Journal of Chromatography, 155 (1978) 198–202

CHROM. 10,924

Note

High-performance liquid chromatography and gas chromatography of organic isothiocyanates and their methanol-isothiocyanate addition compounds*

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(Received January 31st, 1978)

Analysis of organic isothiocyanates is of importance in the study of cruciferous crops. These compounds, amongst others, are formed from glucosinolates which occur in all cruciferous plants[1,2]. Isothiocyanates contribute to the desirable flavour and aroma of common cruciferous vegetables, *e.g.*, cabbage and turnip, or condiments, *e.g.*, mustard and horseradish, but they are also known to be goitrogenic[1].

Glucosinolates and their hydrolysis products have been determined by gas chromatography (GC)[3,4]. High-performance liquid chromatography (HPLC) offers another method of isothiocyanate analysis which has the advantage of speed over GC methods. This report gives details of analysis of organic isothiocyanates by GC and HPLC, and problems encountered when using methanol as sample solvent.

EXPERIMENTAL

Chemicals

Allyl, butyl, heptyl, and benzyl isothiocyanates were obtained from Eastman-Kodak (Rochester, N.Y., U.S.A.); phenylisothiocyanate was from Fluka (Buchs, Switzerland). HPLC grade methanol and "Certified" grade methylene chloride were obtained from Fisher Scientific (Pittsburgh, Pa., U.S.A.). Distilled water was passed through a deionizer before use.

Gas chromatography

GC analyses were performed on a Perkin-Elmer Model 3920 gas chromatograph equipped with dual flame ionization detectors and a Perkin-Elmer Model 56 recorder. The glass columns were 2 m × 2.4 mm I.D. packed with 80–100 mesh Chromosorb W HP coated with 1.5% Apiezon L. The carrier gas was helium with a flow-rate of 25 ml/min; inlet temperature was 220° and detector temperature 230°. The column oven was programmed for an initial isothermal period of 8 min at 50° then increased at 8°/min to 210° where the temperature remained until all products had eluted.

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* Contribution No. 347 from Food Research Institute, Agriculture Canada.

Gas chromatography–mass spectroscopy (GC–MS)

A Finnigan Model 3100D gas chromatograph–mass spectrometer was used. The glass GC column had the same dimensions and packing as in the Perkin-Elmer gas chromatograph. The separator and transfer lines were maintained at 250°. A mass range of 35 to 500 was scanned every second.

High-performance liquid chromatography

The HPLC system consisting of a Varian Model 8500 liquid chromatograph equipped with dual syringe pumps and solvent programming, DuPont Model 837 variable wavelength detector set at 245 nm, and Westronix Model S11B/U recorder was used. The 50 cm × 2 mm I.D. column was slurry packed with LiChrosorb RP18, 10 μm (E. Merck, Darmstadt, G.F.R.). Initial mobile phase conditions were methanol–water (3:1) increasing to 100% methanol at 4%/min immediately after injection. The flow-rate was maintained at 40 ml/h, the pressure required was 100 atm. Samples were injected through a stop-flow injection port.

RESULTS AND DISCUSSION

In recent years sensitive and accurate GC methods for analysis of glucosinolate hydrolysis products have been developed by Daxenbichler and VanEtten[3]. HPLC offers advantages over GC in speed of analysis and in higher capacity. The latter is important since the non-destructive detection of isothiocyanates after separation allows relatively easy collection of pure compounds for further identification and use as standards. The vast majority of glucosinolate hydrolysis products are not commercially available.

The initial experiments with HPLC separation of isothiocyanates showed encouraging results using methanol–water as the mobile phase. To avoid solvent peaks with void volume elution the isothiocyanate standards were dissolved in methanol. However, sample degradation was noticed after a few days storage, the standard peaks were reduced and other peaks appeared on the HPLC chromatograms. Examination by GC showed the same trend and an investigation by GC–MS was initiated. After sample degradation had been observed with methanol, methylene chloride was used as solvent for isothiocyanate standards. No sample degradation was found though elution of methylene chloride in HPLC analysis gave minor baseline instability at high detector sensitivity.

