Journal of Chromatography, 130 (1977) 399–402

CHROM. 9571

Note

Analysis of methionine in plant materials

Improved gas chromatographic procedure*

S. L. MacKENZIE

Prairie Regional Laboratory, National Research Council of Canada, Saskatoon, Sask. S7N OW9 (Canada)

(Received July 14th, 1976)

The rapid, accurate and preferably specific analysis of methionine is important for the improvement of the nutritional quality of crops in which methionine is the nutritionally limiting amino acid and important for the efficient use of the various food products derived from those crops.

Cyanogen bromide reacts with methionyl residues in intact proteins, yielding stoichiometric amounts of methyl thiocyanate[1]. This reaction has been used primarily in protein sequencing studies because of the selective cleavage of methionyl peptide bonds. Inglis and Edman[2] suggested that the reaction might be used to quantitate methionine in proteins. More recently, it was further demonstrated the cyanogen bromide reaction could be used to quantitate methionine in crude samples such as seed meals[3]. The present report describes an improved gas chromatographic (GC) procedure for the determination of methyl thiocyanate. The analysis time has been shortened and the column packing is more stable to aqueous formic acid than that previously described.

EXPERIMENTAL

Reagents

Cyanogen bromide, reagent grade, was obtained from Eastman-Kodak (Rochester, N.Y., U.S.A.). Formic acid was purified by vacuum distillation and had a melting point of 7°. Methyl thiocyanate was obtained from Eastman-Kodak and was further purified by fractional distillation.

Procedure

The reaction mixture consisted of 100 μl of the cyanogen bromide reagent (10% w/v in anhydrous formic acid), 100 μl of an aqueous solution of the internal standard, and 5–15 mg of finely ground sample in a 1-ml screw-cap vial (Pierce Reactivial). After 3–4 h at room temperature, the vial was centrifuged (2000 *g*) for 15 min and 1–2 μl aliquots of the supernatant were injected directly on the chromato-

* NRCC No. 15660.

graphic column using a Hamilton 7010 syringe. The concentration of internal standard —dimethyl formamide or methyl isobutyl ketone— varied, depending on the anticipated methionine content of the sample.

The effect of the cyanogen bromide concentration on the release of methyl thiocyanate from pea meal was determined using concentrations of 0.5, 1.0, 2.0, 3.0, 5.0, and 10.0% in reaction mixtures where all other factors, including the weight of the meal, remained constant.

Gas chromatography

All analyses were performed using a Hewlett-Packard Model 7611 gas chromatograph equipped with a dual flame ionisation detector. The column (40 cm × 2–3 mm I.D., Pyrex) was packed with Porapak QS, 80–100 mesh (Applied Science Labs., State College, Pa., U.S.A.) and baked at 230° for 2 h with a normal carrier gas flow-rate. The GC conditions were as follows: column temperature, 160°; detector temperature, 225°; air flow-rate, 300 ml/min; hydrogen flow-rate, 30 ml/min; carrier gas (nitrogen) flow-rate, 25–30 ml/min. Quantitation and data reduction were performed by a Hewlett-Packard Model 3352B laboratory data system using an internal standard programme.

RESULTS AND DISCUSSION

In an earlier publication describing the analysis of methyl thiocyanate, the GC column showed no deterioration after several hundred analyses[3]. However, the majority of the analyses were performed in initial experiments using anhydrous formic acid, and in later experiments —using 50% or 75% aqueous formic acid— the column

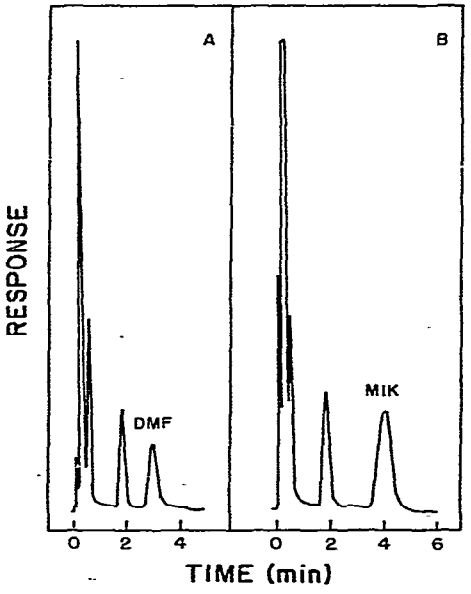

Fig. 1. Chromatographs illustrating the separation of methyl thiocyanate (retention time 2 min) from (A) N,N-dimethylformamide (DMF) and (B) methyl isobutyl ketone (MIK).

deteriorated after about 100 analyses, resulting in tailing and increased variability. Since the reaction of cyanogen bromide with methionine in seed meals progressed more rapidly in aqueous formic acid[4] a more stable GC column packing was sought. Porapak QS was found to be suitable. Typical chromatographs illustrating the separation of methyl thiocyanate on Porapak QS are shown in Fig. 1. The large peak previously observed to elute after methyl thiocyanate[3], and determined to be some product of the reaction of formic acid with ethylene glycol adipate, is not present.

