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Analysis of triazine and triazole herbicides by gradient-elution supercritical fluid chromatography

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SUMMARY

A mixture of substituted triazine and triazole herbicides is separated on an analytical-scale column by gradient elution using supercritical fluid CO_2 with increasing methanol content and flow-rate. High efficiency and fast analysis times are achieved with complete resolution of all the compounds. The effect of CO_2 flow-rate on the separation has been studied and it is shown that an increase in the overall mobile phase flow-rate by a factor of two reduces the analysis time drastically with no loss in resolution. Also, the effects of the oven temperature and the column outlet pressure on the separation have been studied.

INTRODUCTION

Triazines and triazoles are widely used as herbicides and fungicides. Several methods have been developed for the analysis of triazines by both gas chromatography (GC) and high-performance liquid chromatography (HPLC)[1–3]. GC with flame based detectors and GC coupled with spectroscopic detection have been employed for the analysis of volatile components[4–11]. HPLC methods, on the other hand, have been applied to the analysis of high molecular weight and thermally labile herbicides and metabolites[12–17]. Ultraviolet and mass spectrometric (MS) detection in both the positive and negative ion modes have been used for triazine detection. Positive-ion thermospray MS detection appears to be the most common MS method for LC effluent containing triazines.

Supercritical fluid chromatography (SFC) has gained popularity for the analysis of various classes of compounds of industrial interest. Specifically, SFC–MS has been employed to analyze pesticides with a basic triazine structure. In this regard, six triazine derivatives were separated on an analytical-scale packed column with methanol-modified CO_2 and the mass spectrum of each solute was achieved with a thermospray interface[18]. In another study, a triazole fungicide metabolite was separated by SFC on a capillary column with electron-capture detection[19].

None of the above mixtures of herbicides contained both triazine- and triazole-based compounds. The non-volatile nature of triazoles and triazines restrict

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their analysis by GC. Since SFC offers certain advantages, such as faster analysis and higher resolution per unit time over HPLC, SFC has been applied to the analysis of a model mixture (Fig. 1). Since several highly polar components of our synthetic mixture did not elute with 100% CO_2 from either packed or capillary columns, a modified graded mobile phase has been employed for their elution from an analytical scale packed column.

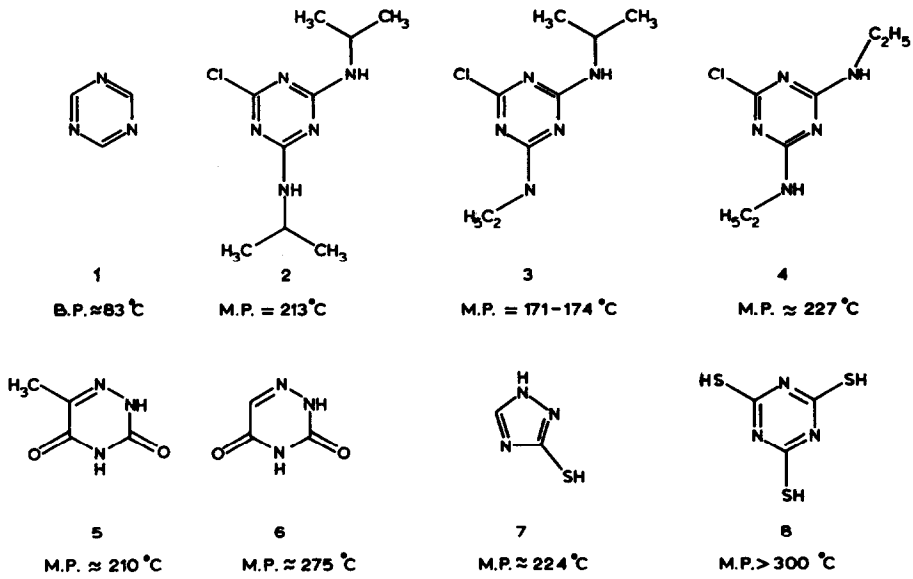

Fig. 1. Structures of triazine and triazole derivatives.

Composition mobile phase gradients were first introduced in SFC by Klesper and Schmitz[20] for effecting the separation of polymeric materials. Its application, however, to SFC has not been widespread . The effect of increasing percent of polar modifier in SCF on the elution and retention of fat-soluble vitamins has previously been reported by Board *et al.*[21]. A mixture of 24 derivatized amino acids has also been separated using a gradient mobile phase of CO_2 and methanol containing tetramethylammonium hydroxide. Nearly complete resolution of 22 derivatives was achieved in 15 min[22]. In this paper we describe the separation of our mixture of herbicides with a CO_2–methanol gradient.

