testa, *J. Chromatogr.*, 69 (1972) 285.
3 H. Frank, G. J. Nicholson and E. Bayer, *J. Chromatogr.*, 146 (1978) 197.
4 F. T. Noggle and C. R. Clark, *J. Forensic Sci.*, 31 (1986) 732.
5 T. D. Doyle, W. M. Adams, F. S. Fry and I. W. Wainer, *J. Liq. Chromatogr.*, 9 (1986) 455.
6 S. Fanali, *J. Chromatogr.*, 474 (1989) 441.
7 M. E. Swartz, *J. Liq. Chromatogr.*, 14 (1991) 923.
8 J. Snopek, H. Soini, M. Novotny, E. Smolkova-Keulemansova and I. Jelinek, *J. Chromatogr.*, 559 (1991) 215.
9 S. Terabe, M. Shibata and Y. Miyashita, *J. Chromatogr.*, 480 (1989) 403.
10 K. Otsuka and S. Terabe, *J. Chromatogr.*, 515 (1990) 221.
11 H. Nishi, T. Fukuyama, M. Matsuo and S. Terabe, *J. Microcol. Sep.*, 1 (1989) 234.
12 K. Otsuka, J. Kawahara, K. Tatekawa and S. Terabe, *J. Chromatogr.*, 559 (1991) 209.
13 H. Nishi, T. Fukuyama and S. Terabe, *J. Chromatogr.*, 553 (1991) 503.
14 A. D. Tran, T. Blanc and E. J. Leopold. *J. Chromatogr.*, 516 (1990) 241.
15 H. Nishi, T. Fukuyama and M. Matsuo, *J. Microcol. Sep.*, 2 (1990) 234.
16 S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya and T. Ando, *Anal. Chem.*, 56 (1984) 113.
17 H. Nishi, N. Tsumagari, T. Kakimoto and S. Terabe, *J. Chromatogr.*, 465 (1989) 331.
18 R. Weinberger and I. S. Lurie, *Anal. Chem.*, 63 (1991) 63.
19 W. L. Hinze, T. E. Riehl, D. W. Armstrong, W. DeMond, A. Alak and T. Ward, *Anal. Chem.*, 57 (1985) 237.
20 A. T. Balchunas and M. J. Sepaniak, *Anal. Chem.*, 60 (1988) 617.
21 I. S. Lurie, A. C. Allen and H. J. Issaq, *J. Liq. Chromatogr.*, 7 (1984) 463.
22 A. Dobashi, T. Ono, S. Hara and J. Yamaguchi, *Anal. Chem.*, 61 (1989) 1986.
23 J. Gorse, A. T. Balchunas, D. F. Swaile and M. J. Sepaniak, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 11 (1988) 554.
24 A. S. Cohen, S. Terabe, J. A. Smith and B. L. Karger, *Anal. Chem.*, 59 (1987) 1021.
25 M. J. Sepaniak and R. O. Cole, *Anal. Chem.*, 59 (1987) 472.
26 D. W. Armstrong and G. Y. Stine, *Anal. Chem.*, 55 (1983) 2317.
27 J. H. Knox, *Chromatographia*, 26 (1988) 329.
28 E. Grushka, R. M. McCormick and J. J. Kirkland, *Anal. Chem.*, 61 (1989) 241.
29 S. Terabe, K. Otsuka and T. Ando, *Anal. Chem.*, 61 (1989) 251.
30 N. Nimura, H. Ogura and T. Kinoshita, *J. Chromatogr.*, 202 (1980) 375.