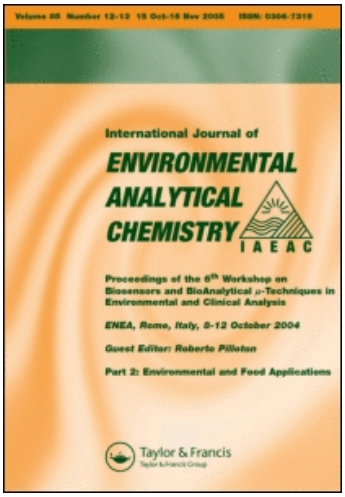
This article was downloaded by:
On: *18 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Determination of Environmental Contaminants in Foods by Liquid Chromatography — An Overview

J. F. Lawrence[a]
[a] Bureau of Chemical Safety, Food Directorate, Health Protection Branch, Ottawa, Ontario, Canada

To cite this Article Lawrence, J. F.(1992) 'Determination of Environmental Contaminants in Foods by Liquid Chromatography — An Overview', International Journal of Environmental Analytical Chemistry, 49: 1, 15 — 29
To link to this Article: DOI: 10.1080/03067319208028123
URL: http://dx.doi.org/10.1080/03067319208028123

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Intern. J. Environ. Anal. Chem., Vol. 49, pp. 15-29
Reprints available directly from the publisher
Photocopying permitted by license only

© 1992 Gordon and Breach Science Publishers S. A.
Printed in the United States of America

DETERMINATION OF ENVIRONMENTAL CONTAMINANTS IN FOODS BY LIQUID CHROMATOGRAPHY - AN OVERVIEW

J. F. LAWRENCE

Bureau of Chemical Safety, Food Directorate, Health Protection Branch, Ottawa, Ontario, K1A OL2, Canada

(Received, 1 October 1991)

Gas chromatography (GC) has long been used for the determination of trace levels of environmental contaminants in many different types of samples. The technique has reached an advanced state of development. However, there are many types of contaminants which are not amenable to GC due to thermal instability or poor volatility and thus high performance liquid chromatography (LC) has become the preferred technique for their analysis. Included in this group are certain agricultural, industrial and natural contaminants which may find their way into food for human consumption. This paper discusses approaches to the LC determination of selected contaminants in foods. The techniques involve extraction and clean-up of the samples followed by direct analysis or pre- or post-column derivatization with either UV absorption of fluorescence detection. The potential of LC-mass spectrometry and chemical derivatization for confirmation of identity is also discussed.

KEY WORDS: Foods, HPLC, pesticides, PAHs, marine toxins.

Downloaded At: 15:25 18 January 2011

INTRODUCTION

High-performance liquid chromatography (LC) has been available for more than 20 years and has established a place for itself in analytical chemistry, particularly for pharmaceutical products and quality control in many industrial applications. However, its use in environmental analysis especially for contaminants at trace (parts per million or less) levels has been slower to develop[1-4]. This has been mainly due to the lack of selective, sensitive detectors compared to those that are available for gas chromatography (GC). However, great technological strides have been made in the last few years to remedy this deficiency. Computer controlled diode array based absorption and fluorescence spectrometric detection systems have greatly enhanced both sensitivity and selectivity of LC. This has led to much improved detection limits for contaminants in environmental samples. The current research efforts by scientists around the world on LC-mass spectrometry is leading to improved interfaces that enable the identification of compounds of widely varying chemical composition, structures and polarities. This exciting research will undoubtedly lead to LC-mass spectrometry becoming much more frequently used for confirmation of results obtained by LC with

Table 1 Chemical contaminants in foods

Pesticides	
Heavy Metals	
Polycyclic Aromatic Hydrocarbons (PAH)	
PCB	
Dioxins	
Natural Toxins-	mycotoxins
	phycotoxins

other detection systems. Such confirmation is very important in environmental analysis particularly where findings can lead to regulatory action by governments.

RESULTS AND DISCUSSION

Types of chemical contaminants in foods

Downloaded At: 15:25 18 January 2011

Table 1 lists examples of the types of chemical contaminants that may find their way into foods. They may roughly be divided into three groups: agricultural chemicals, industrial pollutants and natural toxins. To ensure a safe food supply all three groups must be monitored. The methods required depend upon the substance being determined and its toxicity. Table 2 lists the approximate concentration levels that methods must be capable of quantitating. These depend upon the toxicity of the chemicals with the carcinogens requiring much lower detection limits than acutely toxic substances. For example carcinogenic PAH are of concern at low ng/g levels while pesticides which have acute toxicity but no carcinogenicity are of concern at μg/g levels.

