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Separation of amphetamines by supercritical fluid chromatography

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ABSTRACT

The separation of polar compounds by supercritical fluid chromatography (SFC) is difficult, especially with amine compounds. In this study a derivatization method was used to obtain apolar compounds and also to block the amine functions. The target compounds were amphetamines and the derivatizing agent was the 9-fluorenylmethyl chloroformate. This reagent reacts with primary and secondary amines to form UV-absorbing apolar complexes which permits selective and sensitive detection methods. This method allowed the SFC separation of five amphetamines in less than 5 min.

INTRODUCTION

Supercritical fluid chromatography (SFC) with packed or capillary columns has been developed for the analysis of organic apolar and moderatly polar compounds with carbon dioxide as mobile phase[1], but only a few results have been reported for polar compounds and particularly basic compounds[2], owing to the apolar nature of carbon dioxide. However, the analysis of amines by chromatography is a challenge, as gas chromatography (GC) is limited to volatile and thermally stable compounds and high-performance liquid chromatography (HPLC) involves a long analysis time and lacks a universal detector. SFC could be an efficient alternative technique for the analysis of these compounds[3], because it offers faster separations and higher efficiencies per unit time than HPLC.

The separation of primary and secondary amines is difficult by SFC with carbon dioxide as the mobile phase on a packed column for two reasons: amines react with carbon dioxide and a strong silanol–analyte interaction induces significant peak tailing. There are various ways of overcoming these difficulties. (1) The most commonly used method is the addition of a polar modifier to mask the silanol groups and to enhance the solvent polarity. However, when this is done, SFC may no longer have an advantage over HPLC, and in many instances separation is achieved by subcritical chromatography[4]. (2) The use of columns specially prepared for the separation of basic compounds, such as polymer-encapsulated stationary phases with

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aliphatic groups[5] or cross-linked cyanopropyl-bonded phase silica[6], eliminates the silanol–amine interactions and improves the separation efficiency, but it does not eliminate the carbon dioxide–amine reaction. (3) A polar supercritical fluid, such as ammonia, has been used instead of carbon dioxide, but it requires stationary phases such as *n*-octyl or *n*-nonyl polysiloxane and a special device (sample introduction valve equipped with a rotor made of Valcon H material, ferrules in graphite or Kel-F material, etc.) to work with such a corrosive medium[7–9]. Sulphur hexafluoride (SF_6), alone[10–12] or mixed with ammonia[13] has not been used extensively, owing to its weak solvating power. (4) The derivatization of polar to apolar or less polar compounds by masking the ionizable functions would permit the use of conventional packed columns with carbon dioxide as supercritical fluid[14,15].

In this study, the last-mentioned procedure was chosen for the separation of amphetamines by SFC. The continuing abuse of amphetamine and related compounds as stimulants has led to the development of many GC and HPLC for their determination. Currently, HPLC is the most commonly used technique for amphetamine analysis, as the sample preparation with aqueous samples is not laborious. In order to overcome the weak UV absorbance and the slight natural fluorescence of amphetamines, several derivatization procedures have been reported[16,17]. Both primary and secondary amines react with many reagents, which permits selective and sensitive detection methods. These derivatization procedures can be employed before SFC analysis. The aim of this study was to use 9-fluorenylmethyl chloroformate (FMOC-Cl) as a derivatizing agent for the separation of amphetamines before SFC separation to improve the chromatographic performance and the resolution per unit time relative to conventional HPLC procedures.

EXPERIMENTAL

Apparatus

The apparatus used for this study included a Varian 2500 chromatograph (Varian, Palo Alto, CA, U.S.A.), modified for SFC operations. Cooling of the pump head with cold ethanol (0°C) is necessary to improve the pump efficiency. Temperature control of the fluid and the chromatographic columns was achieved by using an oven (Crocosil; Varian). The injector was a Rheodyne 7125 six-way switching valve with a 10-μl loop. A Varian UV 2550 spectrophotometer was used with a detection cell modified in order to withstand pressures up to 350 bar. The pressure in the system was monitored by a back-pressure regulator (Model 26-3220-24004; Tescom, Minneapolis, MN, U.S.A.). A Kontron (Zurich, Switzerland) Model 414-T pump was used as a modifier pump.

Materials

The carbon dioxide (technical grade) was contained in a cylinder with an eductor tube (Polygaz, Geneva, Switzerland). Methanol, 2-propanol and acetonitrile were of HPLC grade (Romil, Shepshed, U.K.). Solvent mixing of the cabon dioxide and the modifier was accomplished by using a static mixer, incorporated in the liquid chromatograph.

The chromatographic columns were stainless-steel columns (30 cm × 0.39 cm I.D.) packed with Hypersil ODS (10 μm) and Hypersil APS (5 μm) (Shadon, Runcorn,

Cheshire, U.K.) and a commercial Nucleosil-100 bare silica (5 μm) (20 cm × 0.4 cm I.D.) (Macherey, Nagel & Co., Duren, F.R.G.).

