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Short Communication

Capillary supercritical fluid chromatography of primary aliphatic amines using carbon dioxide and nitrous oxide as mobile phases

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ABSTRACT

The chromatographic behaviour of primary aliphatic amines on different capillary columns (OV-1, SB-phenyl-5 and SB-biphenyl-30) is described. The best performance was obtained using the OV-1 column; however, this column showed some peak tailing and losses probably due to adsorption effects. The primary amines heptylamine, undecylamine, tetradecylamine and stearylamine with $k'_{eff} \approx 4 \cdot 10^{-4}$ were eluted from the columns using both carbon dioxide and nitrous oxide as mobile phase, and no difference in chromatographic performance was observed using either mobile phase. The use of flame ionization detection using nitrous oxide as mobile phase is discussed.

INTRODUCTION

In supercritical fluid chromatography (SFC), carbon dioxide is by far the most commonly used mobile phase. Carbon dioxide has been utilized as the mobile phase for a wide variety of compounds but has been shown to be less suitable for polar compounds. It has been claimed that carbon dioxide cannot be used as a mobile phase for the elution of primary amines because of the reaction of carbon dioxide with basic compounds like amines [1,2]. A similar conclusion has been drawn from the results of extractions with supercritical carbon dioxide and nitrous oxide [3]. Some primary amines have, however, been successfully chromatographed using nitrous oxide [4] and sulphur hexafluoride [2] as eluents. Aromatic amines and tertiary aliphatic amines have been reported to be readily eluted from packed columns and capillary columns using neat carbon dioxide [5,6]. The rather low polarity of supercritical carbon dioxide limits its use as a mobile phase for more polar compounds. Nitrous oxide has a permanent dipole moment and is therefore expected to be a better solvent for these compounds. The results from a group separation of crude oil indicated that nitrous oxide was a stronger eluent than carbon dioxide for aromatics

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and polar compounds [7]. However, it has also been shown that supercritical carbon dioxide and nitrous oxide have nearly identical solvation properties and elute most solutes in the same range of densities [8,9].

The aim of this study was to investigate to what extent the rather non-polar fluids nitrous oxide and carbon dioxide can be used for the chromatography of polar compounds, especially primary and secondary amines, on capillary columns. When both carbon dioxide and nitrous oxide are used as the mobile phase, flame ionization detection (FID) can be utilized, even though it is known that nitrous oxide gives a high background signal with this type of detection [3,7,10,11]. It was found that aliphatic amines, also primary aliphatic amines, could be eluted from capillary columns with neat carbon dioxide as well as with nitrous oxide as the mobile phase. The chromatographic behaviour of primary aliphatic amines on different stationary phases is reported, and the possibility of using capillary SFC for the analysis of these compounds is discussed.

EXPERIMENTAL

Equipment

The SFC system consisted of a Model 602 supercritical fluid chromatograph from Lee Scientific (Salt Lake City, UT, USA). This instrument was equipped with a Valco C14W injector with a 200-nl loop; the injector could be operated with timed split injection. In addition, a 500 μm I.D. dynamic splitter [12] was installed inside the oven. Injections were performed at or slightly above ambient temperature, while dynamic splitting occurred under supercritical conditions. The columns were installed into the dynamic splitter at a position 2–3 mm below the injector rotor. The restrictor utilized was a 50-μm frit restrictor (Lee Scientific), while the dynamic split restrictor was either an integral restrictor [13] made from 50 μm I.D. fused silica or a linear restrictor made from 10 μm I.D. fused silica. The split restrictor was heated by an extra heating unit (copper block) with temperature control (Eurotherm, Worthing, UK). The heating unit was installed outside the oven (lower left side). The flame ionization detector was operated at 325°C, and normal flow-rates of hydrogen, air and make-up gas (nitrogen) were utilized. Peak-area determinations were accomplished using Multichrom software (VG Lab. System) on a Micro PDP 11/73 (Digital).

Columns

The following columns were utilized: a 10 m × 50 μm I.D. (0.2 μm film thickness) OV-1 (dimethyl silicone) from Rescom (Kortrijk, Belgium), a 5 m × 50 μm I.D. (0.25 μm film thickness) SB-phenyl-5 (5% phenyl, 95% methyl polysiloxane) and a 10 m × 50 μm I.D. (0.25 μm film thickness) SB-biphenyl-30 (30% biphenyl, 70% methyl polysiloxane) from Lee Scientific.