These different concentration levels impose different requirements on analytical methods. Table 3 lists types of methods required for the determination of various contaminant groups. As can be seen for the organic compounds, GC and LC are most commonly used. Even for organometallic substances combined GC-atomic absorption (AA) or LC-AA have been reported in the literature although the former is in a much more advanced state due mainly to the relative ease of interfacing GC to AA.

Table 2 Levels of contaminants in foods

Pesticides:	0.1 - 20 μg/g
Natural Toxins:	Non-carc. 0.1 - 1 μg/g
	Carc. 1 - 10 ng/g
Industrial Chemicals:	Metals 10 - 100 ng/g
	PCB 1 - 50 ng/g
	PAH 1 - 10 ng/g
	Dioxins pg/g

Table 3 Methods for contaminants

Pesticides	GC	LC
PAH	GC	LC
Natural toxins	GC	LC
PCB	GC	
Dioxins	GC-mass spectrometry	
Heavy metals	atomic absorption (AA)	
	ICP emission	
Organometallics	GC-AA	LC-AA

Choice of LC

How does one decide whether to choose LC or GC? The simplest answer is the one which will provide the easiest analysis at an acceptable cost. Normally, this would mean a method involving a straight-forward extraction with minimal sample clean-up followed by direct analysis without the need for chemical derivatization or expensive specialised detectors. There are many compounds that can be determined in foods by both GC and LC. Anilazine (a triazine fungicide), for example, can be determined by either technique using the same extraction procedure. Figure 1 compares chromatograms obtained by GC using electron-capture or nitrogen-phosphorous detection with LC using UV detection at 275 nm[5]. As can be seen the results are similar at a 1.0 μg/g level in potatoes. Similarly, polycyclic aromatic hydrocarbons (PAH) may be determined by the two chromatographic techniques although fluorescence detection with LC is much more selective than flame ionization commonly used for their detection by GC. In fact GC-FID is not particularly suitable for sub-ng/g

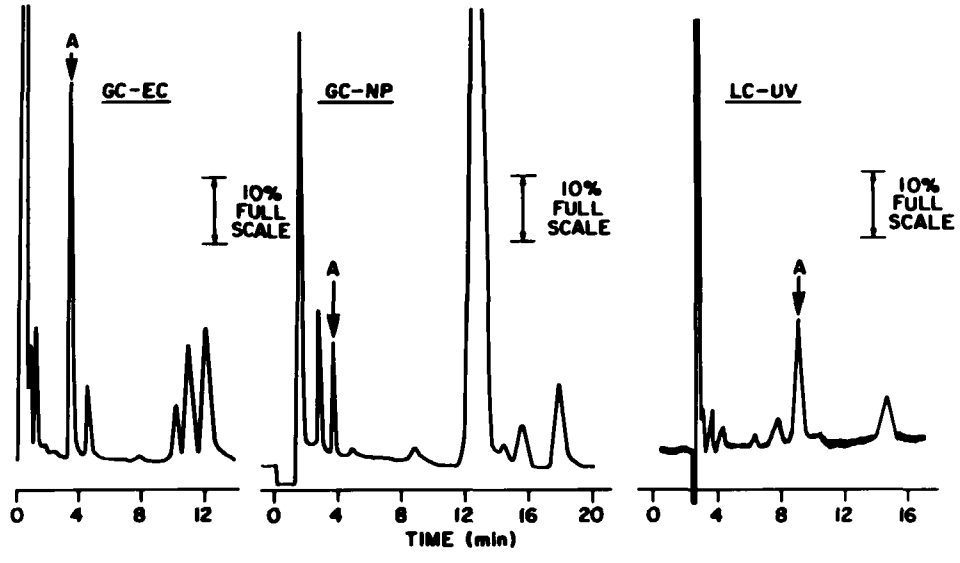


Figure 1 Comparison of GC (EC (electron-capture) and NP (nitrogen-phosphorus detection)) with LC (UV detection) for the determination of anilazine (A) in potato at 1.0 μg/g. Quantities of potato sample injected are: GC-EC, 0.5 mg; GC-NP, 2.5 mg; LC-UV, 25 mg.

