III Advances in the Analysis of Trace Organic Constituents of the Diet, with Particular Reference to Mass Spectrometry

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1 Introduction

What is meant by a trace organic constituent of a food? Each generation of food chemists would give a different answer. For us in 1980 it is appropriate to define 'trace' as meaning constituents present at or below the parts per billion (p.p.b.) level, but because we are primarily concerned with instrumental techniques it is more precise to talk about submicrogram levels of detection. The adequacy of such levels of sensitivity will depend upon the nature of the problem.[1] For example, it may only be necessary to establish that the compound of interest is above or below a specified limit; on the other hand, it may be the minimum possible detectable level that is sought, for monitoring known potently toxic or carcinogenic constituents. From an analytical point of view, achieving the latter presents the greatest challenge, and it is the wide range of different methods available for trace organic analysis that is in need of careful assessment to ascertain which ones are suitable for a particular job.

Usually there are four stages in the estimation of a trace organic compound, sampling, extraction, separation, and detection, and it is important to be able to identify initially the most likely sources of error. Whilst the importance of sampling from biological materials should not be overlooked, it is not normally a serious methodological problem in food analysis, provided that the proper procedures have been implemented. The extraction of the compound of interest from this sample, however, can involve several processes which are difficult to perform accurately because of the physical and compositional complexities of food matrices. Relatively little progress has been made of late in this area, which contrasts sharply with the innovations in the separation and detection stages.

The purpose of this review is to consider the assay as a whole, from sampling through to detection, and to draw upon some specific examples from the literature to illustrate critically both the advantages and shortcomings of alternative techniques that might be considered for a particular trace analysis problem. Throughout, mass spectrometry has been used as a linking theme, not because it

[1] W. Horwitz and J. W. Howard in 'Trace Organic Analysis: A New Frontier in Analytical Chemistry', NBS Spec. Publ. 519, ed. H. S. Hertz and S. N. Chesler, Washington D.C., 1979, p. 231.

can in any way be described as a 'widely used' or 'freely available' technique but because technically mass spectrometry is unquestionably in the forefront as regards recent advances in trace analysis and because as an analytical tool it is unsurpassed both for versatility of application and in terms of specificity and sensitivity.

2 Sample Preparation

The sample submitted to the analytical laboratory may not be representative of the batch of material from which it was taken. Furthermore, even if it were when it was collected, its temporary storage may have seriously affected the level of any trace organic constituent. The losses caused by adsorption onto container surfaces, evaporation, thermal decomposition, and exposure to light can often be great enough to invalidate the results of the analysis.[2] The maximum sample size that can be used for analysis is often determined by the extraction/concentration procedures that follow. It is not always possible to take samples large enough to be truly representative of the bulk material because unacceptable errors would be introduced at the concentration stage if large volumes of solvent have to be used to ensure complete extraction.

It is at the extraction stage that an internal standard can be added to help compensate for these losses and for any further losses that may be attributed to adsorption later on in the assay.[3] The conventional practice of 'spiking' with the standard compound at the extraction stage to determine the recovery has always been viewed with caution because it cannot be proved that spiking truly simulates the physical condition of the trace compound in the biological matrix. But no matter how problematical these procedures are, it is essential to persevere with the quantification of this unsatisfactory aspect of the assay.

Solvent extraction is seldom specific for the analyte, and often trace impurities are introduced from the solvent itself. Depending on the specificity of the detection method employed, some degree of clean-up will be required. The most popular methods are adsorption chromatography, gel permeation, and ion exchange, which can cope with the large volume of solvent, but after an evaporation step (unfortunately prone to large errors) other conventional chromatographic techniques, *e.g.* thin-layer chromatography, have been used. Extra care is required when internal standards are present, to ensure that they pass through the chromatography stage with the compound of interest. The cleaned-up extract is then evaporated to low volume or dryness, depending on the next stage. Even with an internal standard and after deactivation of the glassware with silylating agents, this is still a vulnerable procedure.

There have been few improvements in the isolation and concentration methodology since the pioneering work in flavour chemistry during the 1960s,[4] but the

[2] H. S. Hertz, W. E. May, S. A. Wise, and S. N. Chesler, *Anal. Chem.*, 1978, **50**, 428A.
[3] B. J. Millard, Quantitative Mass Spectrometry in Life Sciences II, Proc. 2nd Int. Symp., Ghent, 13—16 June 1978, ed. A. P. de Leenheer, R. R. Roncucci, and C. van Peteghem, Elsevier S.P. Co., Amsterdam, 1978, p. 83.
[4] J. M. H. Bemelmans, 'Progress in Flavour Research', ed. D. G. Land and H. E. Nursten, Applied Science Publishers, London, 1979, p. 79.

use of organophilic resins, *viz* XAD-2 and Tenax, is worthy of note.[2,5] The improvement of microchemical techniques for trace organic analysis must be the foundation work urgently required to increase the reliability of this stage of the assay.

It may be necessary to alter the molecular structure of the compound of interest in order to provide enhanced volatility for further chromatography or to incorporate a feature which will increase the specificity to detection. The most popular derivatives are those that do both, *e.g.* pentafluorophenyldimethylsilyl ether.[6,7] There is a wide choice of derivatives and derivatization methods,[8] but only those capable of working efficiently on the microscale will be acceptable.