Methylamphetamine chlorohydrate and amphetamine sulphate were obtained from Sigma (St. Louis, MO, U.S.A.) and phenethylamine, ephedrine, norephedrine and FMOC-Cl from Fluka (Buchs, Switzerland). Stock solutions of each amphetamine were 10^{-2} *M* in 0.1 *M* hydrochloric acid and were stored in the dark at 5°C. A stock solution of 150 mg of FMOC-Cl in 100 ml of acetone was stored at 5°C.

Derivatization procedure

The derivatization procedure for the amino acids and the amines has been described in detail by Einarsson and co-workers[18,19]. The sample (250 μl), buffered at pH 9.50 (below pH 9.0 the derivatization of amphetamines is incomplete), was mixed with 250 μl of the FMOC-Cl solution, placed in a 1-ml reaction vial and allowed to react for 10 min, then extracted with dichloromethane. The organic layer, containing the FMOC–amphetamine complexes, was injected into the HPLC system.

RESULTS AND DISCUSSION

In a previous study[14], amino acids were derivatized with FMOC-Cl reagent prior to SFC separation. Here, this reagent was used for amine separation, in particular for the amphetamines, where it forms an apolar complex that can be readily eluted with a supercritical mobile phase. The extraction procedure was modified so as to collect the complex in the organic layer. Dichloromethane was chosen as the solvent, owing to its higher density than water (so no evaporation occurs during the experiment) and its compatibility with carbon dioxide.

Chromatography was performed on bare silica octadecyl-bonded silica and aminopropyl-bonded silica with methanol, 2-propanol and acetonitrile as polar modifiers.

Influence of polar modifier

The results obtained on the three columns showed that a polar modifier is necessary to elute the FMOC–amphetamine complexes, otherwise no elution occurs. The capacity factors decrease with increasing percentage of the polar modifier in carbon dioxide. Methanol was the modifier that increased the polarity of the mobile phase most and yielded the fastest elution times of amphetamine complexes. A methanol concentration in carbon dioxide of 2.4% (v/v) is sufficient to elute and resolve a mixture of five amphetamines in less than 5 min on a bare silica, as shown in Fig. 1. Under the same conditions, 2-propanol has a lower eluting power; further, methylamphetamine and amphetamine are not resolved, even with a low percentage of modifier, as shown in Fig. 2. Acetonitrile was also tested as a modifier, but poor results were obtained (band broadening, long retention times and poor efficiency). These results indicate that modifiers increase the solubilities of solutes in the mobile phase according to their eluting strength (methanol 0.73, 2-propanol 0.63, acetonitrile 0.50)[20]. Further, as shown previously[14], modifiers also play a role as silanol masking agents.

The effects of pressure and temperature were also investigated to modify the mobile phase density (in the presence of a polar modifier). The capacity factors of all

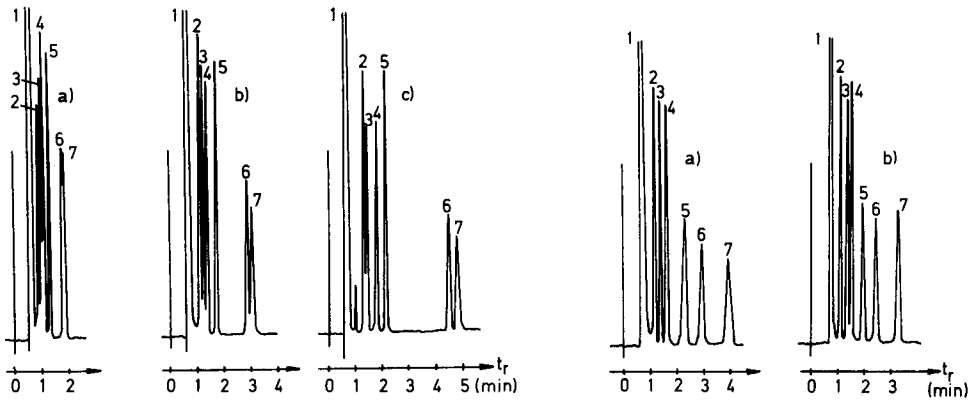

Fig. 1. Separation of five FMOC–amphetamines on Nucleosil-100 (5 μm) bare silica (15 cm × 0.4 cm I.D.) as a function of the percentage of methanol in CO_2: (a) 7.0%; (b) 4.8%; (c) 2.4%. CO_2 flow-rate, 4 ml/min; mean pressure, 200 bar; temperature, 40°C; detector, 269 nm. Solutes: 1 = acetone; 2 = methylamphetamine; 3 = amphetamine; 4 = phenethylamine; 5 = FMOC-Cl; 6 = ephedrine; 7 = norephedrine.

Fig. 2. Separation of five FMOC–amphetamines on bare silica as a function of the percentage of 2-propanol in CO_2: (a) 7.0%; (b) 4.8%. Other conditions and solutes as in Fig. 1.

the solutes were found to decrease with increasing density, whereas the selectivity remained changed. Hence the mobile phase density has a slight influence on the solvating power in comparison with the nature and concentration of the polar modifier.

