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THERMOSPRAY LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY IN FOOD AND AGRICULTURAL RESEARCH

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SUMMARY

A thermospray liquid chromatography–mass spectrometry interface connected to a double-focusing magnetic sector instrument has been applied successfully to the solution of a number of analytical problems in food and agricultural research. Examples of the qualitative analysis of some anti-nutrients, plant growth control hormones, biotechnology products and a plant polyphenol are presented and discussed.

INTRODUCTION

The thermospray (TSP) technique[1,2], which combines the ability to handle high solvent flow-rates with a method for ionising samples directly from solution, is proving to be one of the most fruitful methods of obtaining on-line liquid chromatography–mass spectrometry (LC–MS) data. TSP was developed on quadrupole mass spectrometers but its successful linkage to high-voltage magnetic sector instruments[3] has added another dimension to LC–MS. The work described here was all performed on a commercially available magnetic sector instrument, retro-fitted with a TSP LC–MS interface and ion source. The examples presented were selected from a number of analytical applications which arose during the course of current research programmes in progress at Agricultural and Food Research Council Institutes.

EXPERIMENTAL

Liquid chromatography was conducted using a Gilson System 41 gradient liquid chromatograph and Gilson Holochrome UV detector (Anachem, Luton, U.K.). The TSP interface (which has been described elsewhere[4]) was supplied by Kratos Analytical (Manchester, U.K.) and was connected to a Kratos MS80 RFA mass spectrometer. The TSP vaporiser and source block temperatures were optimised for the particular type of analysis in progress but were typically in the range 230–

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260°C and 200–270°C, respectively. In all cases ions were produced by ensuring that a solution of 0.1 *M* aqueous ammonium acetate was present. Spectra were typically calibrated over the mass range 700–18 daltons at scan speeds of 1 or 3 s/decade, and an interscan time 0.5 s, mass resolution of 1000 (10% valley definition) and accelerating voltage of 4 kV.

RESULTS AND DISCUSSION

Glucosinolates

Over 100 glucosinolates (Fig. 1, R′ = SO_4, R = a wide variety of side chains, often amino acid related) have been isolated from many plants and vegetables, particularly cruciferae[5,6]. These compounds have a very wide range of physiological properties which are attributable to their enzymatically released aglycones. Of particular interest to food and agricultural scientists are their roles as (i) anti-nutrients (*e.g.* in rapeseed), (ii) precursors of pungency (*e.g.* mustard, horseradish). Although there have been considerable advances in methods for the qualitative and quantitative analysis of glucosinolates[7], there is still a strong demand for improvements in methodology. Mass spectrometric studies have already demonstrated the potential of electron ionisation[8], chemical ionisation[9], gas chromatography–mass spectrometry[10] (GC–MS) and fast atom bombardment[11] in the analysis of glucosinolates, desulphoglucosinolates (Fig. 1, R′ = OH) and their volatile derivatives, furthermore an high-performance liquid chromatographic (HPLC) method for the analysis of glucosinolates has been developed[12]. This entails a simple on-column enzyme treatment of a glucosinolate extract with sulphatase, producing desulphoglucosinolates[13,14], which are then separated by reversed-phase gradient elution with UV detection at 230 nm. A logical extension to this analytical scheme is the application of LC–MS as a qualitative analytical technique and this was investigated with the aid of a TSP interface.

A number of desulphoglucosinolate standards were examined by TSP in order to evaluate the LC–MS method and to optimise the performance of the interface. The promising results obtained in these preliminary studies were sufficiently encouraging to prompt us to investigate desulphoglucosinolate mixtures obtained from natural sources. A typical example, which neatly demonstrates the main TSP LC–MS characteristics of desulphoglucosinolates, is the analysis of material derived from seeds of *Brassica campestris*. The total ion current chromatogram (background subtracted spectra) is compared to the UV chromatogram in Fig. 2 where details of the chromatographic conditions are also given. LC with UV detection and LC–MS were performed on columns which contained the same packing material, but yielded slightly different retention times, however the elution order was identical for each column.

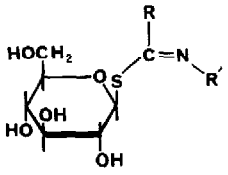


Fig. 1. Structure of glucosinolates and desulphoglucosinolates.

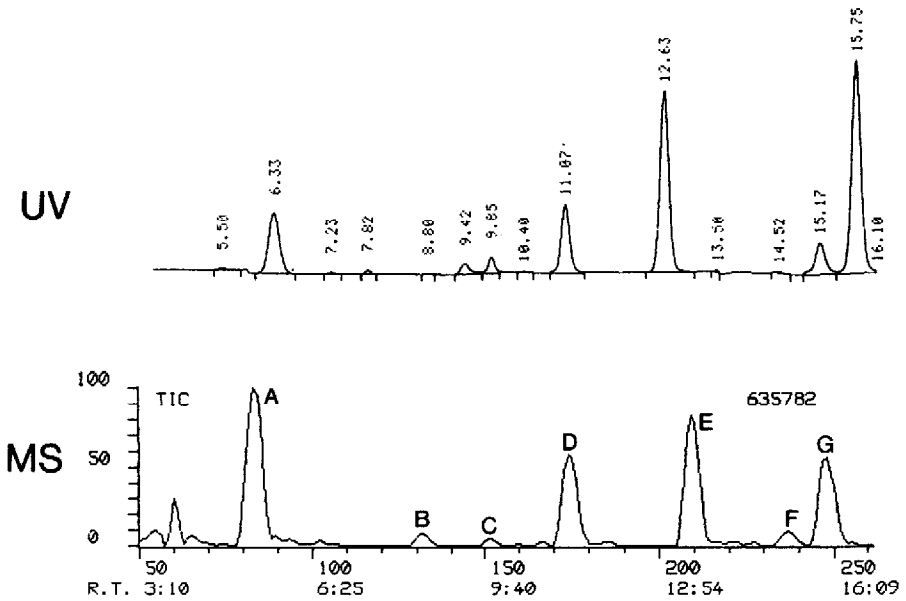


Fig. 2. LC–UV and LC–MS chromatograms of desulphoglucosinolates from *Brassica campestris* seeds. Column, Spherisorb 5 ODS2; flow-rate, 1.5 ml/min; solvent A, 0.1 *M* ammonium acetate; solvent B, acetonitrile–0.1 *M* ammonium acetate (20:80). Gradient: A–B (99:1) for 1 min, then to A–B (1:99) in 20 min.