Methanol as sample solvent

As soon as it was noticed that the isothiocyanates were degrading in methanol the samples were discarded. Fresh samples were made up in methanol, but degradation occurred again. The initial HPLC separation did not give a clear indication of what was happening. The isothiocyanate peaks were reduced in size and other large peaks appeared soon after the void volume had eluted (Fig. 1). By GC a clearer indication was obtained (Fig. 2), three new peaks were observed adequately separated from the standard. By GC–MS the identity of the new peaks was elucidated and mixed peaks were found in two of the standards. By analysing the MS data, compounds of a mass corresponding to each standard isothiocyanate + 32 were found, three of these as new peaks and two as mixed peaks. It is evident that each isothio-

cyanate forms an addition compound with methanol (MW = 32). These compounds are quite stable and are probably formed in a similar manner to thioureas[5]. An example of the MS data is shown in Figs. 3 and 4. The normal fragmentation pattern of allyl isothiocyanate gives peaks at *m/e* 41, 72 and 99 corresponding to $CH_2{=}CH{-}CH_2^+$, $^+CH_2{-}N{=}C{=}S$, and $CH_2{=}CH{-}CH_2{-}N{=}C{=}S^+$, respectively. The mass spectrum of the corresponding methanol addition compound gave major intensity peaks at *m/e* 131 and 116 together with those found with allyl isothiocyanate. Similar patterns of isothiocyanate and methanol addition compounds were observed for each standard.

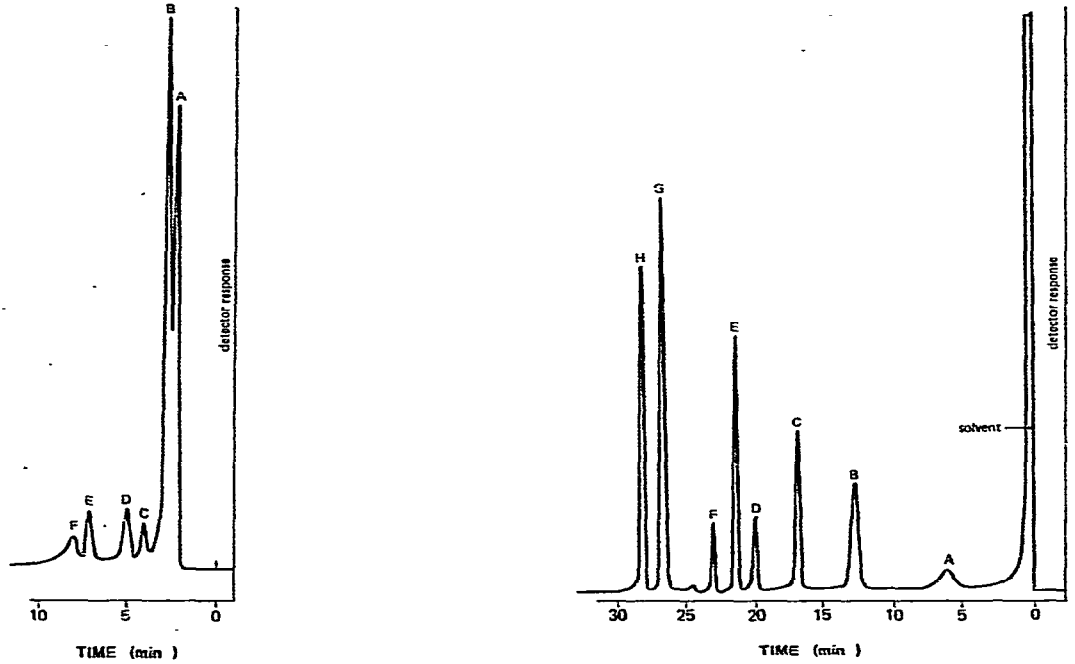

Fig. 1. HPLC separation of isothiocyanates and their addition compounds using methanol as solvent. A and B = addition compounds; C = allyl isothiocyanate; D = butyl isothiocyanate; E = phenyl and benzyl isothiocyanates; F = heptyl isothiocyanate.

Fig. 2. GC separation of isothiocyanates and their addition compounds formed using methanol as solvent. A = allyl isothiocyanate; B = butyl isothiocyanate; C = allyl isothiocyanate–methanol addition compound D = phenyl isothiocyanate + butyl isothiocyanate–methanol addition compound; E = heptyl isothiocyanate; F = benzyl isothiocyanate; G = mixture of phenyl and heptyl isothiocyanate–methanol addition compounds; H = benzyl isothiocyanate–methanol addition compound.