It may be concluded that detection should be as sensitive and selective as possible so that the number and extent of these preliminary stages can be kept to a minimum.

3 Identification and Confirmation of Identity

Compound identification is an obvious prerequisite of any quantitative assay. Mass spectrometry and combined g.c.–m.s. are often the only techniques with sufficient sensitivity for trace organic analysis. Modern rapid-scanning mass spectrometers with on-line computerized data acquisition and processing facilitate the collection of several thousand spectra during the g.c.–m.s. analysis. These can be tested for information content (mass resolved chromatography), the redundant spectra discarded, and the remainder searched, in the forward or reversed mode, using a library collection. Reversed searching using small specialized collections is particularly useful for the semi-quantitative screening of trace compounds.

Alternatively, the mass-spectral data accumulated during g.c.–m.s. runs can be presented as a chromatographic profile by using a particular ion or any selection of ions in the spectrum (mass chromatography) in order to indicate the presence of particular compounds in complex mixtures submitted for rapid testing. In conjunction with the g.c. retention data, a known compound can be recognized from the partial mass spectrum. Typically 10—100 ng samples are required for interpretation.

4 Chromatography with General-purpose Detectors

Typical extracts submitted for analysis require further purification to ensure that the compound of interest is free from substances which could cause chemical interference with its quantitative estimation. General-purpose detectors are invaluable in dealing with mixtures of unknown volatile compounds, and the flame-ionization detector (FID) for gas chromatography has been extensively used in food analysis. However, if a general-purpose detector is to be used, it is

[5] W. Averill, *Chromatogr. Newsl.*, 1978, **6**, 4.
[6] A. J. Francis, E. D. Morgan, and C. F. Poole, *J. Chromatogr.*, 1978, **161**, 111.
[7] A. J. Francis, E. D. Morgan, and C. F. Poole, *Org. Mass Spectrom.*, 1978, **13**, 671.
[8] K. Blau and G. King, 'Handbook of Derivatives for Chromatography', Heyden and Son Ltd., London, 1978.

essential to optimize the selectivity of the chromatographic separation. There have been several developments in this area (see below).

A. The Improved Manufacture of Glass Capillary Columns.—The high adsorption levels previously associated with glass support-coated and stainless-steel wall-coated open tubular columns have been greatly reduced by improved surface deactivation techniques applied to the latest glass and fused-silica columns.[9,10] It is now possible to detect low picogram quantities of, *e.g.*, 2,4-dimethyl aniline and 2,6-dimethylphenol (acid/base test compounds) with a FID.[11]

A particularly effective use of high-efficiency capillary column analysis is in the separation of amino-acid enantiomers with specially prepared chiral stationary phases.[12] The unnatural enantiometers can then be used as internal standards for quantitative studies.

B. New Developments in Capillary Column Injector Design.—The previous inherent disadvantage of capillary columns – the low capacity for injected volumes of solvent – can now be overcome by new injectors utilizing the 'solvent effect'[13,14] (injections of up to 5 μl of solvent), meaning that a wide range of compounds of interest can be accommodated.

C. The Use of Mixed Stationary Phases.—A development with potential application to trace quantitative analysis is the manufacture of columns specially designed to separate a particular component from the rest of the mixture. If two different monophasic columns cannot separate the compound of interest from interfering constituents, it should be possible to calculate the percentage composition of a mixture of the phases and manufacture a column that will produce the desired effect.[15]

The introduction of liquid chromatography (l.c.) to quantitative analysis has extended the range of compounds to include those which are thermally unstable or too involatile for g.c. analysis even when derivatized. Recent advances in this area have been confined largely to the diversification of its application, but the development of microbore columns shows considerable promise.[16]

5 Chromatography with 'Specific' Detectors

Given unlimited time and resources, no doubt chromatography alone could provide the specificity required for use with general-purpose detectors, but in practice it is expedient to use detectors having increased specificity in conjunction with readily available chromatographic methods to provide combinations with

[9] G. Schomburg, H. Husmann, and H. Behlau, *Chromatographia*, 1980, **13**, 321.
[10] K. Grob and G. Grob, *J. High Res.*, 1980, **3**, 197.
[11] R. Self, unpublished observations.
[12] W. A. Konig and G. A. Nicholson, *Anal. Chem.*, 1975, **47**, 951.
[13] K. Grob and K. Grob, jun., *J. High Res. Chromatogr. Chromatogr. Commun.*, 1978, **1**, 57.
[14] K. Grob and K. Grob, jun., *J. Chromatogr.*, 1978, **151**, 311.
[15] R. J. Laub, J. H. Purnell, D. M. Summers, and P. S. Williams, *J. Chromatogr.*, 1978, **155**, 1.
[16] T. Takeuchi and D. Ishii, *J. Chromatogr.*, 1980, **190**, 150.

appropriate selectivity. The enormous variation in the properties of candidate organic compounds precludes any logical plan to simplify the present diversification in methodology. At this time it is only possible to make superficial comparisons (see below).