EXPERIMENTAL

A Hewlett-Packard (Avondale, PA, U.S.A.) 1082B liquid chromatograph modified for supercritical fluids was used to deliver CO_2 (Scott Specialty Gases, Plumsteadville, PA, U.S.A.) to the system. This instrument was equipped with an Hewlett-Packard 78795 variable-wavelength ultraviolet detector. A Suprex (Pittsburgh, PA, U.S.A.) Model 200A micro-LC syringe pump was used to deliver methanol

(Fisher Scientific, Fairlawn, NJ, U.S.A.). Liquid methanol and supercritical CO_2 were introduced in a T-mixing chamber (Lee Co., West Brook, CT, U.S.A.) and the resulting mixed mobile phase was passed on to the column. A back-pressure regulator was used to maintain system pressure. A Deltabond® (Keystone Scientific, Bellefonte, PA, U.S.A.) cross-linked cyanopropyl bonded silica column of 25 cm × 4.6 mm I.D., 5 μm particle size, was used to develop the separation. All triazine- and triazole-based compounds were purchased from Aldrich (Milwaukee, WI, U.S.A.). The injected solution had a concentration of 200 ng/μl of each component prepared in HPLC-grade methanol. An injection volume of 1.0 μl was employed.

RESULTS AND DISCUSSION

The dual-pump system employed in this study is similar to that described previously[22]. A separation of the eight component mixture was unsuccessfully tried on several conventional analytical-scale columns packed with different stationary phases such as amino, cyano and octadecyl modified silicas under isocratic conditions. Various concentrations of methanol and different temperatures were attempted to develop the separation. The complete separation of all the components in the mixture was, however, achieved only using gradient elution. An oven temperature of 60°C and a flow-rate of 2 ml/min of CO_2 on a highly deactivated (Deltabond) cyanopropyl column with an outlet pressure of 4000 p.s.i. was employed. The percent methanol was increased to about 33% by the end of the chromatographic run as shown in Fig. 2 with an analysis time of less than six minutes. Throughout the separation, the flow of CO_2 was maintained constant while the flow of methanol was gradually increased. Unsubstituted *sym*-triazine (1,3,5-triazine) elutes first followed by the chloro-substituted *sym*-triazines. Among these substituted triazines, the one with two propyl groups elutes before the analyte with one propyl and one ethyl followed by the analyte with two ethyl groups. The two *asym*-(1,2,4-)triazines elute next followed by the two thiol containing compounds. The baseline shifted slightly when the amount of methanol in the mobile phase reached approximately 30%.

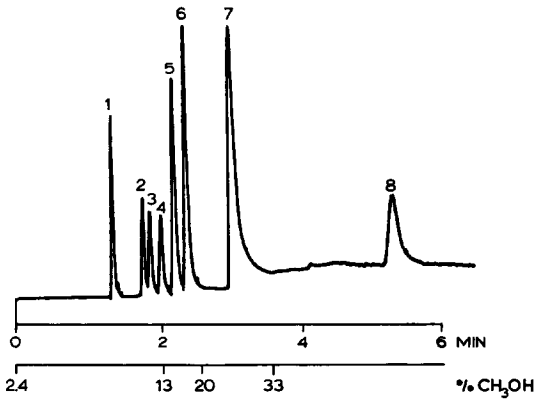

Fig. 2. Separation of triazine mixture at a flow-rate of 2 ml/min of CO_2, 250 × 4.6 mm Deltabond cyanopropyl (5 μm), 60°C, outlet pressure 4000 p.s.i., UV (440 nm). Peak Nos. correspond to the structures in Fig. 1.

The same separation was then carried out under exactly identical conditions but at a CO_2 flow-rate of 4 ml/min (Fig. 3). The flow of methanol in this case was also increased in order to achieve a gradient that was similar to the previous case. Both separations are quite comparable. The resolution between the closely related compounds, namely propazine, atrazine and simazine, is slightly less at the higher flow-rate but the total analysis time is reduced by a factor of approximately two. The peak area for component **8** has decreased somewhat at the higher flow-rate.

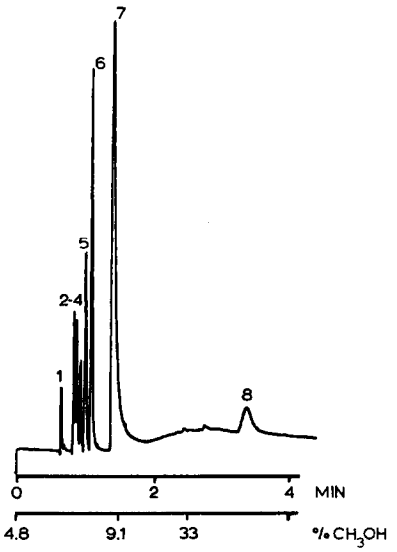

Fig. 3. Separation of triazine mixture at a CO_2 flow-rate of 4 ml/min: Peaks and conditions as in Fig. 2.