LC–MS sensitivity was maintained by decreasing the vaporiser temperature slowly during the LC gradient, thus compensating for the linearly increasing organic content of the solvent. Peak G (Fig. 2) is an internal standard (desulphoglucotropaeolin) used for LC–UV quantification.

The mass spectrum of peak A (identified as desulphoprogoitrin) is shown in Fig. 3 and exemplifies many of the features found in the TSP mass spectra of desulphoglucosinolates. A protonated molecular ion, $[M + H]^+$, is evident at m/z 310 and is accompanied by a small ammonium adduct peak, $[M + NH_4]^+$ at m/z 327 (ammonium adducts are not always present). A peak 179 daltons below the molecular ion is evident in the TSP spectra of all desulphoglucosinolates studied so far and is frequently the base peak in the spectrum, as in this case (m/z 131). This peak probably arises by attack of $[NH_4]^+$ on the intact molecule, yielding a rearrangement peak of general formula $[RNCO + NH_4]^+$. Characteristic peaks related to the glycosidic part of the molecule occur at m/z 180, 198, 214 and 240 (the last peak is often weak or even absent). Fragment ions occurring in the spectra of desulphoglucosinolates are rationalised in scheme 1, however, some of the assignments must be considered tentative until supporting evidence is forthcoming.

Peak D (Fig. 2) presents an interesting example of one of the ways in which LC data can be enhanced by MS detection. In both the UV and total ion current traces this chromatographic peak appears to be a single component. The mass spectrum at the peak maximum (Fig. 4) appears to be an intense spectrum of desulphogluconapin (mol.wt. 293), however a number of anomalous ions are evident, particularly at m/z 177 and 193. These ions are characteristic of desulphoglucoalyssin

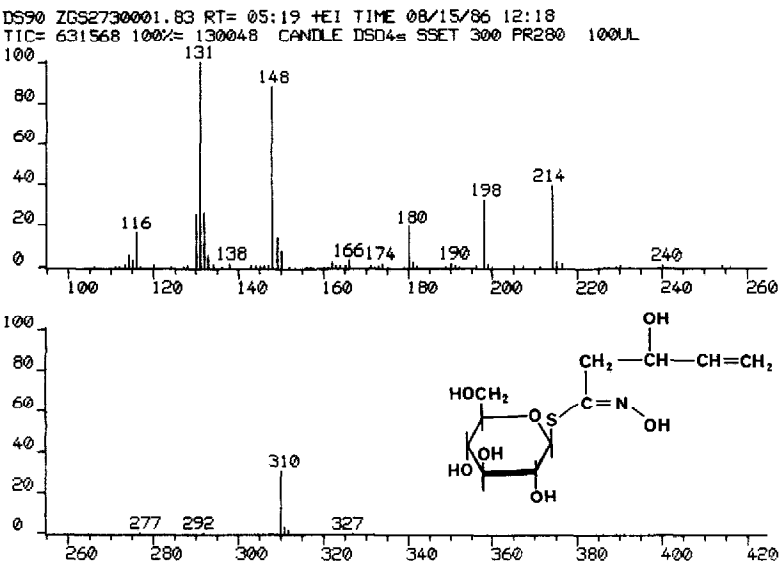


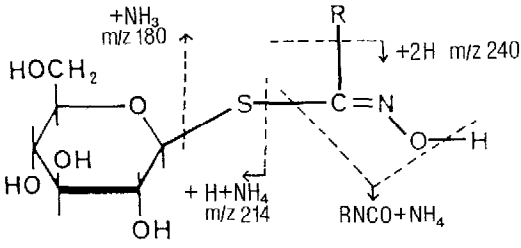
Fig. 3. TSP mass spectrum of desulphoprogoitrin (mol. wt. = 309).

(mol.wt. 371). When a mass chromatogram of ions typical of both suspected components is plotted across peak D (Fig. 5a), it is even clearer that two compounds are present. Computer manipulation of the overlapping components produces two "pure" spectra (Fig. 5b and c) which confirm the identity of the desulphoglucosinolates.

A total of eight glucosinolates were identified from the LC–MS run (Table I). Where protonated molecule ions were absent because of the low intensity of the spectrum (peak B) it was still possible to identify the desulphoglucosinolate from the characteristic fragment ions and retention time.

Interestingly, those desulphoglucosinolates which are most difficult to characterise by GC–MS of their volatile derivatives also yielded low intensity molecular ions in TSP LC–MS mode. When sulphinyl or indolic groups were present, GC–MS of the trimethylsilyl derivatives frequently resulted in weak or absent molecular ions or anomalous chromatographic responses[15]. The reactive indolyl and alkyl sulphinyl R groups appear to influence mass spectrometric behaviour in both types of analytical scheme.

TSP LC–MS clearly shows considerable promise as a general technique for the



Scheme 1. Fragmentation scheme for TSP mass spectra of desulphoglucosinolates.

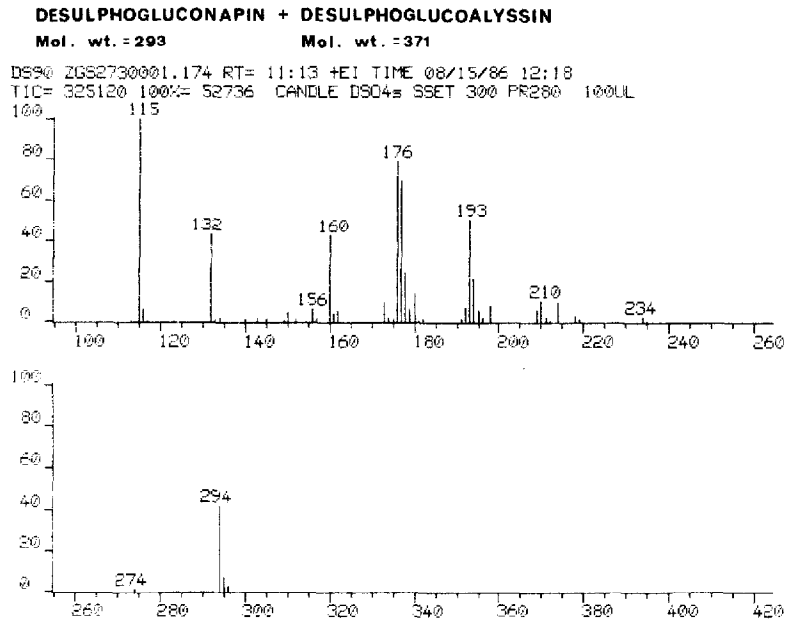


Fig. 4. TSP mass spectrum, Peak D, Fig. 3.