A. Comparison of Gas Chromatography Detectors.—A large number of g.c. detectors are available, of which those shown in Figure 1 are by no means exhaustive (additional examples being photoionization and ultrasonic and i.r.–u.v. spectroscopic detectors). The detection limits shown are very much general guidelines and in actual analyses the smallest detectable amount may be much more or much less, but they serve to give an impression of the relative merits and in particular indicate the position held by mass spectrometry when used for ion monitoring. The recently introduced negative-ion chemical ionization (n.i.c.i.) for quantitative estimation of electron-capturing compounds, or chemically prepared electron-capturing derivatives, offers unsurpassed sensitivity and considerable selectivity. The most selective detector is the thermal-energy analyser (TEA)[17] which was specially built for nitrosamine analysis.[18]

B. Comparison of Liquid Chromatography Detectors.—In Figure 2 the attainable limits of detection for l.c. are compared. There is no equivalent in l.c. to the universal and sensitive FID used in g.c. Refractive index and nephelometric detectors are really too insensitive for trace analysis. U.v. detectors are the most widely used for compounds with a chromophore, utility being extended by monitoring at 190—200 nm with the proper selection of a non-absorbing mobile phase. With fluorescence, one is beginning to approach comparable limits of detection to those of g.c., but this technique is necessarily limited to the few compounds with native fluorescence, *e.g.* aflatoxins, polyaromatics, or those compounds which can be derivatized to incorporate a fluorophore. It is the recent advances in liquid chromatography–mass spectrometry (l.c.–m.s.) coupling[19] which indicate the possible future direction of detection in l.c. The technique is in its infancy and many different approaches are being made. Moving belts were the first commercial system, but orifice types are making progress now. A direct coupling can be made if the l.c. solvent, after vaporization, can be re-used as the reagent gas for chemical ionization mass spectrometry (c.i.m.s.). Other innovations include the use of a microbore column[16] to reduce the quantity of solvent to a level which can be handled by conventional g.c.–m.s. interfaces, again provided that c.i.m.s. is employed. For quantitative analyses c.i.m.s. is not an impediment and, by analogy with g.c. capillary columns, microbore techniques will increase the selectivity (and sensitivity) of the assay. By using the mass spectrometer, it means that for the first time a specific, sensitive, and yet general-purpose detector is available, enabling previously intractable problems in food analysis to be tackled.

[17] D. H. Fine, R. Rufeh, D. Lieb, and D. P. Rounbehler, *Anal. Chem.*, 1975, **47**, 1188.
[18] T. A. Gough, K. S. Webb, and R. F. Eaton, *J. Chromatogr.*, 1977, **137**, 293.
[19] P. J. Arpino and G. Guichon, *Anal. Chem.*, 1979, **51**, 683A.

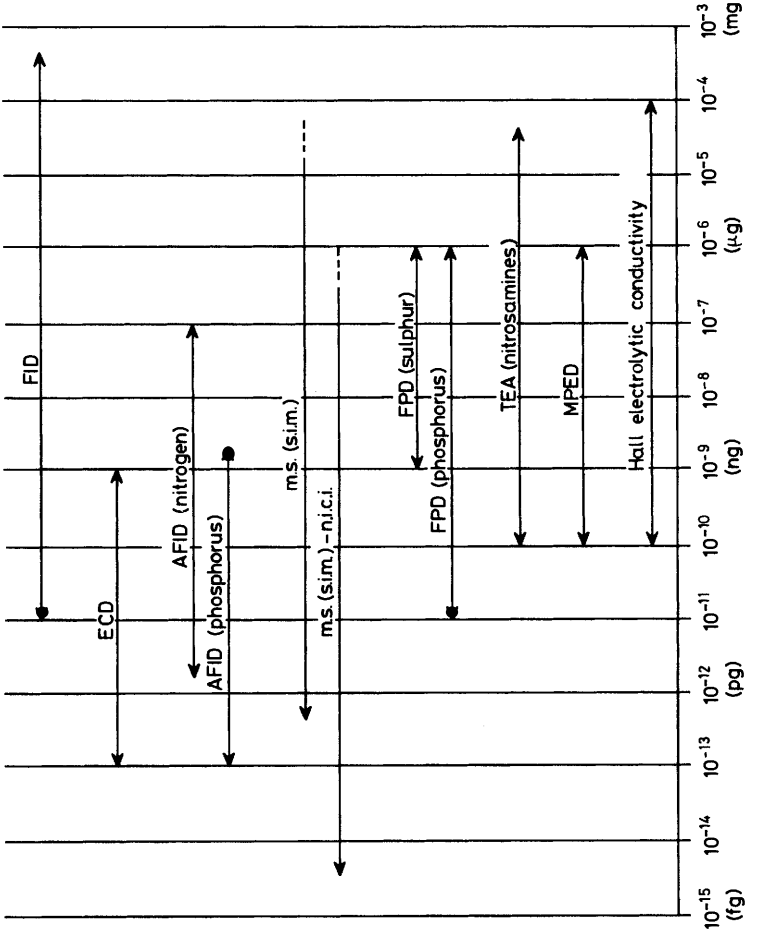

Figure 1 *Comparison of limits of detection and linear response for gas chromatographic detectors [FID = flame-ionization detector, ECD = electron-capture detector, AFID = alkali flame-ionization detector, m.s.(s.i.m.) = mass spectrometry (selected-ion monitoring), n.i.c.i. = negative-ion chemical ionization, FPD = flame photometric detector, TEA = thermal-energy analyser, MPED = microwave plasma-emission detector; the dashed right-hand end of an arrow indicates that the upper limit is uncertain]*