Materials

Carbon dioxide (grade 5.0) was obtained from Aga Norgas (Oslo, Norway), while nitrous oxide (SFC grade) was obtained from Scott Specialty Gases (Plumstedville, PA, USA).

The standards were obtained from different commercial sources and were dissolved at a concentration of 1 mg/ml unless otherwise stated. The solvents used were

high-performance liquid chromatography grade chloroform from Rathburn (Walkerburn, UK) and carbon disulphide (analyzed reagent) from J. T. Baker (Deventer, Netherlands).

RESULTS AND DISCUSSION

Nitrous oxide was the mobile phase of choice since it was expected to give better solvation of polar compounds than carbon dioxide and since it has been argued that primary amines react with carbon dioxide. The elution of the primary aliphatic amines was examined on three different stationary phases. The main conclusion was that the best results with respect to peak shape were obtained with the methyl column. However, the peak shape of a primary amine was inferior to that of a tertiary amine and a normal alkane, as shown in Fig. 1A.

It has been observed [14] that primary aliphatic amines can be eluted from a glass capillary column using neat carbon dioxide as the mobile phase. Therefore the

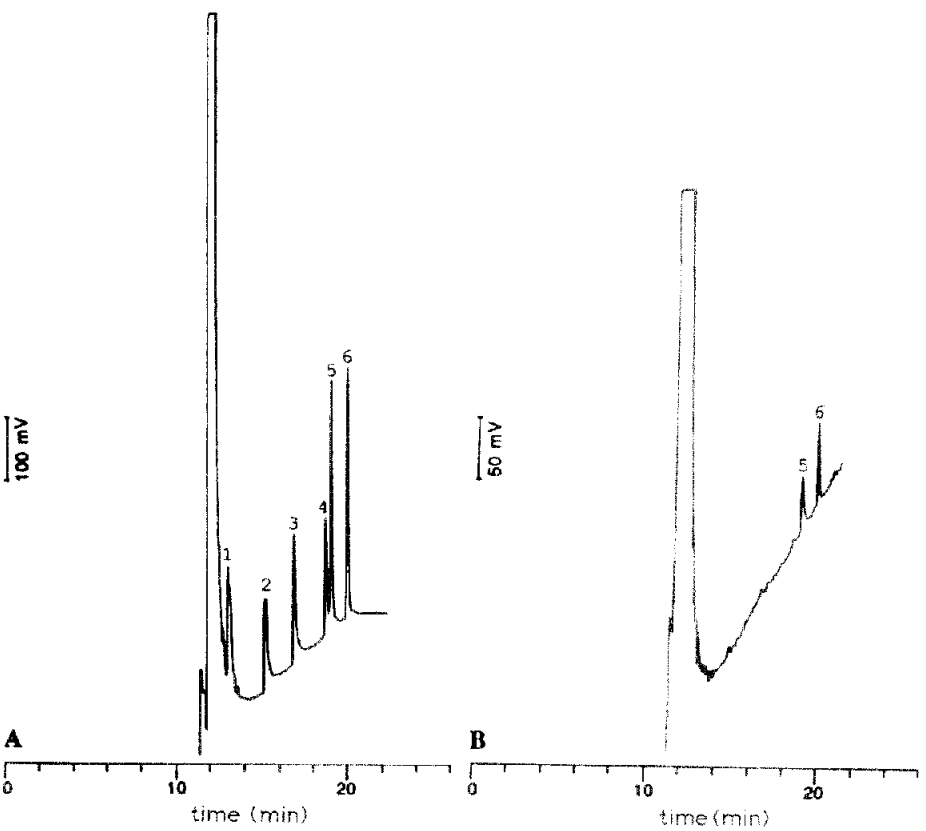

Fig. 1. Separation of heptylamine (1), undecylamine (2), tetradecylamine (3), stearylamine (4), trioctylamine (5) and NP-25 (6) on a OV-1 methyl column using nitrous oxide as the mobile phase at a column temperature of 70°C and using the following pressure programme: 90 bar for 10 min followed by 90 to 210 bar at 10 bar/min. About 15 ng (A) and 2.5 ng (B) of each component were injected onto the column.