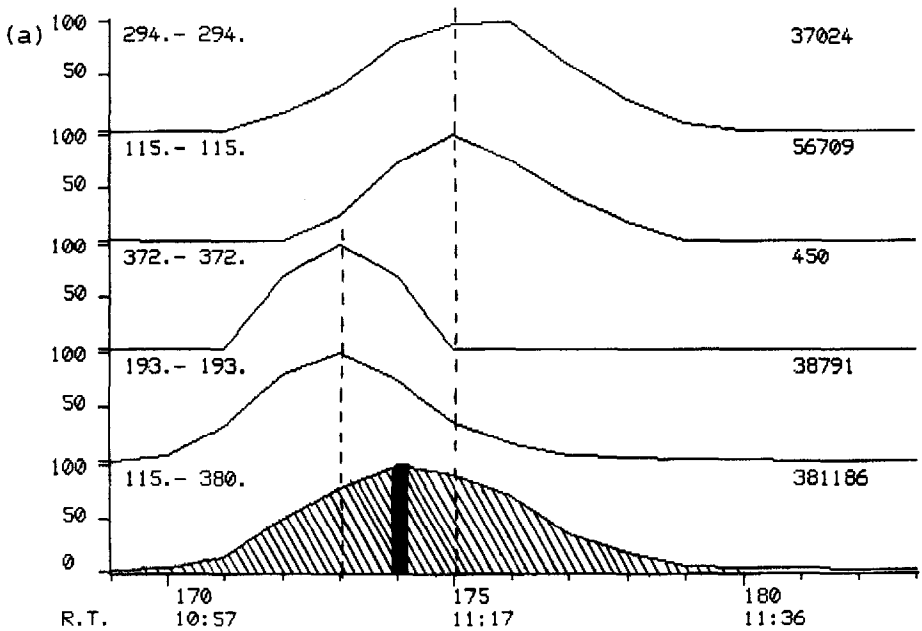


Fig. 5.

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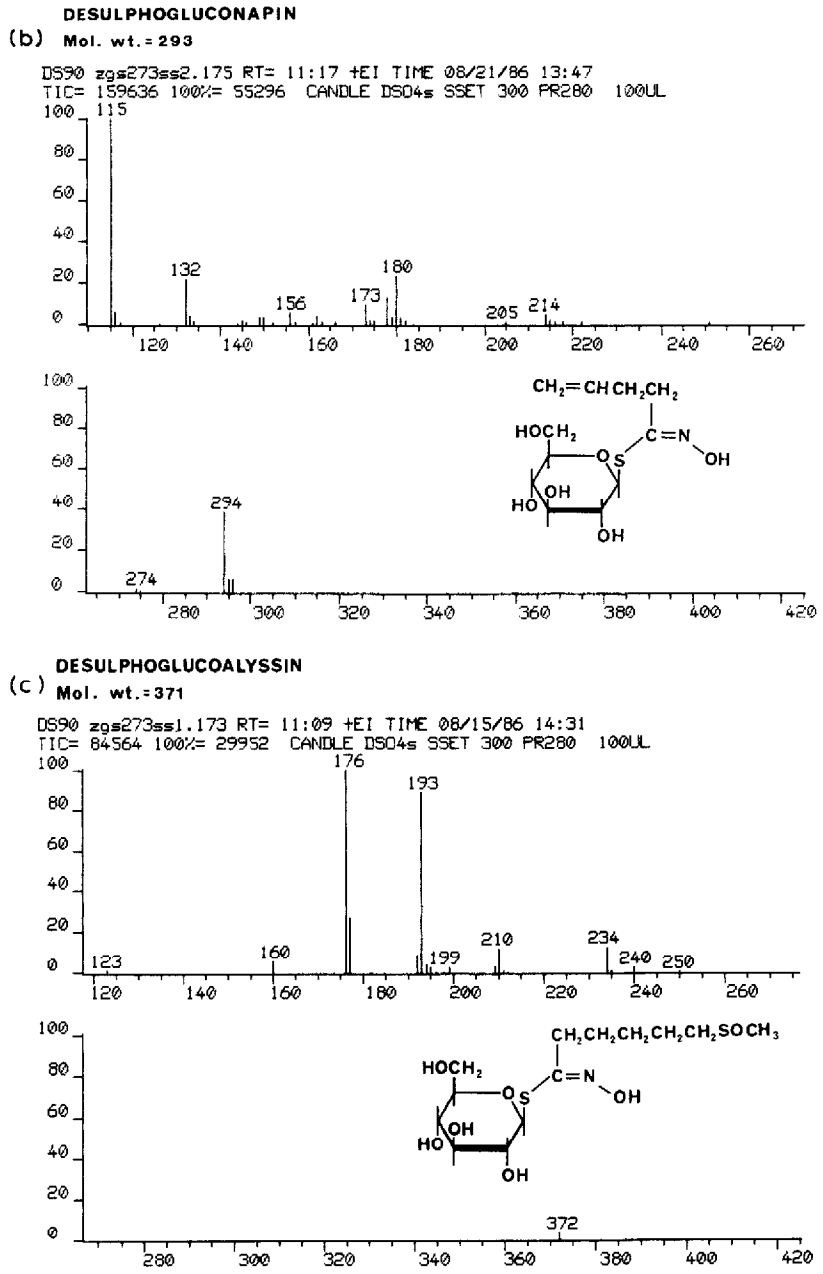