Downloaded At: 15:25 18 January 2011

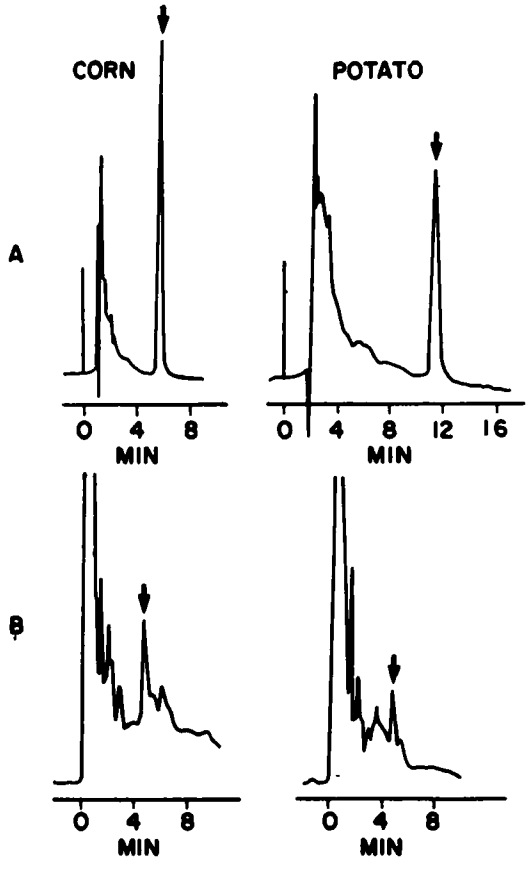


Figure 2 Chromatograms of terbacil in corn (2.0 μg/g) and potato (0.2 μg/g) by LC-UV, 254 nm (A) and GC-EC (B). Arrows indicate terbacil peaks.

Downloaded At: 15:25 18 January 2011

detection of the carcinogenic PAH in foods although GC-mass spectrometry is suitable and can be used as a confirmation of LC results.

In most cases with environmental contaminants individual substances are more suited to one technique than another. For example most organochlorine, organophosphate and triazine pesticides are much more easily determined by GC whereas carbamates and ureas are more amenable to LC determination.

It is important to realize that GC and LC are very complimentary in what they offer to the analytical chemist. They should not be regarded as competitive techniques although it is sometimes difficult for an analyst who has used nothing but GC to try his best to analyze for all substances by that technique even if it requires extensive sample pretreatment and chemical derivatization[2]. For those who have only one or the other technique available to them then there is no choice-you do the best with what you have. However because of the wide diversity in chemical and physical properties of environmental contaminants most laboratories should be equipped with both types of chromatographic systems.

Figure 2 compares results obtained on the same extracts for the herbicide, terbacil, in corn and potato[2]. Here we can see that LC with UV detection is much superior to GC with EC detection. However, if we look at benzoylpropethyl (Figure 3) we see the opposite[6]. The GC results are much superior to those obtained by LC. This illustrates the fact that indeed the techniques are complimentary.

Optimization of LC methods

When LC is selected as the method to use for an analysis, there are a number of factors to consider to make it work best to resolve the analytical problem. Most LC methods now-a-days use reversed-phase chromatography with UV absorption detection. Such a system can be extremely flexible being suitable for the determination of compounds over a wide polarity range with only a change in mobile phase composition. It is this flexibility to determine very lipophilic compounds such as fatty acids and ionic compounds requiring ion-pair chromatography using the same type of reversed phase column that makes LC so useful. Modern computer controlled equipment can generate many different isocratic and gradient programs and can optimize separations for any given analytical problem. Figure 4 compares three different chromatograms for the determination of 2-acetoxy-4-tetrahydroxybutyl imidazole (THI) in a beer sample at low ng/g levels[7]. The change of organic modifier affects the chromatographic pattern due to selectivity in solute-modifier interactions. Subtle differences as this may have important ramifications on the success of an analytical method.