The next goal was to develop the same separation at a different temperature and/or a different pressure and study the effect of the changed parameters on resolution. Fig. 4 showns a separation which was performed at the same conditions as for Fig. 2, but at a temperature of 100°C. This separation at the elevated temperature (at 2 ml/min) was very similar to that achieved at the lower temperature. Peak shapes and retention times were almost identical in both cases. The increase in temperature probably does not change the density and the solvating power of the methanol-modified mobile phase significantly thereby having little effect on the separation.

This observation, of course, precludes any effect of changing linear velocity on the separation. Next, the outlet pressure was reduced to 2000 p.s.i. with the same gradient and flow-rate as shown in Fig. 2, and the separation was carried out at 60°C (Fig. 5). All of the components are retained longer due to the reduced density (2000 *vs.* 4000 p.s.i.) of the mobile phase. Yet, the resolution between peaks is comparable to the previously described separations. It is important to note that the mobile phase is in the subcritical state with reference to both temperature and pressure in the initial stages of the separation even with 2.4% methanol. The *sym*- and *asym*-triazines required a higher percentage (20%) of methanol for elution at the lower pressure. The most

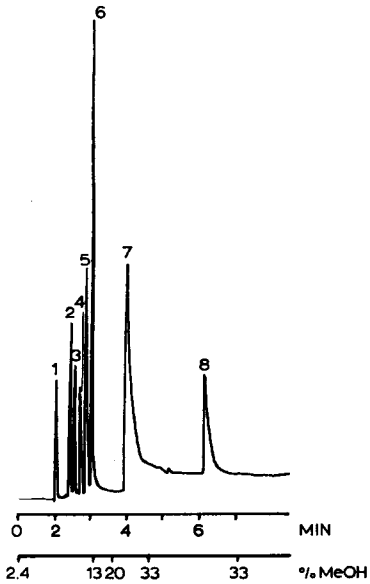

Fig. 4. Separation of triazine mixture at 4000 p.s.i. outlet pressure and 100°C. Peaks and other conditions as in Fig. 2. MeOH = Methanol.

polar component of the mixture, trithiocyanuric acid, is little affected by changes in both temperature and pressure.

The addition of tetramethylammonium hydroxide to methanol was found to

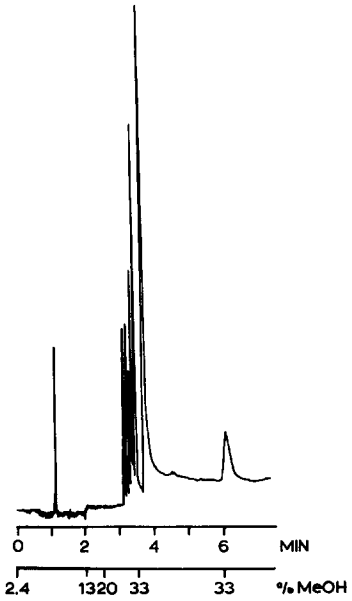

Fig. 5. Separation of triazine mixture at 2000 p.s.i. outlet pressure and 60°C. Peaks and other conditions as in Fig. 2.

have a great effect on the gradient mobile phase elution of acidic and basic phenylthiohydantoin-amino acids as reported previously by Berger *et al.*[22] The same base was added to methanol (0.001 *M*) and the separation of triazines was attempted. The addition of base in this case was found to have a slightly negative effect on the elution. Hexanol instead of methanol was also tried for the triazine. The kinetics of mixing of CO_2 and hexanol were, however, found to be very slow with our instrumental set-up. The failure to achieve a homogeneously mixed mobile phase manifested itself in a very unstable and noisy baseline probably due to air bubbles introduced into the flow cell.

In summary, it can be concluded from these experiments that little apparent loss in resolution occurred with a 100% increase in flow-rate. Further, if pressure is relatively high, the separation under subcritical conditions is quite comparable to the separation achieved under supercritical conditions. Gradient mobile phase elution SFC using a high percentage of modifier appears to bridge SFC and HPLC with the added advantage that faster analysis times can be achieved relative to HPLC. Furthermore, higher mobile phase flow-rates in gradient SFC with packed columns reduce the analysis time drastically with little change in resolution, thereby making this a viable technique for routine analysis.

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