Fig. 5. Mass chromatogram (a) and resolved spectra (b and c) of the two components in peak D, Fig. 3.

qualitative analysis of desulphoglucosinolates. Detection limits for full scan spectra, including the molecular ion, were very dependent on the nature of the R group but were generally in the range 200 ng to 2 μg. Diagnostic fragments were sufficient to identify those molecules which yielded weak molecular ions, however, and in such

TABLE I

COMPOUNDS IDENTIFIED IN A DESULPHATED EXTRACT OF SEEDS OF *BRASSICA CAMPESTRIS*

Peak (Fig. 1)	*Mol. wt.*	*Characteristic ions (% base)*		*Identification*
		$[M + H]^+$	$[RNCO + NH_4]^+$	
A	309	37	100	Desulphoprogoitrin
B	357	–*	42	Desulphoglucoraphanin
C	323	24	100	Desulphonapoleiferin
D (i)	379	3	89	Desulphoglucoalyssin
(ii)	293	39	100	Desulphogluconapin
E	384	2	10**	Desulpho-4-hydroxyglucobrassicin
F	307	100	12	Desulphoglucobrassicanapin
G	329	100	74	Desulphoglucotropaeolin***

* Protonated molecule not seen. Peak identified by characteristic fragment ions and retention time.
** Base peak is *m/z* 189, assigned to $[RNCO + H]^+$.
*** Internal standard.

cases the detection limits would drop to the lower end of the range given. Improvements in TSP ion source design (better ion extraction efficiencies, improved design of the sampling cone etc.) are expected to produce still lower detection limits. Ion-pair LC methods for the examination of intact glucosinolates have also been developed[16] and it may be possible to modify these to ensure compatibility with TSP LC–MS. This will be investigated in the near future.

The cytokinins

The study of plant growth control hormones and their mode of regulation is an important area of agricultural research, not least because it could lead, via chemical application or genetic engineering, to improved crop yields and resistance to disease and environmental stress. The cytokinins are one group of phytohormones which are of considerable interest for the above reasons. Mass spectrometric methods for the qualitative and quantitative determination of cytokinins have largely relied on GC–MS of volatile derivatives[17]. A more elegant and less time-consuming approach would be to employ on-line LC–MS of free cytokinins, consequently we have investigated the potential of TSP LC–MS in the qualitative analysis of several zeatins (Fig. 6). Zeatin and dihydrozeatin yield intense TSP spectra (detection limits *ca.* 10 ng, full scan spectra) containing $[M + H]^+$ peaks and no detectable fragments. Detection limits were approximately an order of magnitude worse for the ribosidic zeatins. The TSP LC–MS spectrum of a standard solution of dihydrozeatin riboside (Fig. 7) displays a protonated molecule ion and a fragment which corresponds to the protonated base. The drop in detection limit may be partly attributable to thermal decomposition of the molecule; some tailing of the basic fragment was evident. We have frequently observed that an overall loss of TSP sensitivity occurs (compared to that found for the parent molecule) when a thermally or chemically labile group is attached to an otherwise stable molecule.

The results obtained with cytokinin standards were sufficiently encouraging for us to investigate samples obtained from natural sources. Purified extracts of sap

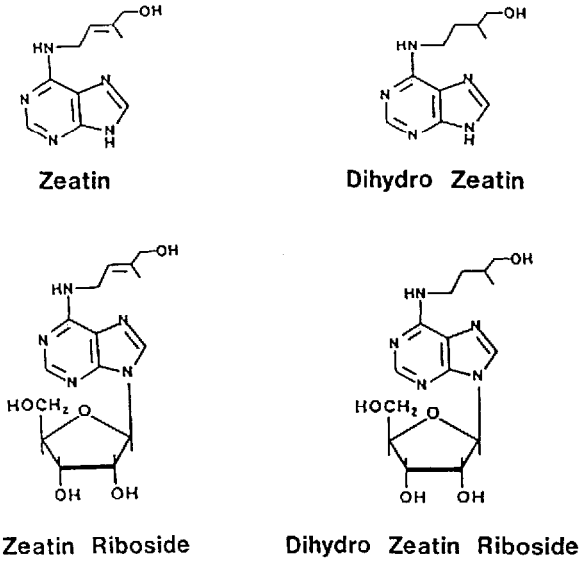


Fig. 6. The structures of the zeatins.

obtained from *Phaseolus vulgaris* L. (commonly known as the French or kidney bean) were fractionated and subjected to TSP LC–MS analysis. A representative chromatogram is shown in Fig. 8. The spectra of the two main peaks (Fig. 9), although not identical to standards run under ideal conditions were sufficiently similar to spectra of authentic zeatin riboside and dihydrozeatin riboside, run on the same day, to confirm the presence of these two cytokinins in this particular fraction of the sap extract. The differences between the spectra of dihydrozeatin riboside shown in Figs.

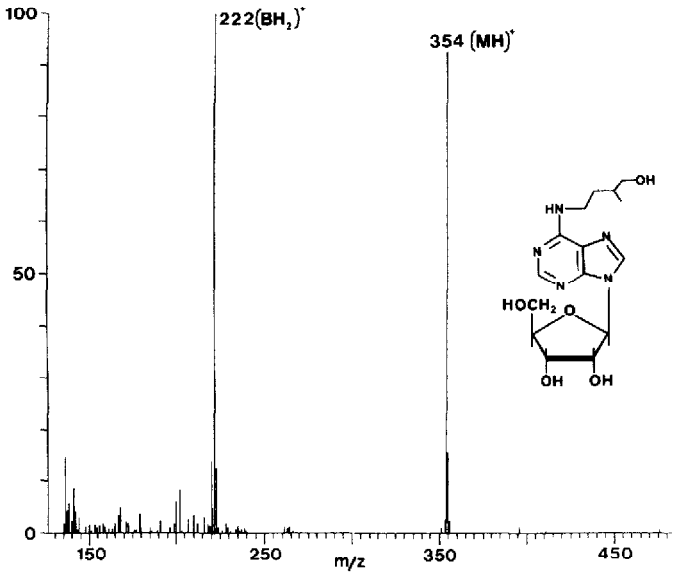


Fig. 7. TSP spectrum of dihydrozeatin riboside.