Methylene chloride as sample solvent

The separation of the mixture of standard isothiocyanates was achieved in a much shorter time by HPLC than GC (Figs. 5 and 6). Also the high-pressure liquid chromatograph could be returned to starting conditions much faster than cooling the gas chromatograph back to starting temperature. Under isochratic conditions, methanol–water (3:1), elution of heptyl isothiocyanate was slow forming a broad

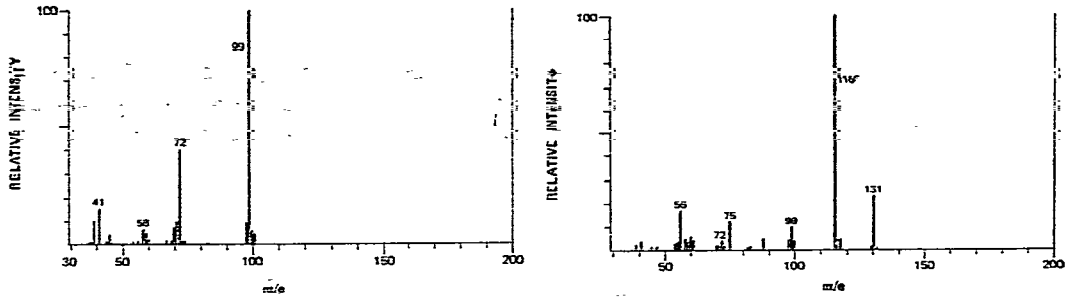

Fig. 3. Mass spectrum of allyl isothiocyanate (CH_2=CH-CH_2-N=C=S; MW = 99).

Fig. 4. Mass spectrum of allyl isothiocyanate–methanol addition compound (MW = 131).

peak, solvent programming improved peak shape and retention time. Phenyl and benzyl isothiocyanates could not be separated by the system used, even with a much higher proportion of water in the mobile phase. Satisfactory separation would require a different solvent system and/or column packing. On the strength of these results HPLC separation of isothiocyanates, derived from naturally occurring glucosinolates, has been attempted in these laboratories. Initial results are encouraging, good separations have been made.

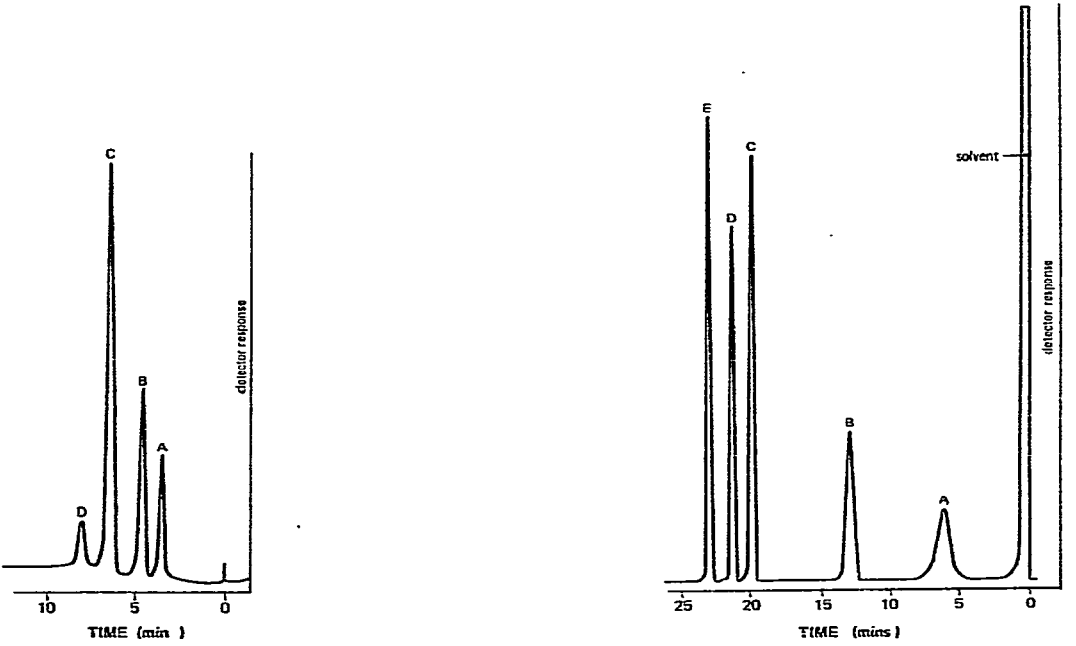

Fig. 5. HPLC separation of isothiocyanates dissolved in methylene chloride. A = allyl; B = butyl; C = benzyl + phenyl; D = heptyl isothiocyanate.

Fig. 6. GC separation of isothiocyanates dissolved in methylene chloride. A = allyl; B = butyl; C = phenyl; D = heptyl; E = benzyl isothiocyanate.

CONCLUSION

Preliminary results of HPLC analysis of organic isothiocyanates indicate that this method would be of use in the analysis of glucosinolate hydrolysis products. The use of methanol in extraction and preparation of isothiocyanates from glucosinolates should be avoided.

ACKNOWLEDGEMENT

The GC–MS analyses were performed by S. Skinner and D. Dobson, C.B.R.I., Agriculture Canada.

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