Journal of Chromatography, 348 (1985) 317–320
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 167

Note

Elemental sulphur analysis using high-performance liquid chromatography on 10-μm rigid polymer particles

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(First received July 31st, 1985; revised manuscript received September 10th, 1985)

Elemental sulphur analysis is of interest in several fields including fertiliser research and advice[1], food analysis[2] and in heavy-water production[3]. Most available methods are laborious or suffer from poor selectivity and/or sensitivity[4]. Gas chromatography has been used[5] but in our experience neither electron-capture nor flame photometric detection provided a sufficient linear range for practical use.

The method of Cassidy[3] which employs high-speed liquid chromatography with styrene–divinylbenzene packing material has significant advantages including that of selectivity. It does not seem, however, to have been applied to any extent. Possible reasons are that an experimental column was used, and a single set of operating conditions was not recommended. Most non-specialist laboratories, and indeed many experienced liquid chromatography laboratories, are not confident in the packing of columns, in particular those with steric exclusion packing material.

Our initial investigations followed the method of Cassidy[3] except that a column packed with Bio-Beads S-X8 in place of Poragel was used. Attempts to buy a suitable commercial column packed with modern efficient steric exclusion material were unsuccessful. No supplier was prepared to provide a column packed in the selected mobile phase of methanol–chloroform (30:70).

We are now able to report that a commercially available column packed with small (10 μm) rigid particles of styrene–divinylbenzene and sold as a reversed-phase column can be used for elemental sulphur analysis. The columns may be subjected to relatively extreme changes in solvent composition without any apparent degradation. The performance of this column is compared with others packed with either Bio-Beads or with conventional reversed phase material.

EXPERIMENTAL

Materials and reagents

Solvents used for high-performance liquid chromatography (HPLC) were methanol and chloroform (Ajax HPLC grade) while the chloroform used for elemental sulphur standards and for soil extracts was Ajax analytical grade. Standard solutions were prepared from both analytical and agricultural grades of sulphur. Extracts of air-dry soil (10 g) were prepared by shaking overnight with chloroform (20 ml).

0021-9673/85/$03.30

Apparatus

HPLC was performed using Spectra-Physics 740B pumps, a Rheodyne 7120 manual injector or Micromeritics 725 autosampler, both fitted with fixed loops of either 10 or 20 μl and a Shimadzu SPD-2A variable-wavelength detector set at 254 nm. Data acquisition was by peak height using either a chart recorder or a Spectra-Physics SP4270 integrator. The analytical column was preceded by a 2-μm in-line filter (Rheodyne).

Preferred HPLC conditions

A PRP-1 reversed-phase column (15 cm × 4.1 mm I.D.) (Hamilton, Reno, NV, U.S.A.) containing 10-μm particles of a styrene–divinylbenzene copolymer was used. The mobile phase was methanol–chloroform (50:50) at 1 ml/min. The column was supplied containing water–acetonitrile (10:90). Before changing to the mobile phase it was flushed with acetonitrile and then methanol. After each use the column was flushed and then stored with methanol. The retention time for sulphur was 200 sec, capacity factor (k') = 5.2. Samples were injected in chloroform.

Alternative HPLC conditions

A home-made column (50 cm × 3.1 mm I.D.) was packed with Bio-Beads S-X8, 200–400 dry mesh size, in the mobile phase of methanol–chloroform (30:70). The particles were previously cleared of fines and swollen in the mobile phase. Packing pressure was about 900 p.s.i. Analysis flow-rate was 2 ml/min which gave a retention time for sulphur of 182 sec, k' = 4.1. Samples were injected in chloroform.

Reversed-phase columns were used with methanol mobile phase at 1 ml/min. They were Nova-Pak C_{18} (15 cm × 3.9 mm I.D.) (Waters Assoc., Milford, MA, U.S.A.); CP-Spher C_8 (25 cm × 4.6 mm I.D.) (Chrompack, Middelburg, The Netherlands) and a home-made column (10 cm × 4.0 mm I.D.) of Hypersil ODS (5 μm) (Shandon Southern, Astmoor, England). Retention times for sulphur for these columns were 214, 162 and 140 sec respectively, with k' values of 4.9, 2.5 and 3.4, respectively. Samples in chloroform were diluted with methanol to no more than 50% chloroform before injection.