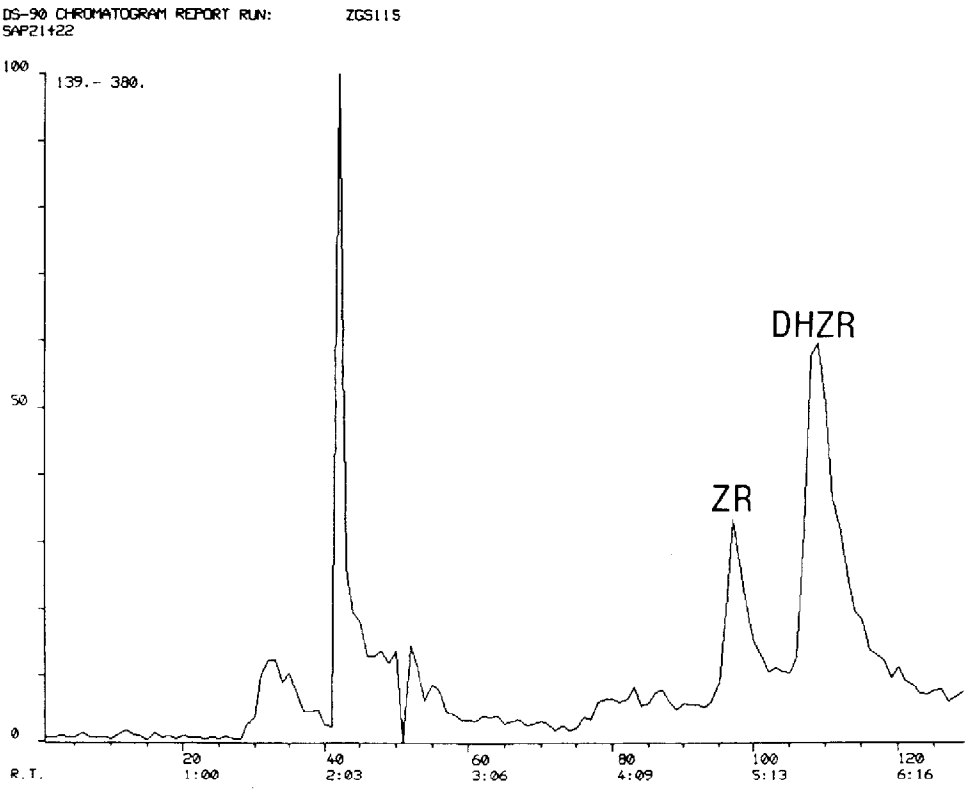


Fig. 8. LC–MS chromatogram of cytokinins from sap of *Phaseolus vulgaris* L. Column, Hypersil ODS; flow-rate, 1 ml/min; solvent A, acetonitrile–0.1 *M* ammonium acetate (15:85); solvent B, acetonitrile. Gradient, 100% A to A–B (55:45) in 20 min. Masses summed: *m/z* 139–380.

7 and 9 are an extreme examples of changes in the day-to-day reproducibility of TSP spectra. The reduced performance was in this case almost certainly caused by a build up of involatile material in the heated capillary, causing the spray to emerge at an acute angle. However, even under these conditions it was still possible to obtain useful data.

Biotechnological applications

The use of plant cell cultures to produce secondary products of commercial value to food manufacturers is under active investigation at the Institute of Food Research, Norwich Laboratory[18] and the potential of linked scanning mass spectrometry for identifying alkaloids produced by cell suspension cultures has been explored[19]. Although it was possible to identify major components in crude extracts by solids probe mass spectrometry and B/E scanning, complete analysis of the complex mixtures produced by the cell cultures was not possible. LC–MS of cinchona alkaloids using a belt interface has already proved to be successful[20], consequently it was decided to investigate the potential of TSP LC–MS in the analysis of cell culture product.