Downloaded At: 15:25 18 January 2011

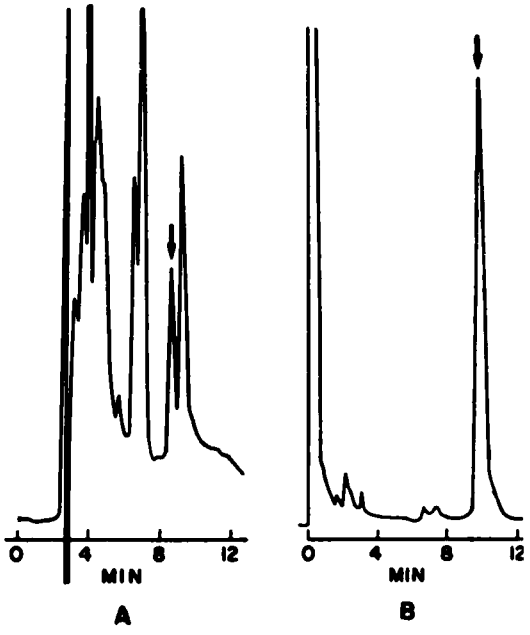


Figure 3 Chromatograms of benzoylpropethyl in corn at 0.2 μg/g. A. LC-UV (254 nm). B. GC-EC. Arrows indicate peaks corresponding to benzoylpropethyl.

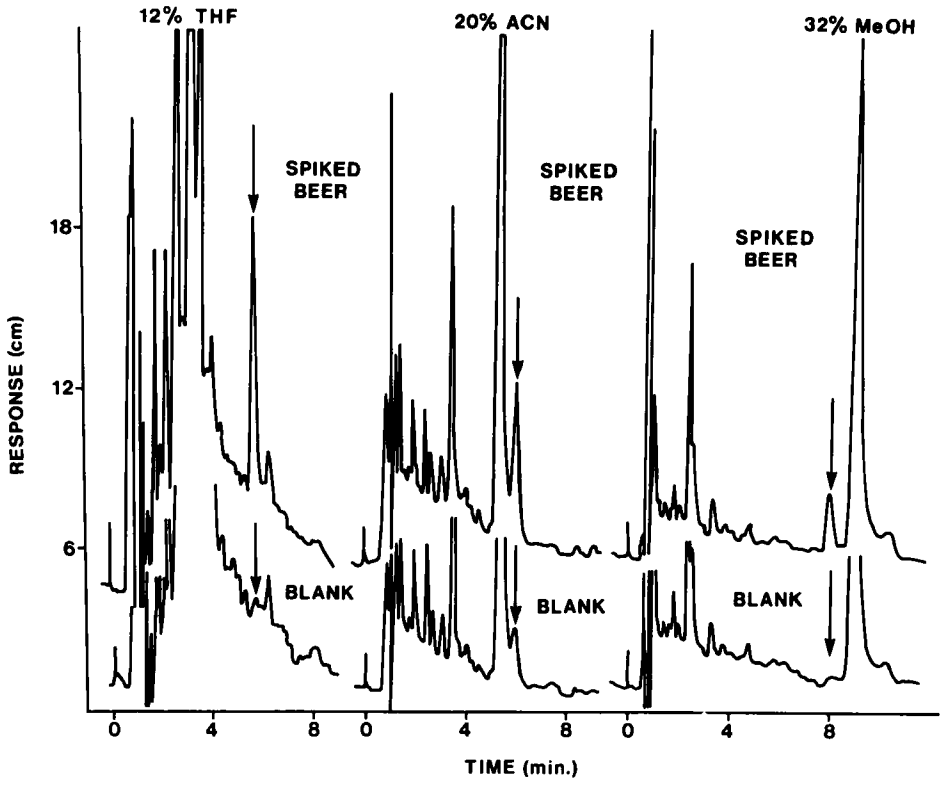


Figure 4 Comparison of chromatograms of THI in beer obtained with three different mobile phases for the same sample extract (blank and spike, 200 ng/ml). Injection volumes, 20 µl for acetonitrile (ACN) and methanol (MeOH) and 50 µl for tetrahydrofuran (THF) mobile phases.

The technological advances made in detection systems particularly in terms of sensitivity and flexibility have greatly improved the capabilities of LC for environmental analysis. We now use diode array UV detection for both routine applications and research work. It has proven to be a valuable confirmatory tool in the absence of mass spectrometric confirmation. Figure 5 shows some chromatographic results obtained for the marine toxin domoic acid in a serum sample from a human patient who was poisoned by eating contaminated mussels[8]. The direct UV analysis showed a peak exactly at the retention time of the toxin while the rest of the chromatogram was relatively clean. It was not until we carried out a selective additional column cleanup on the extract that we found it was not domoic acid. We reanalyzed the original extract by LC with diode array detection and generated a UV spectrum of the peak that immediately showed that it was not domoic acid. If we had performed the analysis with the diode array detector in the first instance we would not have had to perform the additional cleanup.