same mixture as above was chromatographed with carbon dioxide as the mobile phase. The resulting chromatogram (Fig. 2A) resembled the results obtained with nitrous oxide. The amount injected was the same in both cases, and was about 15 ng of each compound (on column). It has been argued [15] that the low recovery of primary aliphatic amines on capillary columns using carbon dioxide as the mobile phase is due to the reaction with carbon dioxide. The peak areas of undecylamine and tetradecylamine were compared with the peak area of the normal alkane NP-25 in both nitrous oxide and carbon dioxide. The same amount of each compound was injected, however the peak areas of the two primary amines were both only about 70% of the peak area of NP-25. Even though we expect a lower FID response of the nitrogen-containing amines compared with a normal alkane [16], the whole difference cannot be explained by this. Therefore there must be some loss of the primary amines in the chromatographic system. Interestingly the same ratio was found in both carbon dioxide and nitrous oxide. We therefore concluded that the lower recovery was not caused by any reaction with carbon dioxide, but rather may be caused by losses to the column or possibly the injector. When a 1:10 diluted solution of the same mixture was chromatographed at the same conditions, the peak heights for the primary amines became very low compared with those of the tertiary amine and NP-25 (Fig. 2B). The

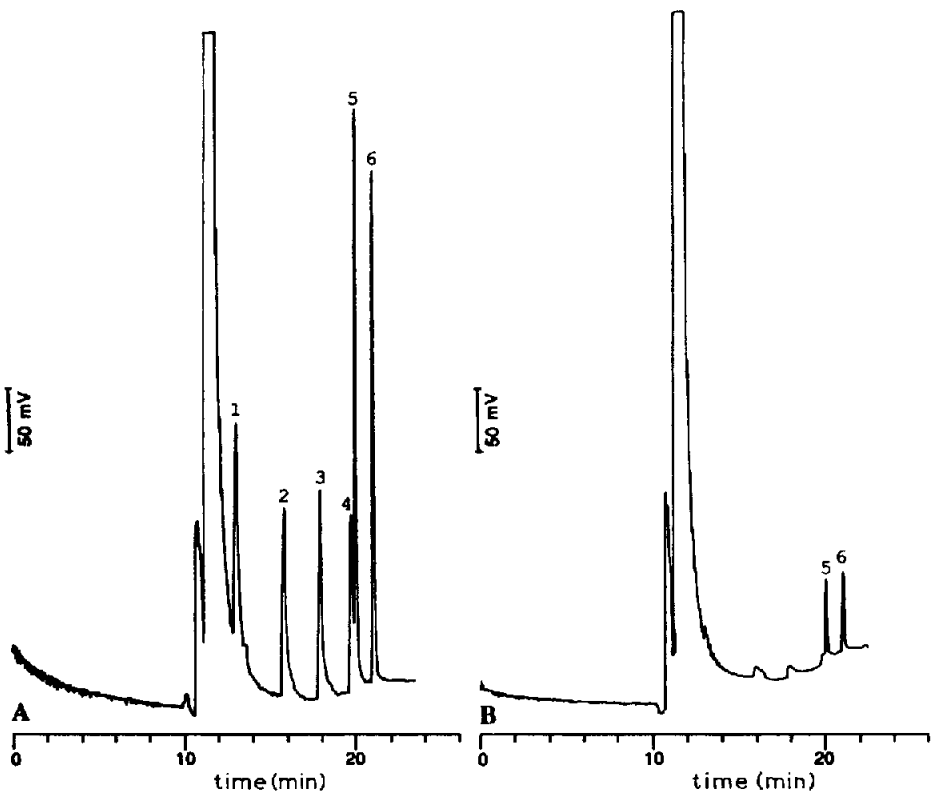

Fig. 2. Separation of heptylamine (1), undecylamine (2), tetradecylamine (3), stearylamine (4), trioctylamine (5) and NP-25 (6) on a OV-1 methyl column using carbon dioxide as mobile phase. Conditions and labeling as in Fig. 1.

same result was obtained with nitrous oxide as the mobile phase (Fig. 1B). Unfortunately the high background level with nitrous oxide prevented easy integration of the peaks at the low concentration (Fig. 1B), but from the inspection of the chromatograms it seems likely that the same reduction in peak height, area was found with both mobile phases. The high background level with nitrous oxide as the mobile phase is due to nitrous oxide itself [11]. The background level became smaller when the amount of hydrogen to the flame ionization detector was reduced, however the signals for the injected compounds also decreased, giving no overall gain in sensitivity. The mass sensitivity of the detector with nitrous oxide as the mobile phase was checked by determining the peak areas of a series of *n*-alkanes using gradient elution and comparing the results with a similar gradient using carbon dioxide as mobile phase. Identical relative areas confirmed the mass sensitivity of the flame ionization detector using gradients of nitrous oxide as the mobile phase. The detection limit with nitrous oxide was about ten times higher than with carbon dioxide at the mobile phase flow-rates used in this study.