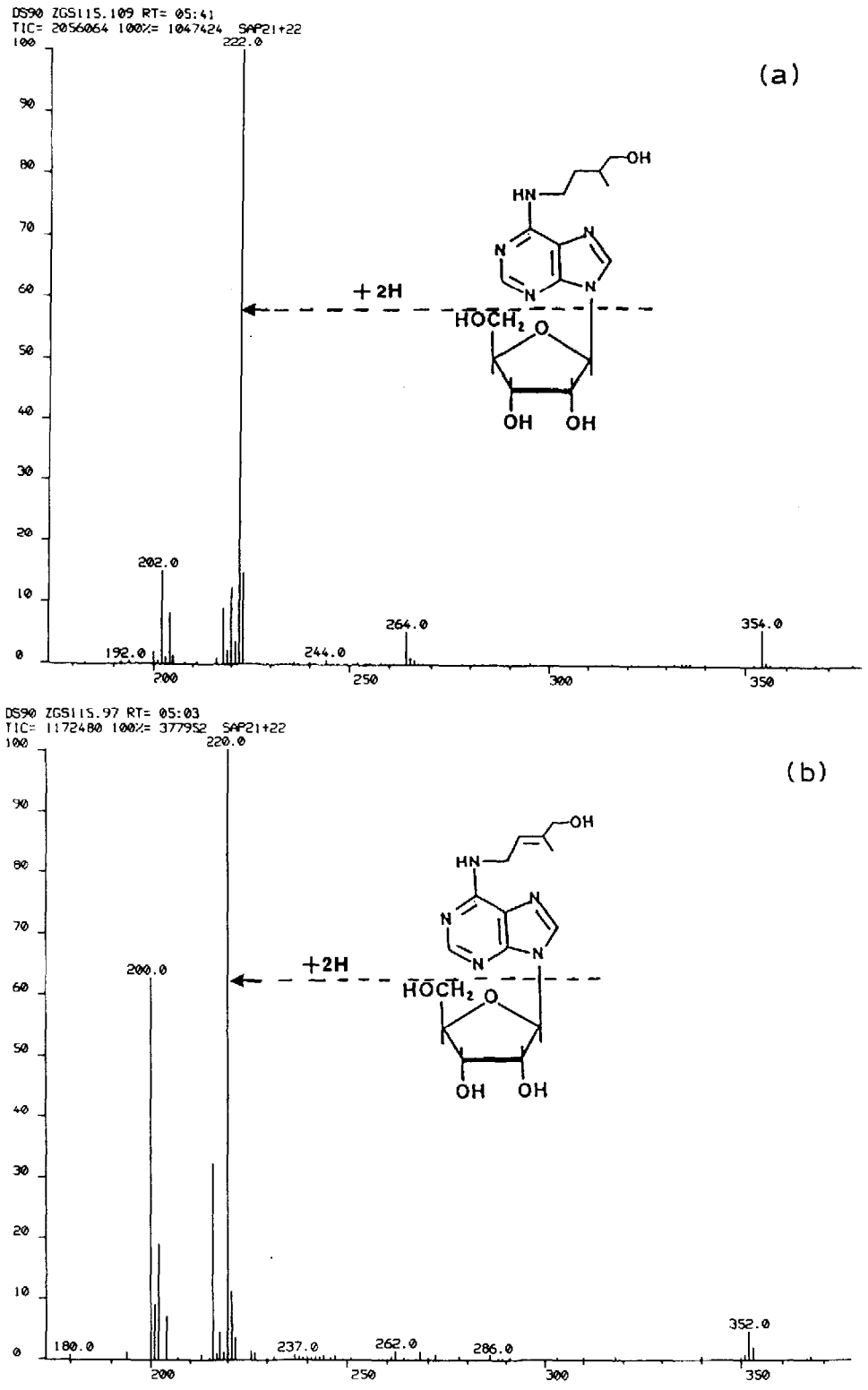
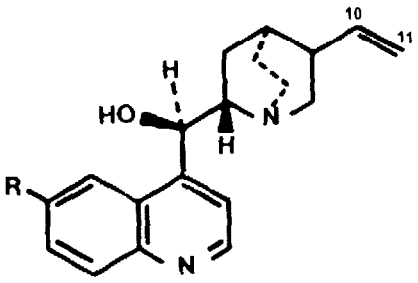
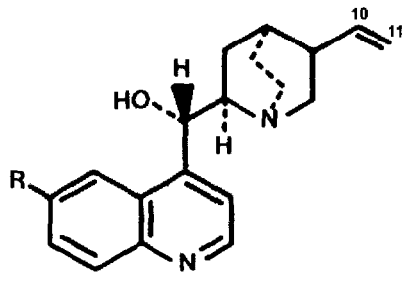


Fig. 9. TSP spectra of peaks ZR (b) and DHZR (a) in Fig. 8.



R = CH_3; Quinine
R = OCH_3, 10,11 = Dihydro; Quinidine
R = H; Cinchonine
R = H, 10,11 = Dihydro; Dihydrocinchonine



R = OCH_3; Quinidine
R = OCH_3, 10,11 = Dihydro; Dihydroquinidine
R = H; Cinchonidine

Fig. 10. Structures of the cinchona alkaloids.

Cinchona alkaloids (Fig. 10) yielded very simple TSP mass spectra containing only protonated molecules and associated isotope peaks. The mass spectrum of cinchonine (Fig. 11) is typical (low mass peaks are unsubtracted background ions). A mass chromatogram obtained from an isocratic TSP LC–MS run on authentic cin-

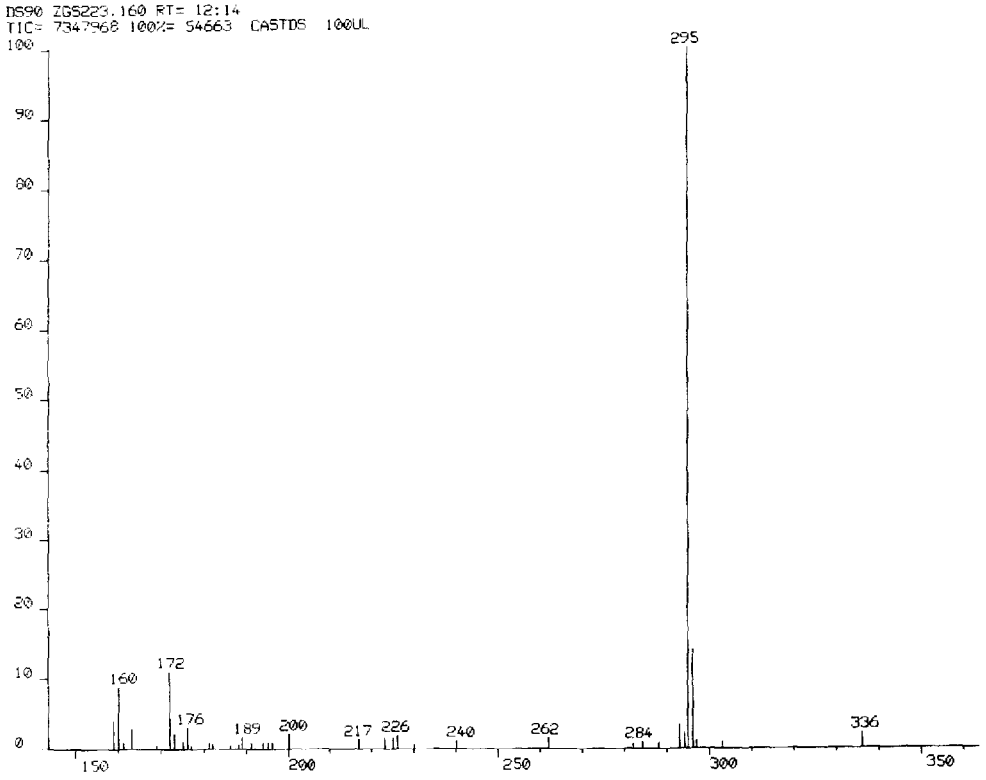


Fig. 11. TSP spectrum of cinchonine.

chona alkaloids is shown in Fig. 12. A similar run (Fig. 13) on a mixture obtained from a cell suspension culture confirmed the molecular weights of the extracted alkaloids (full scan spectra were also examined to ensure the absence of other components). The TSP LC–MS technique does not yield as much information as belt LC–MS[20] but is nevertheless useful for confirming molecular weights and is being used routinely for analysing cell cultures producing datura, nicotine and other classes of alkaloid. TSP LC–MS in conjunction with collisional activation and linked scanning at constant B/E may provide more useful structural information and this will be explored in the near future.