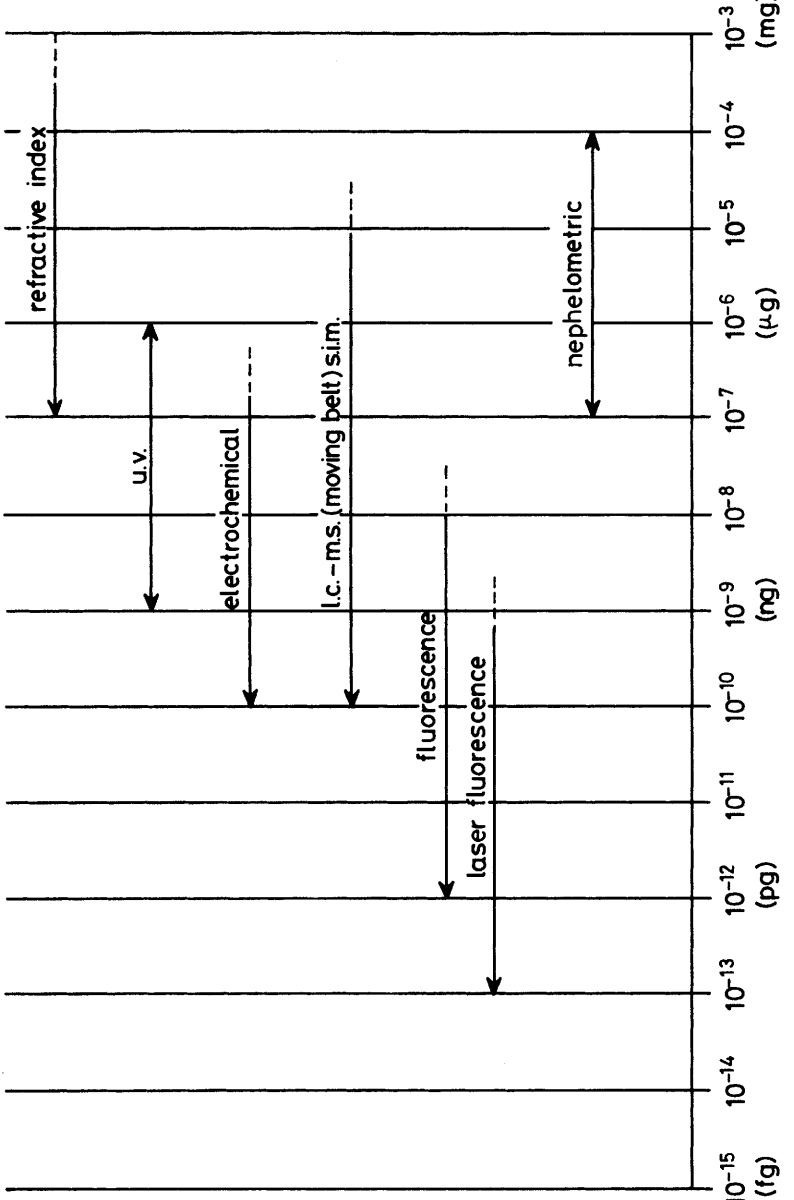

Figure 2 *Comparison of limits of detection and linear response for liquid chromatographic detectors (the dashed right-hand end of an arrow indicates that the upper limit is uncertain)*

6 Chromatography with the Mass Spectrometer as Detector

Some of the detectors mentioned above allow a choice to be made, *e.g.* among the different wavelengths, at which a compound may absorb radiation, so that selectivity can be maximized. This type of flexibility is extended by using the mass spectrometer as detector. The multitude of ionic species created from the sample and its chemical background (interference) are separated into discrete ion beams which, at low resolution, can contain mixtures of both isomeric and isobaric species but at optimum resolution contain only a mixture of isomeric ions. The species considered to offer the greatest specificity for the compound of interest are selected for detection – selected-ion monitoring (s.i.m.). There are two types of s.i.m.:

(*a*) single-ion monitoring, carried out with the mass spectrometer in the static mode, affords maximum sensitivity at optimum resolution but excludes the use of both isotopically labelled internal standards and multicomponent analyses, where two or more characteristic ions have to be detected, and

(*b*) multiple-ion monitoring (m.i.m.) with the mass spectrometer in the switched mode, which, although free from the above exclusions, can only be used with limited resolution and sensitivity.

S.i.m. methods in general are susceptible to instrument instability (drift).

The method of displaying on the oscilloscope the regions of the spectrum containing the selected ion (selected mass scanning) prevents drift errors and also gives a visual warning of any chemical interference from ions of closely similar mass. This has been the method of choice for some nitrosamine and mycotoxin assays.

