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DETECTION OF GOITRIN AND ITS HEPTAFLUOROBUTYRYL DERIVATIVE BY GAS–LIQUID CHROMATOGRAPHY WITH ELECTRON CAPTURE, ELECTROLYTIC CONDUCTIVITY AND SULFUR DETECTORS

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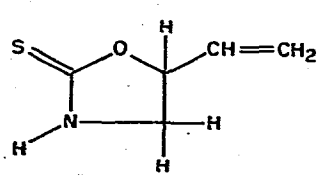
SUMMARY

The separation of goitrin by two gas–liquid chromatographic columns, the response of different detectors and the use of the heptafluorobutyric (HFB) anhydride derivative of goitrin to improve sensitivity was investigated. The non-polar 3% OV-1 and the intermediate 4% SE-30/6% SP-2401 on 80–100 mesh Chromosorb W HP gave comparable results and were considered interchangeable. The sensitivities of the electron capture, sulfur 394 nm emission, chlorine and nitrogen Coulson electrolytic conductivity detectors were inadequate for goitrin *per se*. Chromatography and sensitivity of all detectors to goitrin were greatly improved by using by using the HFB derivative. It was possible to detect 1 to 60 ng of goitrin-HFB in standard solutions. Application of the technique to goitrin-spiked (2 ppm) milk, cleaned up by high-performance liquid chromatography, encountered no problems and was considered satisfactory for all four detectors.

INTRODUCTION

Goitrin, 1,5-vinyloxazolidine-2-thione (Fig. 1), is the aglycone hydrolysis product of progoitrin, a glucosinolate found in rape, turnip and cabbate[1–4]. Goitrin is similar to thiouracil in antithyroid activity[1]. The presence of this compound in foods for human consumption is of some concern to health authorities since residues may occur in milk and other products from animals fed with rapeseed meal protein supplements[5–7].



GOITRIN

Fig. 1. Chemical structure of goitrin.

Analytical procedures for glucosinolates have been reported that are based on ultraviolet (UV) measurements[1,3,4], infrared (IR)[8] and gas–liquid chromatography (GLC) with flame ionization detection[6,10–13]. The latter technique is preferred as it separates and detects various glucosinolates or their hydrolytic products. However, these methods are not adequate for milk as they do not have the necessary sensitivity and specificity for regulatory purposes. There is a need to improve the method.

Recently, we undertook to investigate the response of other detectors such as the ^{63}Ni electron-capture (ECD), Coulson electrolytic conductivity (CECD, nitrogen and halogen modes) and Melpar flame photometric emission (MFPD, sulfur, 394 nm) for goitrin and its haptafluorobutyryl (HFB) derivative. This report describes our results which includes the application of the HFB derivatization technique to milk samples spiked at 2.0 ppm with goitrin.

EXPERIMENTAL

A goitrin standard was prepared from a crude extract of rapeseed meal by thin-layer chromatographic (TLC) isolation techniques. Identity and an indication of purity were established using UV, IR, high-resolution mass spectrometry and nuclear magnetic resonance procedures. A second portion of purified goitrin was obtained from Lancaster Synthesis (Lancaster, Great Britain). Goitrin from both sources were identical as based on the above-mentioned tests. Stock solutions were prepared in methylene chloride and aliquots were serially diluted with the same solvent to the desired concentrations.

All organic solvents were purchased as glass-distilled residue-free grade.

Trimethylamine (anhydrous, Eastman-Kodak, Rochester, N.Y., U.S.A.) in glass ampoules was cooled in ice water and added with stirring to cool, tared benzene to produce a 0.5-*M* solution.

Heptafluorobutyric anhydride (HFBA, PCR Research Chemicals, Gainville, Fla., U.S.A.) was used as received from the supplier.

Gas–liquid chromatography

Two columns were selected for evaluation in resolving goitrin; a non-polar, 3% OV-1, and an intermediate one consisting of 4% SE-30/6% SP-2401, each coated on 80–100 mesh Chromosorb W HP. The stationary phases were packed in approximately 110 cm × 4 mm I.D. borosilicate glass columns and conditioned at temperatures ten degrees below the specified operating maximum of their liquid phases until a consistent response was obtained.

The CECD, MFPD and ECD were fitted to a Tracor Microtek MT220 gas chromatograph. Operating conditions for the CECD in the nitrogen or halogen mode were: pyrolysis furnace, 800°; transfer unit, 235°; d.c. bridge potential, 30 V; hydrogen flow-rate 37 ml/min. The ECD temperature was 280°, while the nitrogen purge gas flow-rate was 42 ml/min. The MFPD block temperature was 175° and the flame gases were: hydrogen (200 ml/min) and air (200 ml/min). The CECD and MFPD used helium as a carrier gas (flow-rate, 40 ml/min) while the ECD system used nitrogen (flow-rate, 40 ml/min). Inlet and column temperatures for all systems were 200 and 149°, respectively.