Fluorescence detectors have also substantially improved in the past few years. Computer controlled systems are capable of changing wavelengths during a run to obtain optimum sensitivity for individual compounds. Some are also capable of stopped flow scanning which

Downloaded At: 15:25 18 January 2011

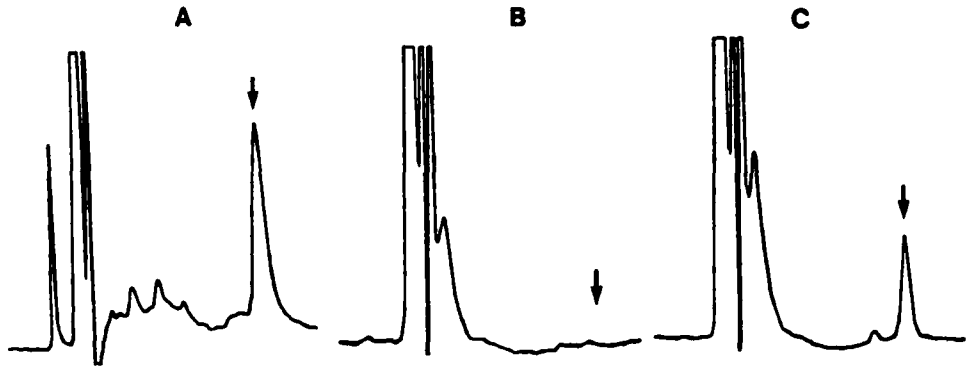


Figure 5 Chromatograms of a human serum sample, before (A) and after (B) solid phase extraction cleanup. C, spiked serum after cleanup. Arrow indicates domoic acid retention time.

can be useful for identifying peaks. Figure 6 shows a chromatogram of an oxidized extract of lobster hepatopancreas containing paralytic shellfish toxins (PSP) with a large unknown peak eluting near the retention time of the oxidation product of a known toxin, neosaxitoxin. However, when we performed a stopped-flow spectral scan on the peak we found that the spectrum was slightly different from those obtained from PSP toxins and, upon reanalysis of the same extract without oxidation, the peak was still present indicating that it was a naturally fluorescent sample constituent.

Figure 7 shows another application of LC-fluorescence for environmental analysis[9]. In this case PAH are analysed in a variety of food products. Although a very selective clean-up was used, the chromatograms are still rather complex and only a few of the peaks corre-

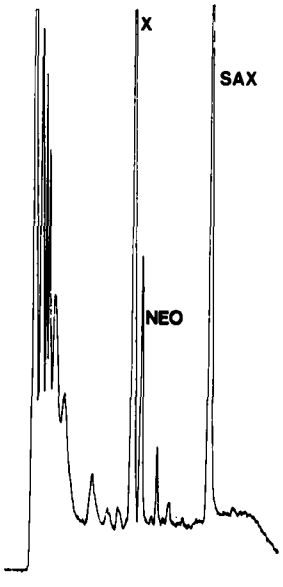


Figure 6 Chromatograms of an oxidized extract of lobster hepatopancreas containing paralytic shellfish toxins. X = unknown, NEO = neosaxitoxin, SAX = saxitoxin.