It is generally accepted that single-ion monitoring will give higher sensitivity than m.i.m. at the same resolution, but the trade-off between sensitivity and resolution[20] and between precision under m.i.m. and high-resolution single-ion monitoring must be carefully 'balanced' against the requirements of every individual assay.

Faced with the problems of whether to use ion ratios or peak areas (heights), labelled or unlabelled internal standards, low-resolution g.c. with high-resolution mass spectrometry or *vice versa*, the analyst has resorted to trial and error methods, and only recently have attempts been made to rationalize the design of an assay from first principles.

Methods of ionization other than electron impact (e.i.) may provide both extra selectivity and increased sensitivity in certain cases, and there have been important recent advances in these areas (see below).

A. Chemical Ionization.[21,22]—Chemical ionization mass spectra are characterized by fewer fragment ions and intense even-electron pseudo-molecular ions.

[20] B. J. Millard, 'Quantitative Mass Spectrometry', Heyden and Son Ltd., London, 1978, p. 135.
[21] K. R. Jennings, 'Gas Phase Ion Chemistry', Academic Press, 1979, Vol. 2, p. 123.
[22] R. E. Mather and J. F. J. Todd, *Int. J. Mass. Spectrom. Ion Phys.*, 1979, **30**, 1.

This simplification of the spectrum means that m.i.m. of simple mixtures is then possible by direct-probe m.s. The enhancement of the molecular ion may also provide increased sensitivity, and an intensification of the ions of higher mass increases the specificity by reducing interference from chemical background ions which are usually of lower mass. The use of g.c.–c.i.m.s. is increasing for both rapid screening and accurate assays, especially for those compounds which are subject to thermal and e.i. induced decomposition.

B. Negative-ion Chemical Ionization.[23]—Two approaches to n.i.c.i. are possible: (*a*) for compounds of high electron affinity the use of conventional reagent gases (*e.g.* nitrogen, methane, or argon) to form electron attachment spectra or (*b*) for compounds of low electron affinity the use of reagent gases which themselves form negatively charged ions (*e.g.* Freons, methylene chloride, nitrous oxide, or even sulphur hexafluoride), whose role is anion-substrate formation through charge exchange or cluster formation.[24] By judicious choice of reagent gases ion–molecule reactions can be manipulated to yield structural information or to provide high-sensitivity detection by s.i.m. The increased sensitivity attainable with n.i.c.i. compared with positive-ion c.i. is due to the greater total ion current achieved for one specific ion (often 1000 times more negative ions than positive ones[24]) and through the much reduced and simplified background.

For the future, the application of n.i.c.i. in trace food analysis will most likely be to problems where confirmation or quantification of electron-capturing compounds is required. In particular one thinks of pesticide analysis[25] where the complexity of other interfering compounds of environmental origin can cause problems of detection by conventional electron-capture detector, but it remains to be seen whether because of the increased specificity of n.i.c.i.–m.s. reduced clean-up procedures could be adopted.

7 The Mass Spectrometer as Its Own Chromatograph

Even under optimized g.c.–m.s. conditions, s.i.m. suffers from chemical interference introduced by residual sample impurities, chromatographic bleed, and the increasing problem of environmental pollution, and these problems will increase as lower detection levels are needed. Metastable-ion mass spectrometry offers a partial solution. Methods have been developed, *e.g.* mass-analysed ion kinetic-energy spectrometry[26] and various forms of linked-scanning,[27] which detect metastable ions free from normal ion interference, *i.e.* free from 'chemical' noise. The acronym m.s.–m.s. has been coined to suggest two distinct mass spectral separations in tandem.[28] Therefore it is possible to work with relatively crude extracts, largely eliminating the time-consuming and error-prone sample

[23] D. F. Hunt and F. W. Crow, *Anal. Chem.*, 1978, **50**, 1781.
[24] H. Brandenberger, *Recent Dev. Mass Spectrom. Biochem.*, 1979, **2**, 227.
[25] D. W. Kuehl, M. J. Whitaker, and R. C. Dougherty, *Anal. Chem.*, 1980, **52**, 935.
[26] M. H. Bozorgzadeh, R. P. Morgan, and J. H. Beynon, *Analyst (London)*, 1978, **103**, 613.
[27] A. P. Bruins, K. R. Jennings, and S. Evans, *Int. J. Mass Spectrom. Ion Phys.*, 1978, **26**, 395.
[28] F. W. McLafferty, *Acc. Chem. Res.*, 1980, **13**, 33.

clean-up and pre-chromatographic stages.[29] Although metastable peaks are of relatively low intensity compared to normal ions, the low background facilitates maximum signal amplification, and already picogram-level analyses have been described.[30] High selectivity has been demonstrated with crude extracts,[31] but the possibility remains that identical precursor ions could originate from more than one compound in the mixture. However, the successful differentiation of isomeric species generates considerable optimism for the widespread application of the method. Work is just beginning on the optimization of mass-spectral conditions for the production of intense metastable ions. Collision chambers[32] and longer field free regions will help this process, as will the new high-performance instruments that are being introduced.[33]

The present sensitivity of these techniques for quantitative analysis is described as similar to that obtained using high-resolution mass spectrometry (h.r.m.s.) for elemental analysis.[34] The most likely application in food science will be for rapid assays of halogenated contaminants, possibly in conjunction with n.i.c.i.[35] The general application will be to the estimation of unusual compounds in bland matrices.