Derivative preparation[14]

Aliquots containing 20–100 μg of goitrin in methylene chloride were transferred to 15-ml glass stoppered or PTFE-lined screw-cap centrifuge tubes. The solutions were taken to dryness by evaporation at room temperature, with the assistance of a gentle stream of nitrogen gas. To each tube was added 1 ml benzene, 20 μl HFBA, followed by 0.1 ml of a 0.5-*M* TMA solution, and thorough mixing. The reaction was allowed to proceed for 30 min at room temperature. At the end of this time 12 ml distilled water were added to each tube, and the mixture thoroughly shaken for 1 min. The water phase was separated from the benzene by centrifuging for 5 min at 500 *g*, then discarded. Aliquots of the washed benzene phases were then analyzed under the various GLC conditions reported.

Analysis of milk samples

Homogenized milk samples were extracted according to Benns *et al.*[15] for analysis by high-pressure liquid chromatography (HPLC). Briefly, the milk was extracted with ether, which was evaporated to dryness. Warm water was added to the residue which dissolved the goitrin. This was extracted with hexane to remove the lipid then finally extracted with ether again for evaporation and analysis by HPLC. An aliquot of the final extract equivalent to 25 g of milk was spiked with 50 μg goitrin and taken to dryness, then dissolved in 1 ml of benzene. The solution was treated exactly as described above for the derivatization, then analysed by GLC with the four detectors. Recoveries through this extraction method have been shown to be $>75\%$ at the 2.0 ppm level of spiking[15].

RESULTS AND DISCUSSION

The direct GLC of goitrin was found to be unsuitable for application to its dermination in milk samples at low ppm levels. The sensitivities of the various detectors to goitrin itself were very poor and could not be used for residue purposes. Electron capture detection proved to be the most sensitive but still required 200–300 ng to produce a 10-cm peak (2.5 min retention time) at $16 \cdot 10^{-2}$ sensitivity setting. The other detectors required microgram quantities for a response.

To improve chromatography and increase detector sensitivity, we sought to prepare a suitable derivative. The HFB derivative proved to be very satisfactory providing much superior response to all detectors evaluated. Fig. 2 illustrates the reaction. The HFB group attaches to the nitrogen atom of the goitrin molecule. Some chromatographic results are shown in Fig. 3 for the four detectors studied. Both OV-1 and SE-30/SP-2401 column packings gave good resolution and were considered interchangeable. Two peaks were present in all chromatograms except those obtained with the MFPD which showed only one derivative peak. This peak corresponded to the latter peak of the other chromatograms and thus was used for quantitative measurements in the milk samples. Although the absolute yield of product was not calculated, the observed sensitivities indicate a high conversion the derivative. The yield wss very consistent producing linear results over a range of 10–100 μg reacted.

Fig. 4 shows some response plots of HFB-goitrin obtained with the four detectors using the mixed column for chromatography. It can be seen that the ECD was the most sensitive to the derivative. The CECD in the halogen mode was the

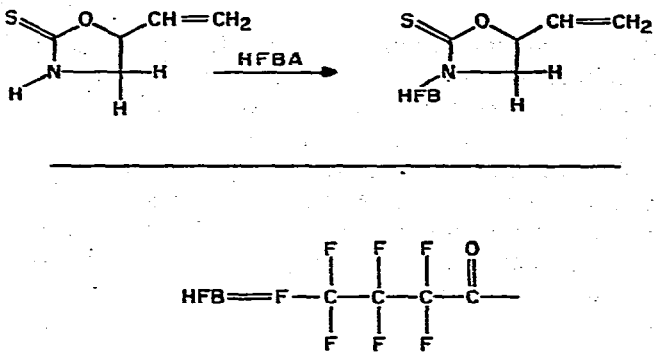


Fig. 2. HFBA reaction with goitrin.

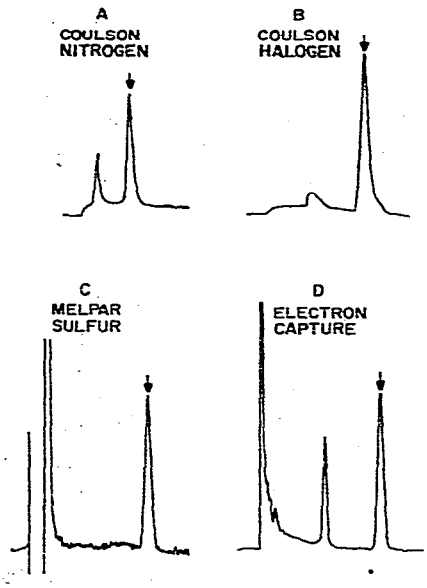


Fig. 3. Detection of HFB-goitrin by A, CECD, nitrogen mode; 130 ng injected; 1 × attenuation; t_R = 6.5 min; B, CECD, halogen mode; 38 ng injected; 1 × attenuation; t_R = 6.8 min; C, MFPD, sulfur; 45 ng injected; 2 × attenuation·10^{-3}; t_R = 6.9 min; D, ECD, 0.6 ng injected; 8 × attenuation·10^{-2}; t_R = 6.9 min. All chromatograms obtained on the 4% SE-30/6% SP-2401 column.

second most sensitive. This is attributed to the large number of fluorine atoms in the HFB derivatives. The nitrogen detector was the least sensitive of the four.