Polyphenols

Polyphenols (*syn.* vegetable tannins) determine the characteristics of many plant products, influencing their taste, palatability, nutritional value, pharmacological and toxic properties and microbial decomposition. A comprehensive investigation of the fast atom bombardment mass spectrometry of polyphenols has been published recently[21]. We have undertaken some preliminary studies of the TSP mass spectra of this important group of compounds and report here a brief comparison of the negative ion TSP (loop injection in 0.1 *M* ammonium acetate) and FAB spectra of a procyanidin isolated from cider apples (Fig. 14). An $[M - H]^-$ ion is visible in both spectra, together with fragment ions indicative of the mass of the monomer (*m/z*

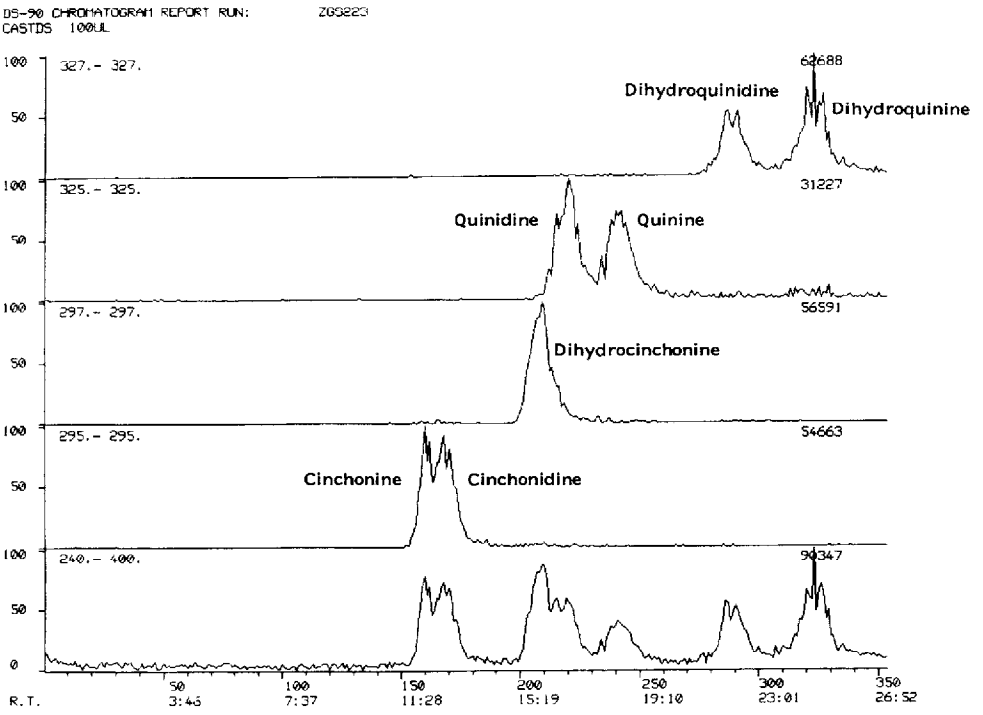


Fig. 12. TSP mass chromatogram of authentic cinchona alkaloids. Column, Waters μBondapak C_{18}. Solvent, 0.1 *M* ammonium acetate–acetic acid–acetonitrile–tetrahydrofuran (86.5:3:10:0.5). Flow-rate, 1.5 ml/min.

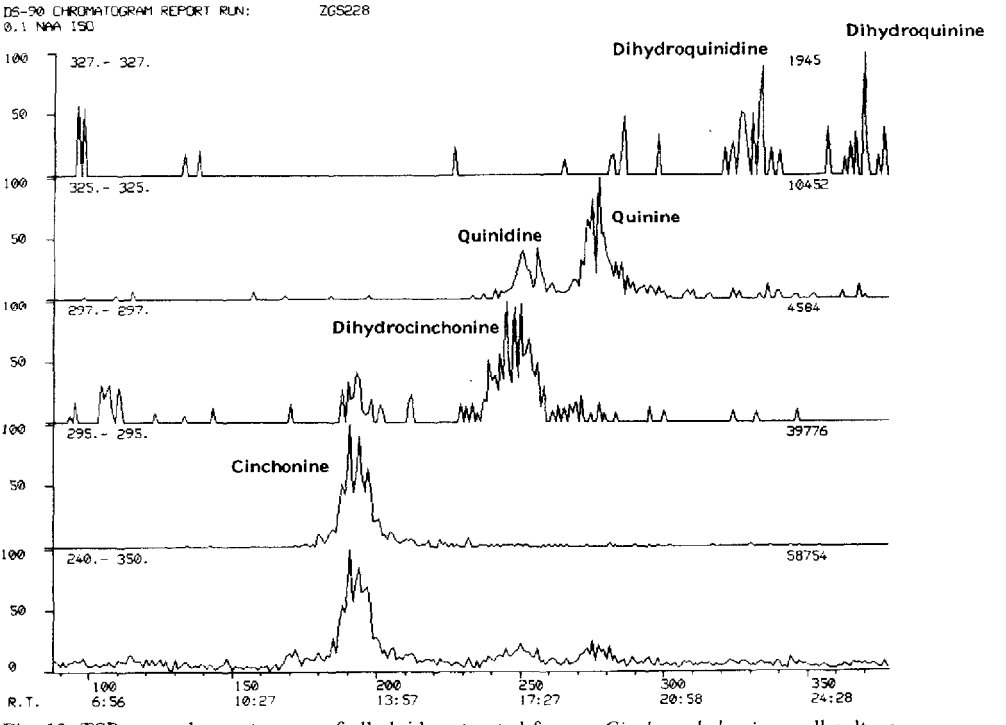


Fig. 13. TSP mass chromatogram of alkaloids extracted from a *Cinchona ledgeriana* cell culture.