8 Immunological Assays as an Alternative to Mass Spectrometry

Many of the methods described so far would be considered to be too expensive for routine quality-control laboratories, and cheaper methods are sought. Immunological assays have been claimed to be more cost-effective, but do they provide true values? Because g.c.–m.s.–s.i.m. methods in clinical chemistry are further advanced than in other fields and have entered the stage of being described as 'definitive' methods, a number of comparisons have been made with radioimmunoassays (r.i.a.).[36–39] The present situation would seem to be that for r.i.a. the preparation of the antiserum is difficult and most antisera demonstrate some degree of cross-reactivity, lowering the specificity.[36] The added inconvenience of having to synthesize the radiolabelled analogue and calculate the compensation factor for its use contrasts with an assay methodology which has been described as both simple[39] and convenient.[37] The sample preparation procedure is also described as simple but should be qualified by 'but extensive'.

Whereas it is clear that extensive clean-up is advisable for r.i.a.,[38] more work is required to determine the optimum level of preparation of samples for s.i.m.

[29] R. W. Kondrat and R. G. Cooks, *Science*, 1978, **199**, 978.
[30] S. J. Gaskell and D. S. Millington, *Biomed. Mass Spectrom.*, 1978, **5**, 557.
[31] T. L. Kruger, R. G. Cooks, J. L. McLaughlin, and R. L. Ranieri, *J. Org. Chem.*, 1977, **42**, 4161.
[32] R. S. Stradling, K. R. Jennings, and S. Evans, *Org. Mass Spectrom.*, 1978, **13**, 429.
[33] F. W. McLafferty, P. J. Todd, D. C. McGilvery, and M. A. Baldwin, *J. Am. Chem. Soc.*, 1980, **102**, 3360.
[34] S. J. Gaskell, R. W. Finney, and M. E. Harper, *Biomed. Mass Spectrom.*, 1979, **6**, 113.
[35] R. W. Kondrat, G. A. McClusky, and R. G. Cooks, *Anal. Chem.*, 1978, **50**, 1222.
[36] S. Baba, Y. Shinohara, and Y. Kasuya, *J. Chromatogr.*, 1979, **162**, 529.
[37] K. H. Powers and M. H. Ebert, *Biomed. Mass Spectrom.*, 1979, **6**, 187.
[38] K. Fotherby, *J. Steroid Biochem.*, 1979, **10**, 121.
[39] V. W. Winkler, J. M. Strong, and R. A. Finley, *Steroids*, 1977, **29**, 739.

It is likely that h.r.s.i.m. in particular will require much less pre-purification and therefore may not be any slower when the total analysis time is considered. Notwithstanding this, most commentators agree that s.i.m. is superior to r.i.a. in specificity and is viewed as the most reliable confirmatory method.

9 Selected Examples of Applications in Trace Analysis

A. Polychlorinated dibenzodioxins (PCDDs).—The PCDDs are a group of highly toxic compounds found as stable contaminants in commercial herbicides and which have additionally, through industrial accidents, been released to the environment. The possible contamination of soil and water through both routes gives rise to concern about the appearance of these compounds in the food chain. There are 75 isomers of PCDDs containing one to eight chlorine atoms (and 22 tetrachloro-positional isomers), which vary greatly in their acute toxicity and biological activity.[40,41] Hence a methodological requirement is the need to be able to distinguish between these similar compounds, and furthermore the extreme toxicity of 2,3,7,8-TCDD necessitates monitoring for it at p.p.t. (10^{-12} g g^{-1}) levels in foods. An added problem is the frequent presence in biological samples of much larger amounts of halogenated aromatics (PCBs, PBBs, and DDE), possibly at a 10^6-fold excess over the TCDD and having only fractional differences in molecular weights from the compounds of interest. Analytical procedures therefore have necessarily involved extensive clean-up to remove potential interference and/or sophisticated detection to achieve both high sensitivity and the desired specificity.

Early work on TCDDs involved analysis of the commercial herbicides for the individual isomers, and techniques used have included capillary column g.c.–m.s.[42] for identification and g.c.–m.s.–s.i.m.[43–45] at m/z = 320 for quantification (m/z = 322, although a more intense ion in 2,3,7,8-TCDD was not used in order to avoid PCB interference). For the analysis of TCDD in Vietnamese fish[46] a high-resolution signal-averaging technique has been described which enhances the signal-to-noise levels of the dioxin peak. The sum of 60 scans presented as a high-resolution mass spectrum showed a dramatic increase in sensitivity and signal quality. The detail of the clean-up for dioxins has been scrutinized, and neutral clean-up procedures have been described for milk[47] and seafood.[48] However, for beef, liver, and milk samples,[49] using solvent extraction and column chroma-