Downloaded At: 15:25 18 January 2011

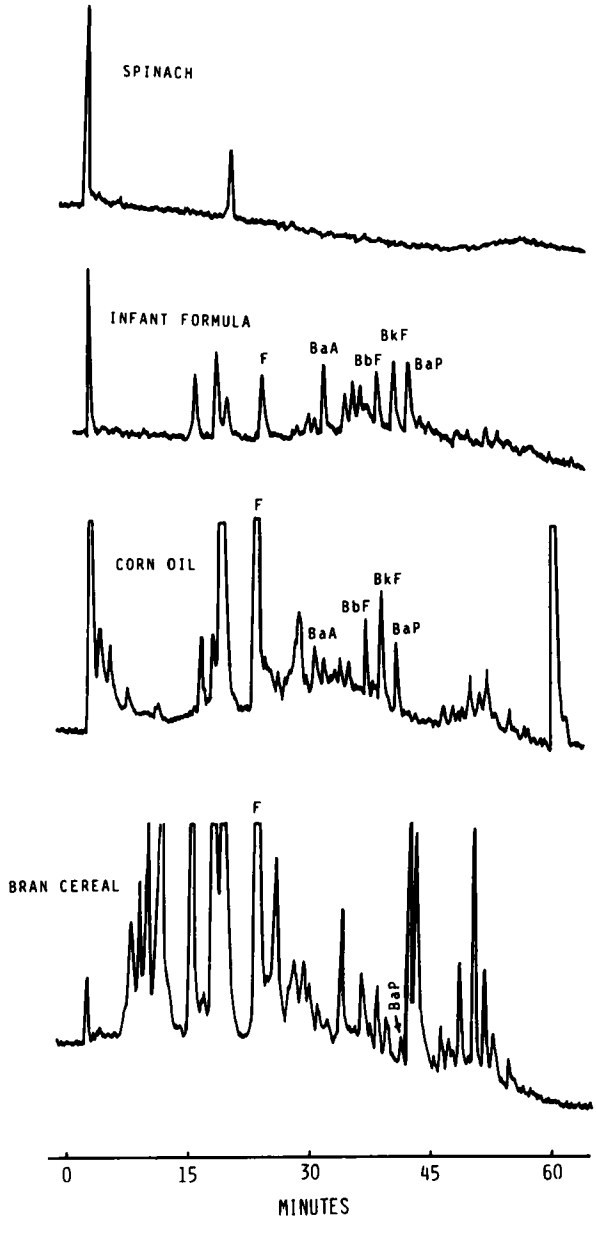


Figure 7 Chromatograms obtained for various food products. Labelled peaks confirmed by GC-MS. F = fluoranthene, BaA = benz(a)anthracene, B(b)F = benz(b)fluoranthene, B(k)F = benz(k)fluroanthene, B(a)P = benz(a)pyrene.

sponded to the standards. However, it is likely that all or most of the unknowns are PAH derivatives since they are very non-polar and are fluorescent.

Electrochemical detection in LC can also be very useful for determining environmental contaminants in foods. Sulfur dioxide for example is very selectively and sensitively

Downloaded At: 15:25 18 January 2011

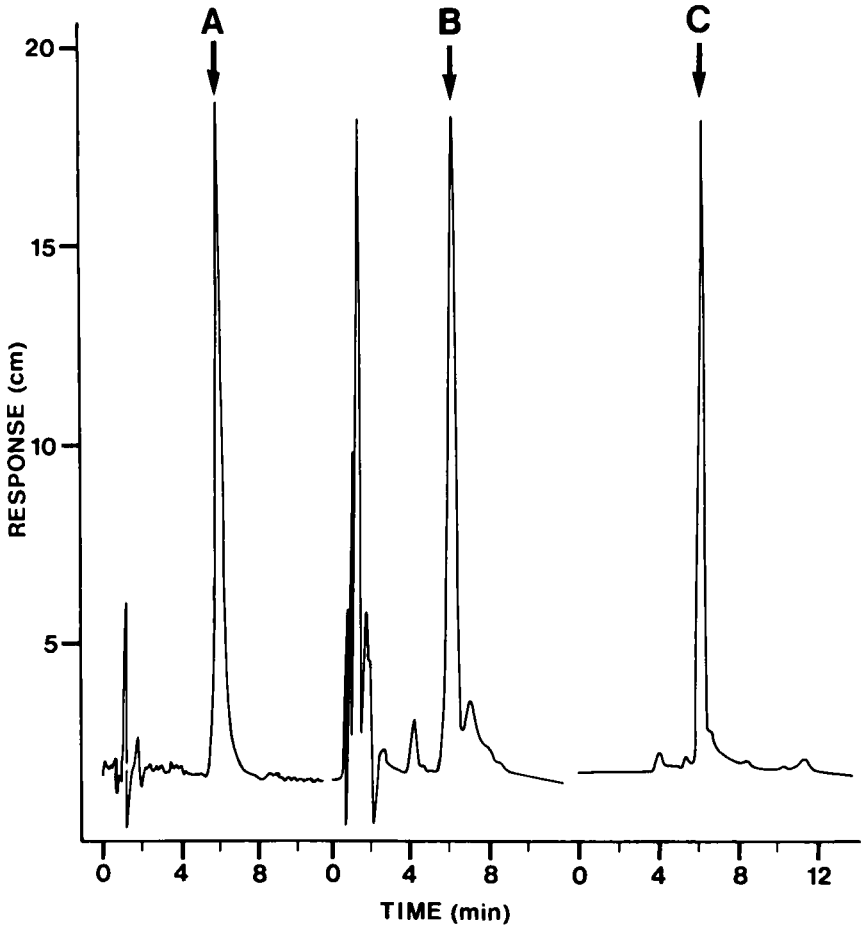


Figure 8 Chromatograms of a white wine sample containing about 100 μg/ml sulfite. A, headspace-LC; B, direct ion exchange LC; C, direct ion exclusion LC. Electrochemical detection (amperometric), A and B with glassy carbon electrode and C with platinum electrode. Voltage was +0.7V for all three results.