Application of this derivatization technique to spiked milk extracts proved to be very satisfactory. A goitrin concentration of 2 ppm was easily detected in the milk samples. No problems were encountered in carrying out the reaction in the presence of milk co-extractives. Fig. 5 shows some representative chromatograms of spiked milk samples, obtained with the four detectors studied. All detectors proved to be satisfactory for the analysis, although the ECD was sensitive enough to keep the quantity of sample injected to less than one-tenth that of the other detectors. This

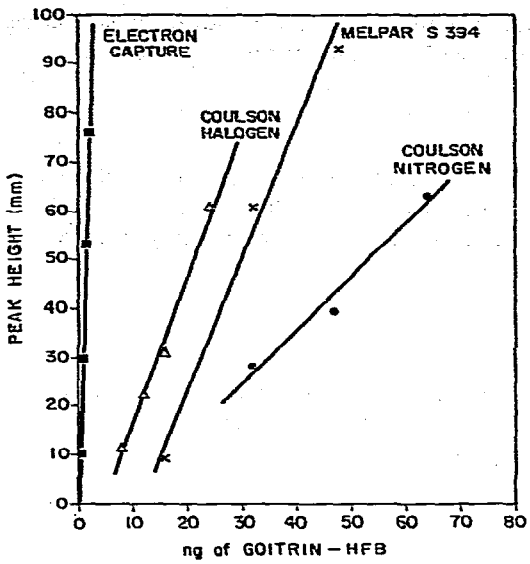


Fig. 4. Response plots for HFB-goitrin obtained with the various detectors used at sensitivity setting described in Fig. 3.

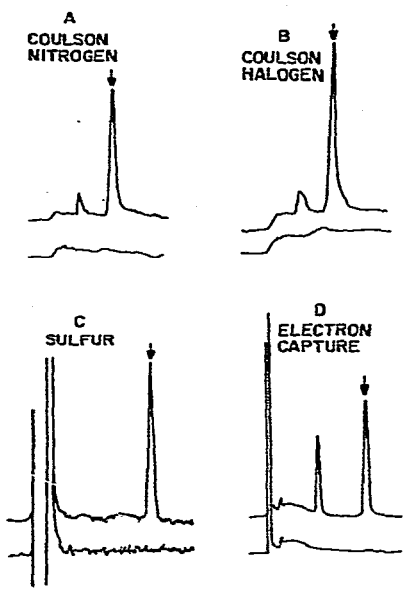


Fig. 5. Chromatograms of homogenized milk extracts containing 2.0 ppm goitrin. A, CECD, nitrogen mode, 62.5 mg injected; B, CECD, halogen mode, 39 mg injected; C, MPFD, sulfur; 32.5 mg injected; D, ECD, 0.29 mg injected. Top chromatogram, spiked milk; bottom chromatogram, blank milk.

may be of some importance if goitrin analysis is carried out on a routine basis, since column contamination would be much reduced with smaller quantities injected.

CONCLUSION

The GLC analysis of goitrin as a HFB derivative shows much promise for application to ppm or lower levels in foods. The various sensitivities and selectivities of the detectors to the derivative should prove to be very useful in development of an analytical method.

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REFERENCES

1 E. B. Astwood, M. A. Geer and M. G. Ettlinger, *J. Biol. Chem.*, 181 (1949) 121.
2 M. T. Lo and D. C. Hill, *Can. J. Physiol. Pharmacol.*, 50 (1972) 373.
3 F. L. Austin, C. A. Gent and I. A. Wolff, *Can. J. Chem.*, 46 (1968) 1507.
4 M. R. Altamura, L. Long, Jr. and T. Hasselstrom, *J. Biol. Chem.*, 234 (1959) 1847.
5 A. Arstila, F. E. Krusius and P. Peltola, *Acta Endocrinol.*, 60 (1969) 712.
6 M. Kreula and M. Kiesvaara, *Acta Chem. Scand.*, 13 (1959) 1375.
7 Z. Madjeski, *Bromatologia Chem. Toksykol.*, 6 (1973) 329.
8 M. E. Daxenbichler, C. A. Van Etten and I. A. Wolff, *Biochemistry*, 5 (1966) 692.
9 M. E. Daxenbichler, G. F. Spencer, R. Kleiman, C. A. Van Etten and I. A. Wolff, *Anal. Biochem.*, 38 (1970) 374.
10 E. W. Underhill and D. F. Kirkland, *J. Chromatogr.*, 57 (1971) 47.
11 S. Persson, B. Toregard and R. Ohlson, *personal communication.*
12 W. Thies, *Fette, Seifen, Anstrichm.*, 76 (1976) 231.
13 M. E. Daxenbichler and C. H. Van Etten, *J. Ass. Offic. Anal. Chem.*, 60 (1977) 950.
14 J. F. Lawrence and J. J. Ryan, *J. Chromatogr.*, 130 (1977) 97.
15 G. Benns, J. F. Lawrence, M. L'Abbe and J. Beare-Rogers, *J. Agr. Food Chem.*, (1978) submitted for publication.