[40] E. J. McConnell, J. Moore, J. Haseman, and M. Harris, *Toxicol. Appl. Pharmacol.*, 1978, **44**, 335.
[41] A. Poland and E. Glover, *Mol. Pharmacol.*, 1977, **13**, 924.
[42] H.-R. Buser, *J. Chromatogr.*, 1975, **114**, 95.
[43] H.-R. Buser and H. P. Bosshardt, *J. Chromatogr.*, 1974, **90**, 71.
[44] H.-R. Buser and C. Rappe, *Chemosphere*, 1978, **7**, 199.
[45] W. W. Blaser, R. A. Bredeweg, L. A. Shadoff, and R. H. Stehl, *Anal. Chem.*, 1976, **48**, 984.
[46] R. Baughman and M. Meselson, *Environ. Health Perspect. (Exp. Issue)*, 1973, **5**, 27.
[47] P. W. O'Keefe, M. S. Meselson, and R. W. Baughman, *J. Assoc. Off. Anal. Chem.*, 1978, **61**, 621.
[48] K. Fukuhara, M. Takeda, M. Uchiyama, and H. Tanabe, *Eisei Kagaku*, 1975, **21**, 318.
[49] L. A. Shadoff and R. A. Hummel, *Biomed. Mass Spectrom.*, 1978, **5**, 7.

tography clean-up (1000:1 concentrations for a 10 g sample) of the TCDD and selected mass scanning at *m/z* 320 and 322, a sensitivity of 10 p.p.t. was achieved,[49] and criteria for positive results were taken as (*a*) correct g.c. retention time and (*b*) correct ratio 320/322. If an incorrect ratio was observed at low resolution, then the assay was repeated first at medium and then at higher resolution if required. More recently it has been shown[50] that, with a clean-up procedure designed specifically to handle samples with high lipid content and excess amounts of chlorinated aromatics (alkaline sample digestion and extraction, sulphuric acid column chromatography, and reversed-phase h.p.l.c. final clean-up), it was possible to attain the desired specificity and sensitivity with packed-column g.c. and with m.i.m. of the molecular-ion cluster of TCDD.[50]

TCDDs present an example of one of the most demanding assays to be encountered at the present time, one where sophisticated m.s. detection systems (high-resolution s.i.m. and/or low-resolution m.i.m.) are an essential prerequisite, but additionally multi-stage clean-up procedures and great attention to detail are required in order to produce accurate and reliable data.

The application of n.i.c.i.–s.i.m. illustrates the increased sensitivity of the technique over e.i.m.s. Of 63 liver assays, 49 were shown to contain measurable amounts of TCDD by n.i.c.i., while only 24 could be detected by e.i.m.s. under similar experimental conditions.[51] Still further improvement was predicted for the use of capillary columns.

B. Analysis for Nitrosamines in Foods.—Volatile nitrosamines found originally in cured meats[52] but more recently in alcoholic beverages[53] are generally extracted by an initial distillation, chemically separated from extraneous constituents by a somewhat lengthy clean-up procedure, and then finally concentrated in an organic solvent by perhaps a 1000-fold over the initial concentrations. Analysis is normally by g.c. (packed columns most frequently used) with detection by either specific nitrogen g.c. (Coulson or Hall), chemiluminescence (TEA), or mass spectrometry.

Nitrogen-specific Detectors. For nitrosamines, nitrogen-specific detectors are now relegated to the role of preliminary screening of samples, for example in a large-scale survey where it is necessary to minimize the use of m.s. instrument time. For primary measurements it is now generally accepted that nitrogen detectors are not sufficiently specific for nitrosamines, and this can be clearly illustrated from the results of a survey of 154 food samples where about half the positive results for *N*-nitrosodimethylamine (NDMA), using the Coulson detector, were shown to be false by m.s.[54] Hence, the two recognized detectors for nitrosamines are now the TEA and mass spectrometer.

[50] L. L. Lamparski, T. J. Nestrick, and R. H. Stehl, *Anal. Chem.*, 1979, **51**, 1453.
[51] J. R. Hass and M. D. Friesen, *Ann. N.Y. Acad. Sci.*, 1979, **320**, 28.
[52] T. A. Gough, *Analyst (London)*, 1978, **103**, 785.
[53] B. Spiegelhalder, G. Eisenbrand, and R. Preussman, *Food Cosmet. Toxicol.*, 1979, **17**, 29.
[54] K. Goodhead and T. A. Gough, *Food Cosmet. Toxicol.*, 1975, **13**, 307.

Chemiluminescence Detectors. TEAs, in contrast, have considerable selectivity for nitrosamines and were designed exclusively for this particular determination. The TEA is an expensive detector compared to conventional g.c. detectors but is only one fifth of the cost of h.r.m.s. Operator skills are not needed at the same level as for m.s., but the TEA is not a versatile detector – its application is limited exclusively to nitrosamines.

The selectivity of the TEA is based on three factors: (i) choice of suitable pyrolysis conditions for N—NO bond cleavage, (ii) selective cold trapping of NO free from potential interfering species, and (iii) NO excitation and chemiluminescent emission. Sensitivity is of the order of 50 pg of NDMA injected.[55] There have been very few erroneous results; one case was reported of a false positive non-nitrosamine chemiluminescent species,[56] and a second case[55] (one out of 98 samples analysed) gave a high NDMA with a TEA but could not be confirmed by m.s.