RESULTS AND DISCUSSION

Typical chromatograms of a sulphur standard (1 μg/ml) and soil extracts (0.15–5.7 μg/ml) containing elemental sulphur are shown in Fig. 1 for three of the columns tested. Note that for both styrene–divinylbenzene packings the sulphur peak was the last to elute. This further verifies the selective nature of these types of packing for elemental sulphur as reported by Cassidy[3]. The PRP-1 column gave greater efficiency than the Bio-Beads column and used less than half the volume of solvent for each analysis. The C_{18} reversed-phase material produced a sharp peak for sulphur providing the injected sample contained less than 50% chloroform, but co-extractive peaks eluted both close to and at greater retention than sulphur. The overall analysis time was longer than for the PRP-1 column and the selectivity not as good. The other C_{18} column, Nova-Pak, gave similar chromatograms to that shown in Fig. 1g, but with significantly greater retention of sulphur and the co-extractives. The C_8 column had insufficient retention of elemental sulphur.

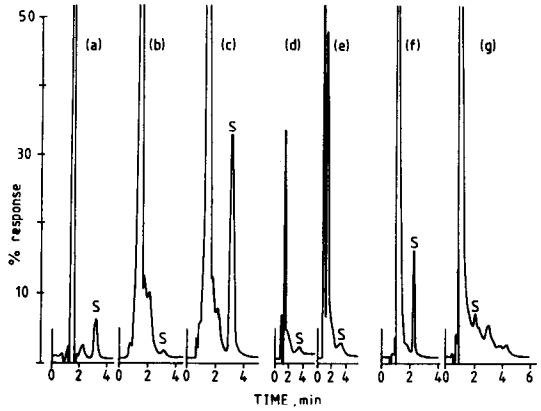

Fig. 1. Analysis of elemental sulphur in standard solutions and soil extracts on three different columns. Detection at 254 nm and 0.04 (or 0.02) a.u.f.s. and 20- (or 10-) μl injection. Primary experimental conditions are described in the text. a, b, c: PRP-1 column, standard, 1 μg/ml S; extract, 0.2 μg/ml S; extract, 5.7 μg/ml S. d, e: Bio-Beads S-X8 column, standard, 1 μg/ml S; extract, 1.1 μg/ml S. f, g: Hypersil ODS column: standard. 1 μg/ml S; extract, 0.15 μg/ml S.S = Sulphur peak.

Five commercial sources were examined for the sulphur species soluble in chloroform and methanol, comprising four of sublimed sulphur (three for agricultural use and one laboratory reagent) and one from a hydrothermal deposit containing 20% S. Mass spectra (direct probe, 70 eV) and HPLC measurements [C_{18} column, methanol–water (96.5:3.5) eluent[6]; PRP-1 column, methanol–chloroform (70:30)] showed the major species was S_8 (99%), with no more than 1% from a peak assigned to S_5 or S_6. Other species were less than 0.5% of the total. The single peak obtained using the PRP-1 column and mobile phase of methanol–chloroform (50:50) was therefore from S_8 with probably a trace of S_5 or S_6, the species in the sulphur investigated. Some larger rings may elute later, and this would need checking if their presence in important amounts were suspected[6].

Detection limits, linearity and selectivity

The detection limit for the PRP-1 column was at least five times better than for the Bio-Beads column. With 20-μl injection, a 1-μg/ml S solution in chloroform gave a 11.5% deflection at 0.02 a.u.f.s. Thus a readily attainable detection limit was 2–4 ng. In these examples injections were made in chloroform to allow direct injection of soil extracts. Improved column efficiency and peak shape, and hence greater resolution and a 10–20% improved detection limit were obtained when samples were injected in methanol or in the mobile phase. Solutions containing very low concentrations of sulphur can therefore be measured more accurately by evaporating and taking up in reduced volumes of methanol. In this instance the relatively low solubility of sulphur in methanol (less than 100 μg/ml) is not a serious limitation.

The linear range of sulphur was found to extend from the detection limit up to at least 10 μg, in agreement with earlier work[2,3].

Over 1000 extracts from pastoral soils of several different types and location have been analysed using the PRP-1 column. In only one soil did another peak begin

to interfere with sulphur analysis, and that only at levels of less than 0.2 ppm of sulphur in the extract solution. The column retained its resolution, and operating pressure did not increase during these analyses.

CONCLUSION

A commercially available PRP-1 column packed with rigid styrene–divinylbenzene particles has been found suitable for routine analysis of elemental sulphur from less than 10 ng up to at least 10 μg. The short analysis time allows 300 samples per day to be analysed with the aid of an automatic injector. The column has been subjected to relatively large changes in solvent composition and large numbers of soil extracts without any apparent loss in performance. It gives a single sharp peak which is well separated from soil co-extractives and provides the basis for a sensitive, rapid and versatile method for elemental sulphur analysis in soils.

ACKNOWLEDGEMENTS

We thank A. Lee, M. P. Agnew and G. C. Milligan for assistance, and P. T. Holland for mass spectra.

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