quantitated using LC with amperometric detection at a silver electrode. The compound is not only an air pollutant of concern but as the sulfite salt is used as a preservative in foods such as wines and dried fruits. Since many people particularly some asthmatics have severe life-threatening allergies to the substance, the Department of Health and Welfare in Canada has required that most foods containing any added sulfite be labelled. Figure 8 shows chromatographic results comparing three different LC methods[10]. Although some differences are observed they all can detect low μg/g levels of sulfite in most sulfited foods.

Chemical derivatization

Often in environmental analysis difficulties are encountered in not being able to detect the

Downloaded At: 15:25 18 January 2011

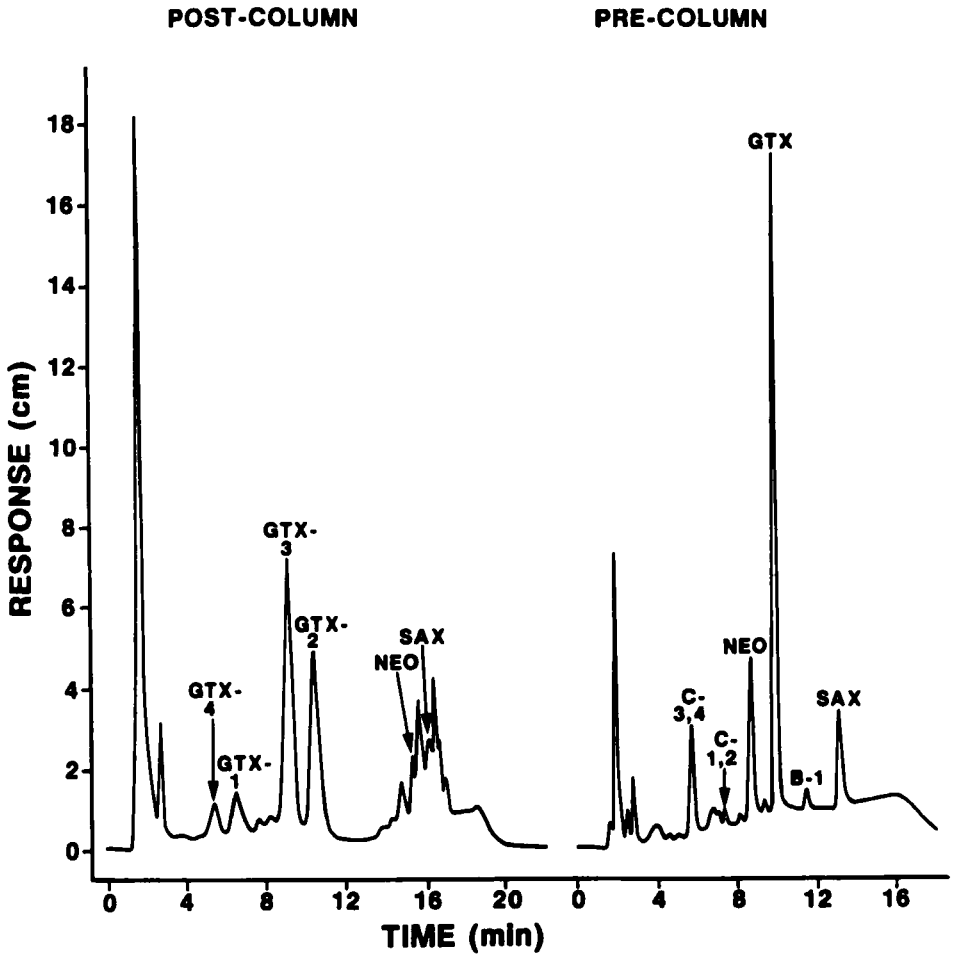


Figure 9 Chromatograms of a contaminated mussel extract, 2 μg/g total PSP. (A) Post-column oxidation. (B) Prechromatographic oxidation with periodate.

analyte of interest with enough sensitivity or selectivity. If a compound cannot be analyzed directly by either GC or LC then chemical derivatization may be useful[11,12]. This involves making a derivative of the analyte which can be determined in the environmental sample of interest. Chemical derivatization requires chemical treatment of the sample extract which involves additional sample manipulation leading to increased error, losses and potential interferences from the derivatizing reagents. This may be time consuming and necessitate additional sample cleanup before LC analysis. Thus the technique should be used as a last resort.