A further requirement to improve the earlier procedure and to simplify the injection of samples on to the column was the addition of an internal standard. The choice of the internal standard was governed by the need to satisfy requirements such as solubility in water, stability in formic acid, and a suitable retention time. A further requirement was that the boiling point of the internal standard be close to that of methyl thiocyanate to minimise differential evaporation of the sample components during injection, particularly from the liquid retained in the syringe needle. Several alcohols had suitable retention times and solubility but reacted with formic acid; several acids had suitable stability, retention times and solubility but produced tailing peaks. Methyl ethyl ketone satisfied most of the above requirements and eluted before methyl thiocyanate when a 45-cm column was used operated at 150°. However, because of its low boiling point the coefficient of variation of the internal standard to methyl thiocyanate ratio could be reduced to the desired level of $<2\%$ only by injecting the samples rapidly. The resultant emphasis on injection technique was felt to be unsatisfactory for routine analysis. Two suitable internal standards are N,N-dimethylformamide (Fig. 1A) and methyl isobutyl ketone (Fig. 1B), both of which are readily separated from methyl thiocyanate on a 40-cm column of Porapak QS operated at 160°.

Initially, excessive variability in the ratio of internal standard to methyl thiocyanate was observed after about 100 analyses. This variability was due to tailing in the internal standard peak, the methyl thiocyanate peak being unaffected, and was caused by the deposition of non-volatile material on the front of the column. This material consisted partly of fine solids remaining in suspension after the centrifugation and a marked improvement was obtained by increasing the centrifugation time. Assuming on-column injection, this problem was minimised by using more glass wool than normal (about 1 in.) at the injection end of the column, thus restricting contamination to the glass wool. When contamination occurred, the glass wool could be quickly removed and replaced —at the end of each day, if necessary— without requiring repacking of the column. Provided the above precautions were taken as required, no evidence of deterioration of the column packing or in the separation of the components was observed after several hundred analyses.

The effect of cyanogen bromide concentration on the release of methyl thiocyanate from pea meal is shown in Fig. 2. Initial concentrations of 0.5–3% were insufficient to promote complete release of methyl thiocyanate unless the reaction time was unnecessarily prolonged. With concentrations of 5 and 10% the reaction was complete in 2–3 h and the values were unchanged after 24 h. For all determinations of the methionine content of pea meal a 5% solution of cyanogen bromide was used. This concentration corresponded to about a 150-fold excess. A greater concentration may be required for other plant materials and the kinetics of the reaction would have to be established for each source of material.

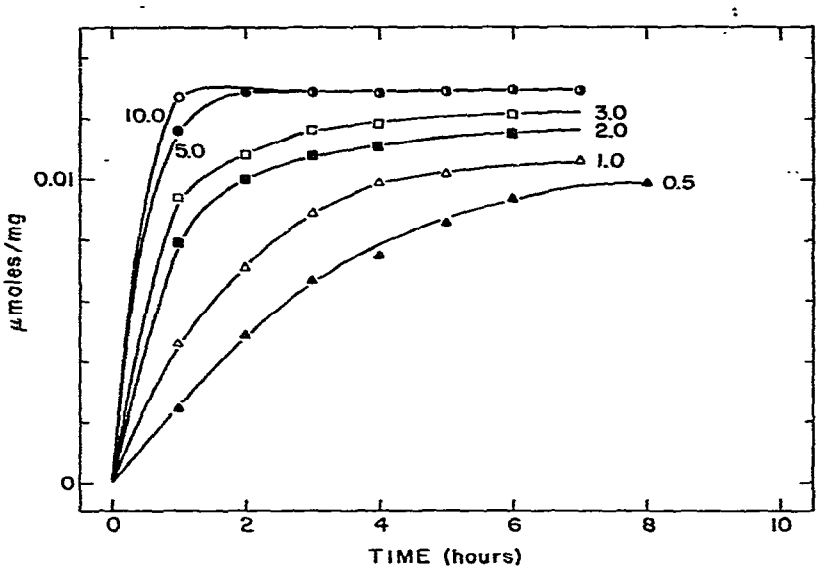

Fig. 2. Graph illustrating the release of methyl thiocyanate from pea meal when varying concentrations of cyanogen bromide are used.

ACKNOWLEDGEMENTS

The excellent technical assistance of Mr. D. Tenaschuk is gratefully acknowledged.

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