Influence of stationary phase

As mentioned earlier, satisfactory separations of amphetamines are obtained on bare silica with methanol or 2-propanol as polar modifiers. A comparison of these results with those of HPLC[16,17], obtained with derivatization, on bare and reversed-phase silicas shows that SFC separation gives a shorter analytical time (3 min instead of 10 min to separate the five amphetamines) and a higher selectivity per unit time (*i.e.*, α divided by the mean retention times), as shown in Table I. On octadecyl-bonded silica, all compounds are eluted very rapidly by SFC, and no resolution was obtained even with 2-propanol as polar modifier. This result may be explained by the smaller number of silanol groups available on this stationary phase.

Aminopropyl-bonded silica gave results similar to those obtained with bare silica and good amphetamine separations. In this instance, with both modifiers (methanol and 2-propanol), all compounds were eluted and resolved in less than 4 min, as shown in Fig. 3. Further, in comparison with results obtained on bare silica, ephedrine and norephedrine are better separated (Table I). The amino functions bonded to the silica induce an additional interaction with the hydroxyl groups of ephedrine and norephedrine. Thus, aminopropyl silica is preferably used for ephedrine–norephedrine separation; otherwise, bare silica and methanol give efficient and rapid separations, as shown previously.

The order of elution is identical on both stationary phases and follows the adsorption energies, as in normal-phase liquid chromatography[20]; only the selec-

TABLE I

RETENTION TIMES (t_r) IN MINUTES AND SELECTIVITIES (α) OF AMPHETAMINES, DERIVATIZED WITH FMOC-Cl, AS A FUNCTION OF THE STATIONARY PHASE AND THE POLAR MODIFIER UNDER OPTIMUM CHROMATOGRAPHIC CONDITIONS

Optimum conditions: CO_2 flow-rate, 4 ml/min; modifier concentration, 4.8%; mean pressure, 200 bar; temperature, 40°C; detection, 269 nm.

Solute[a]	*Parameter*	*Bare silica*		*Aminopropyl silica*	
		Methanol	*2-Propanol*	*Methanol*	*2-Propanol*
MA	t_r	1.12	1.25	1.10	1.33
	α	1.21	1.00	1.66	1.55
AMP	t_r	1.22	1.25	1.35	1.64
	α	1.28	1.45	1.24	1.38
PEA	t_r	1.38	1.50	1.50	1.97
	α	3.05	3.81	2.15	2.77
E	t_r	2.88	3.75	2.40	4.09
	α	1.07	1.06	1.48	1.60
NE	t_r	3.03	3.92	3.20	6.09

[a] MA = methylamphetamine; AMP = amphetamine; PEA = phenethylamine; E = ephedrine; NE = norephedrine.

tivities are slightly different, as shown in Table I. This may be due to the fact that the functional group bonded to the silica induces a steric hindrance of some silanol functions and modifies slightly the main silanol–analyte interaction.

These findings indicate that the separation of amphetamines by SFC is governed principally by an interaction with the silanol groups of the silica, which can be partically hindered with a functional group or masked by a polar modifier. Polar

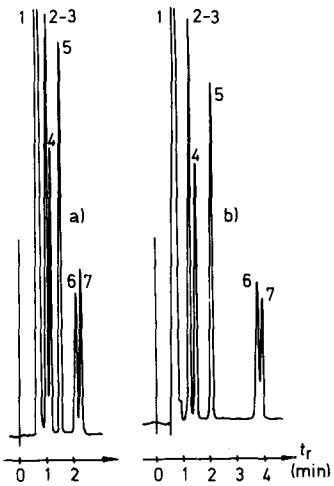

Fig. 3. Separation of five FMOC–amphetamines on Hypersil APS (5 μm) aminopropyl-bonded silica (30 cm × 0.39 cm I.D.) with (a) 7.0% of 2-propanol and (b) 4.8% of methanol. Other conditions and solutes as in Fig. 1.

modifiers also play the role of solubilizing agents for the complexes in the mobile phase.

All chromatograms show the presence of a FMOC-Cl excess peak. This excess can be easily removed by adding a hydrophilic amino acid, such as valine, which consumes this reagent during the derivatization process. The FMOC–valine charged complex may then be extracted in the aqueous layer. The injection of the aqueous solution shows that FMOC–amphetamine complex peaks are absent in this layer. This procedure was not used routinely, because the FMOC-Cl peak does not interfere with any amphetamine peaks.

In conclusion, these preliminary results obtained for amphetamine separations by SFC are satisfactory. Very rapid, efficient elution (no peak tailing) of the uncharged FMOC–amphetamine complexes, on conventional polar silicas is possible under supercritical conditions. The derivatization of amines to apolar complexes therefore has great potential in SFC. These qualitative results must now be developed for quantitative analyses.

The FMOC-Cl reagent was chosen because of the simple derivatization procedure and because, on addition of a methyl group, it becomes a chiral reagent, which is commercially available as FLEC[14]. Work is now in progress to separate amphetamine enantiomers in the same way.

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