Many comparisons for nitrosamine assays have been made both between use of a TEA and m.s.[55,57,58] and among the various options available for determining NDMA by s.i.m.[58] The low molecular weight (74) of NDMA causes particular problems for s.i.m., and by low-resolution m.i.m., even when, for example, three ions of m/z 30, 42, and 74 are monitored, commonly occurring fragment ions in co-eluted components in food extracts can interfere, causing signal suppression or enhancement compared with the pure standard. For h.r.s.i.m. the molecular ion at 74.0480 for NDMA is present at high relative abundance, but $^{29}SiMe_3$ is a potential interferent (arising both from antifoam tablets and certain types of silicone rubber septa),[59,60] and the compound from which this fragment is derived is eluted at a retention time close to that of NDMA. A resolution of greater than 7000 is required for the separation of this and other ions of the same nominal mass.[55] The degree of refinement in m.s. methodology which has been achieved for nitrosamine assays is well illustrated by the fact that even the mode of operation of s.i.m. to be employed (peak matching compared with precise ion monitoring) has been strongly argued.[58]

The current situation appears to be that the high cost of the TEA as a single-purpose detector can be justified, and many are in routine operation for surveillance of nitrosamines in foods and the environment. The clean-up requirements are less rigorous for the TEA than for m.s., and its specificity of detection has been clearly proven in the field. Nevertheless, when high levels of nitrosamines or unexpected positives are found by TEA, the usual procedure would be to repeat the assay by m.s.(s.i.m.) – not, as normally would be the case, for confirmation (which presumes m.s. to be superior) but as a cross-check using a detection method based on a different physical principle.

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C. Mycotoxins.—Microfungal attack on food plants during growth and subsequent storage gives rise to contamination with mycotoxins (fungal metabolites), a diverse group of compounds, many of which are highly toxic. These compounds are unique in the wide range of techniques which are currently being employed for their analysis, ranging from thin-layer chromatography (t.l.c.) to h.r.s.i.m. T.l.c. (and more recently h.p.t.l.c.)[61,62] is perhaps the most commonly used method for routine monitoring of aflatoxins[63,64] where, although there is the disadvantage of a lack of quantitative precision (coefficients of variation range from 15 to 70%),[65] the native fluorescence of certain aflatoxins allows a detection limit of 0.5 ng[66] (under long-wavelength u.v. light). The method is inexpensive and is suitable for the screening of large numbers of samples. H.p.l.c. has tended to replace t.l.c. for mycotoxin analysis[67,68] because the associated techniques of silica-gel packed cells[69] and laser-fluorescence detection[70,71] offer improved sensitivity and precision, but the rather low selectivity makes extensive sample clean-up compulsory. R.i.a. techniques are said to offer the advantage over t.l.c. and h.p.l.c. of needing less sample clean-up (ideal for routine survey or quality-control work). Using a simple solvent extraction, aflatoxin B_1 has been determined in foods with good reproducibility (14—16%)[72,73] and a sensitivity of the order of 1 p.p.b.[73]

Most mycotoxins are too involatile for g.c., but g.c. and g.c.–m.s. have found application for the appropriate derivatives of trichothecenes[74,75] and patulin[76] in foods. M.s. has found extensive application for mycotoxin analysis and has been reviewed elsewhere.[77] However, the point to emphasize regarding m.s. (and the theme of this review) is the possibility of carrying out analyses for mycotoxins by m.s. directly on foods or on crude extracts. Some published work[78] has shown that by direct insertion probe m.s., where the limitation to sensitivity was the sample capacity of the probe (100 μg), it was possible to detect by h.r.s.i.m.

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(resolution = 5000) 0.03 ng of aflatoxin B_1. Surprisingly the limit of detection was better in extracts than standard solutions owing to irreversible bonding effects on glass surfaces.

Finally, l.c.–m.s. is a technique with enormous potential for application to the field of mycotoxins.

9 Concluding Remarks

In this review we have attempted to make an appraisal of the role of m.s. in trace organic analysis by comparing its sensitivity and specificity with that attainable by other techniques and to examine some of the recent advances in instrumentation. We have used some examples from areas of food chemistry where m.s. has been proved to be useful at subnanogram levels, *e.g.* in the analysis of aflatoxins, dioxins, and nitrosamines. For the future it is clear that n.i.c.i. will be widely applied for the ultra-sensitive detection of trace electron-capturing components, and examples are already emerging from the literature. L.c.–m.s. coupling is very much in its infancy, and not until more of the technical difficulties have been resolved will its full potential in food chemistry be realized. However, once established, it will not only enable identification of the less volatile components separated from mixtures, but it will open new frontiers in areas of trace analysis where l.c. has been inapplicable through lack of a suitable detector. Further ahead still is m.s.–m.s., an interesting academic tool so far, but potentially one which could lead the way to high specificity in trace quantitative measurements made directly on foods.