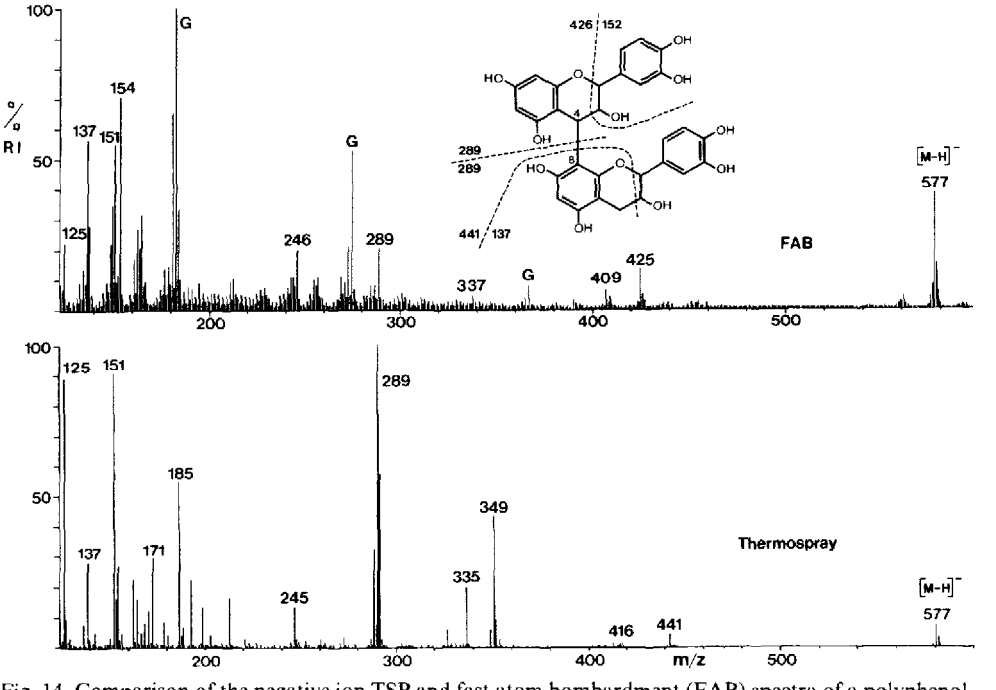


Fig. 14. Comparison of the negative ion TSP and fast atom bombardment (FAB) spectra of a polyphenol.

289) and other moieties in the molecule. An acetic acid adduct of the monomer is also visible in the TSP spectrum at *m/z* 349. Molecular weight information was only obtained in negative ion mode; positive ion operation failed to produce significant ions above the mass of the protonated monomer (*m/z* 291). These initial data clearly demonstrate the potential of negative ion TSP LC–MS in the analysis of plant polyphenols.

CONCLUSIONS

TSP LC–MS on a magnetic sector mass spectrometer has produced useful analytical data in several disparate areas of natural product research in agricultural and food chemistry. In all cases LC conditions could be adapted very simply to ensure compatibility with LC–MS operation. Molecular weight information was complemented by diagnostic fragmentation when thermally or chemically labile molecules were examined. Improvements in ion source design are expected to improve TSP detection limits, which are not yet comparable to GC–MS, and extend the range of compounds amenable to LC–MS analysis.

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REFERENCES

1 C. R. Blakley, J. J. Carmody and M. L. Vestal, *J. Am. Chem. Soc.*, 102 (1980) 5931.
2 M. L. Vestal and G. J. Ferguson, *Anal. Chem.*, 57 (1985) 2373.
3 M. L. Vestal, *Anal. Chem.*, 56 (1984) 2590.
4 J. R. Chapman, *J. Chromatogr.*, 323 (1985) 153.
5 A. Kjaer, *Chem. Org. Naturst.*, 18 (1960) 122.
6 G. R. Fenwick, R. K. Heaney and W. J. Mullin, *CRC Crit. Rev. Food Sci. Nutr.*, 18 (1983) 123.
7 D. I. McGregor, W. J. Mullin and G. R. Fenwick, *J. Assoc. Off. Anal. Chem.*, 66 (1983) 827.
8 G. R. Fenwick, J. Eagles, R. Gmelin and D. Rakow, *Biomed. Mass Spectrom.*, 7 (1980) 410.
9 J. Eagles, G. R. Fenwick, R. Gmelin and D. Rakow, *Biomed. Mass Spectrom.*, 8 (1981) 265.
10 J. Eagles, G. R. Fenwick and R. K. Heaney, *Biomed. Mass Spectrom.*, 8 (1981) 278.
11 G. R. Fenwick, J. Eagles and R. Self, *Org. Mass Spectrom.*, 17 (1982) 544.
12 E. A. Spinks, K. Sones and G. R. Fenwick, *Fette. Seifen. Anstrichmittel*, 86 (1984) 228.
13 I. Minchinton, J. Sang, D. Burke and R. J. W. Truscott, *J. Chromatogr.*, 247 (1982) 141.
14 E. A. Spinks, G. R. Fenwick and W. T. E. Edwards, *Cruciferae Newsletter*, 8 (1983) 48.
15 J. Eagles and R. K. Heaney, personal communication.
16 P. Helboe, O. Olsen and H. Sørensen, *J. Chromatogr.*, 197 (1980) 199.
17 L. M. S. Palni, S. A. B. Tay and J. K. McLeod, in H. F. Linskens, B. D. Sanwal and M. V. Tracey (Editors), *Modern Methods of Plant Analysis: Gas Chromatography–Mass Spectrometry*, Vol. 3, Springer, Berlin, 1986.
18 R. J. Robins, J. Payne and M. J. C. Rhodes, *Planta Med.*, (1986) 220.
19 F. A. Mellon, R. J. Robins and M. J. C. Rhodes, *Biomed. Env. Mass Spectrom.*, 13 (1986) 155.
20 D. E. Games, C. Eckers, E. Lewis, K. R. N. Rao, M. Rossiter and N. C. A. Weerasinghe, in A. Quayle (Editor), *Advances in Mass Spectrometry*, Vol. 8, Heyden, London, 1980, p. 1396.
21 R. Self, J. Eagles, G. C. Galletti, I. Mueller-Harvey, R. D. Hartley, A. G. H. Lea, D. Magnolato, U. Richli, R. Gujer and E. Haslam, *Biomed. Environ. Mass Spectrom.*, 13 (1986) 449.