## **Short communications**

# Kurze Mitteilungen

# Separation of enantiomers of amphetamine and related amines by HPLC

### J. Pfordt

Institut für Rechtsmedizin der Universität, Versbacher Straße 3, D-8700 Würzburg, Federal Republic of Germany

Trennung von Enantiomeren von Amphetamin und verwandten Aminen durch HPLC

### Introduction

The separation of enantiomers by liquid chromatographic methods can be achieved applying different strategies. Chiral stationary and mobile phases have been successfully employed; derivatization with chiral derivatizing reagents prior to chromatography is also a widespread technique.

One of the compounds whose enantiomeric separation is of special forensic interest is amphetamine, because pure enantiomers and racemic mixtures are treated differently by national and international drug laws.

Thus, a number of separation methods have been developed for amphetamine and related amines; most of them include a derivatization step yielding diastereomeric derivatives. For example, N-acyl-L-prolyl chlorides were reported as reagents prior to HPLC [1, 2] or capillary GC [3, 4] separations.

However, most of these compounds are sensitive to moisture and require precautions during storage and derivatization to avoid hydrolysis. Furthermore, most of them are not commercially available.

d-Camphor-10-sulfonyl chloride is a reagent lacking the high sensitivity to moisture which has been successfully used for the separation of enantiomeric amino acids [5]. Its application to amphetamines and ephedrines is reported here.

### Materials and methods

Reagents. d-Camphor-10-sulfonyl chloride was purchased from Fluka (Buchs, Switzerland) and stored at  $+4^{\circ}$ C. d- and l-amphetamine, d- and l-ephedrine, and d- and l-pseudoephedrine were of 95–99% purity and were employed without further purification. All solvents were of analytical grade. Diethyl ether was distilled before use.

Sample preparation. 0.2 mmol amine (hydrochloride or free base), 0.5 mmol d-camphor-10-sulfonyl chloride, 10 ml ether and 10 ml aqueous NaOH (1N) were vigorously stirred for one hour at ambient temperature. After acidification with 1N HCl, the reaction mixture was twice extracted with ether. The combined organic layers were washed with  $\rm H_2O$ , dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated. The residue was taken up in methanol (100  $\mu$ l), and aliquots were submitted to liquid chromatography.

Chromatography. The HPLC equipment consisted of a Perkin-Elmer Series 4 liquid chromatograph (Überlingen, FRG) with a Perkin-Elmer LC-75 UV detector, a Rheodyne No. 7125 S sample valve, and a 6  $\mu l$  injection loop. The camphor sulfonamides were separated on 25 cm  $\times$  4.6 mm I. D. columns with  $C_{18}$  or  $C_8$  reversed-phase, chemically bonded to 10  $\mu m$  silica (C-18-SIL-X-10 and C-8-SIL-X-10, Perkin-Elmer). Mixtures of methanol and water were used as mobile phase. The flow rate was 1.5 ml/min, the detector was operated at 260 nm. All analyses were performed at ambient temperature. A Sigma 10 data system (Perkin-Elmer) was used for recording

Present address: Staatliches Chemisches Untersuchungsamt, Philosophenweg 36, D-2900 Oldenburg, Federal Republic of Germany

# A<sub>260</sub> 1 2 5 10 15 20 t(min)

Fig. 1. Chromatogram of the d-camphor-sulfonyl derivatives of amphetamine on a  $C_{18}$  column. Mobile phase: methanol-water 65:35 (v/v) (1 = l-form, 2 = d-form)

Table 1. Separation of the d-camphor-sulfonyl derivatives of amphetamine on  $C_{18}$  reversed-phase columns

k' (l-form)	k' (d-form)	α
1.95	2.01	1.03
2.91	3.15	1.08
4.81	5.36	1.11
7.75	8.83	1.14
12.38	14.40	1.16
20.66	24.51	1.19
	1.95 2.91 4.81 7.75 12.38	2.91 3.15 4.81 5.36 7.75 8.83 12.38 14.40

**Table 2.** Separation of the d-camphor-sulfonyl derivatives of amphetamine on  $C_s$  reversed-phase columns

Mobile phase	k' (l-form)	k' (d-form)	α
80% CH <sub>3</sub> OH - 20% H <sub>2</sub> O	1.26	1.26	1
$70\% \text{ CH}_3\text{OH} - 30\% \text{ H}_2\text{O}$	2.26	2.32	1.03
$65\% \text{ CH}_3\text{OH} - 35\% \text{ H}_2\text{O}$	3.64	3.90	1.07
$60\% \text{ CH}_3\text{OH} - 40\% \text{ H}_2\text{O}$	6.13	6.72	1.10

chromatograms and retention data. Capacity factors k' and separation factors  $\alpha$  were calculated in the usual manner.

### Results and discussion

Amphetamine and related amines can be derivatized with dcamphor-10-sulfonyl chloride as described for amino acids [5]. As the aromatic ring system of the amines is suited for UV detection, the additional introduction of chromophoric groups (as is usual when derivatizing amino acids) is unnecessary. No racemization of the amines was observed under the reaction conditions described.

Figure 1 shows the resolution of the enantiomers of amphetamine as their camphor-sulfonyl derivatives. With pure enantiomers, the d-form possessed the higher k' value. The capacity factors and separation factors that were obtained when the separation was performed on a RP-18 column with mixtures of methanol and water as mobile phase are summarized in Table 1. With RP-8 instead of RP-18 columns the degree of separation was decreased (cf. Table 2).

625