Since the peak heights of the primary amines relative to the peak heights of NP-25 became smaller when the injected amount became smaller, we anticipate that this is caused by adsorption effects, most probably to active sites in the column. That this adsorption effect is most probably caused by the column and not by the injector or the mobile phase is also indicated by the fact that different peak shapes were observed on the different columns examined. Both the methyl column and the biphenyl column were new, while the phenyl column had been used for some time before this study was started. Better peak shapes were also obtained on the methyl column than on the phenyl column for a mixture of chlorinated phenols. Therefore adsorption onto active sites in the column is the most likely reason for the peak tailing and loss of primary amines. However, reaction of small amines with carbon dioxide cannot be ruled out. Pyrrole ($K_b \approx 10^{-13}$) was eluted with very short retention time on the methyl column with carbon dioxide as the mobile phase and could be detected when carbon disulphide was used as solvent instead of chloroform. However, pyrrolidine ($K_b \approx 10^{-3}$) could not be detected. This compound has been reported to react with carbon dioxide [17], and also reacts rather violently with carbon disulphide. The primary amine butylamine could not be detected when injected on the methyl column. This may be due to the short retention time but is most probably due to reaction with carbon dioxide, since this compound also reacts with carbon disulphide (at a concentration of 25 mg/ml). Thus we cannot rule out the possibility that some primary amines react with carbon dioxide. It has been argued that the reaction between carbon dioxide and primary and secondary amines requires a carbon dioxide:amine ratio of less than 1:2 [18]. However, in chromatography the carbon dioxide:amine ratio is usually not favourable for carbamate formation. Owing to the somewhat unpredictable behaviour of the different amines, authentic reference materials should always be utilized when using this method. For successful chromatography it is also important that the free amines are injected. After desalting, the primary amine phenylpropanolamine was eluted from the phenyl column, as a rather broad peak, using either mobile phase, while this compound could not be detected when the salt form of the amine was used for injection. The same was observed for the secondary amine propranolol. The peak shape of the free base of propranolol was quite reasonable using either mobile phase on this column. These compounds are relatively polar compounds and solubility problems in the mobile phases can also be expected.

Unfortunately, the methyl column, which initially showed the best performance for the separation of primary amines, rather quickly (after more than 50 injections of amines and other compounds) deteriorated with respect to the chromatographic behaviour of the primary amines. The peaks became smaller, with more tailing, relative to the chromatograms shown in Figs. 1 and 2, even at the level of 15 ng injected on the column. At the same time no difference in column efficiency was detected for normal paraffins and chlorinated phenols. Thus, the possible explanation may be an increase in accessible active sites in the column either because of a small but immeasurable stripping of the stationary phase or because of absorption of compounds onto the stationary phase. It has also been observed by others [2] that the column lifetime may be important for the successful chromatography of amines. Recently, Gyllenhaal and Vessman [19] examined the chromatographic behaviour of some aliphatic amines using carbon dioxide and nitrous oxide as mobile phases. They concluded that a 5% phenyl methyl siloxane column was superior to a 25% cyanomethyl siloxane and a Carbowax 20M column for these compounds because of its greater inertness. However, poor and varying symmetry was found for the primary aliphatic amine octylamine on this column, which had a film thickness of 0.5 μm.

The conclusion from this work is that primary aliphatic amines such as heptylamine and larger (with $K_b \sim 4 \cdot 10^{-4}$) can be chromatographed with carbon dioxide as well as nitrous oxide as the mobile phase on rather non-polar capillary columns. However, peak tailing and losses, probably due to adsorption onto active sites in the columns, indicate that this system is not well suited for quantitative analysis of small amounts of amines. A better deactivated column and/or a thicker film could perhaps provide a better chromatographic performance.

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