In LC, chemical derivatization can be carried out before chromatography, that is to create a derivative of the analyte and to do the chromatographic separation on the derivative, or the derivatization can be performed after chromatographic separation via a continuous on-line reaction before the column effluent enters the detector. Both approaches have their

Downloaded At: 15:25 18 January 2011

advantages and disadvantages. Pre-column derivatization is advantageous when derivatization is required only for a few samples on an occasional basis, the only requirement being that the reagents for the reaction be on hand when needed. No specialized reaction systems are required. Post-column reactions are more appropriate for on-going routine work since the set-up of the post-column reactor and associated pumps as well as optimization of reaction time and temperature may be relatively time consuming if only a few samples are to be analysed. Figure 9 illustrates differences obtained between pre and post-chromatographic determination of PSP toxins in a contaminated shellfish extract[13]. The pre-chromatographic reaction is simpler to perform and is more sensitive than the post-column method. However the latter gives results for all individual toxins in a PSP mixture whereas the pre-column method requires some data interpretation since several of the individual toxins yield the same product after oxidation. Because the pre-column reaction is fast (3 min), simple and requires only an ordinary HPLC system, it is well suited to providing results rather quickly. If details on the individual components of the PSP mixture are required, then the post-column reaction would be preferred, although much more time would be needed if the equipment is not already up and running[14,15].

The PSP oxidation procedure is an example of a selective derivatization which requires no additional sample cleanup either before or after the reaction. Unfortunately, most types of derivatizations are not so easy. Figure 10 shows chromatograms of a domoic acid contaminated mussel extract comparing a direct analysis with one that was performed after

Downloaded At: 15:25 18 January 2011

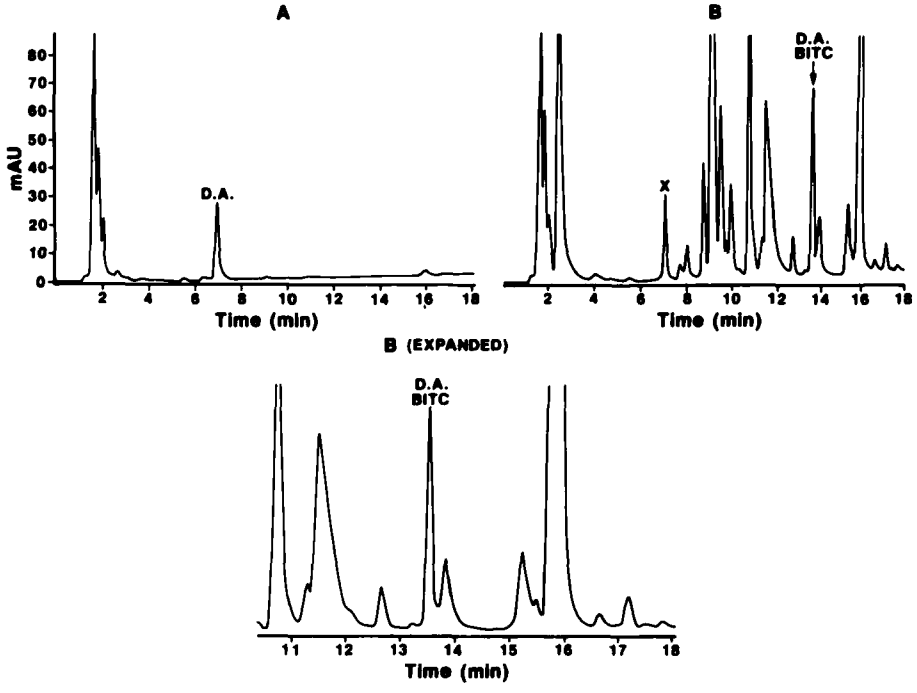


Figure 10 Chromatograms of domoic acid in a naturally contaminated mussel extract (538 μg/g) before (A) and after (B) butylisothiocyanate derivatization. X = reagent peak.

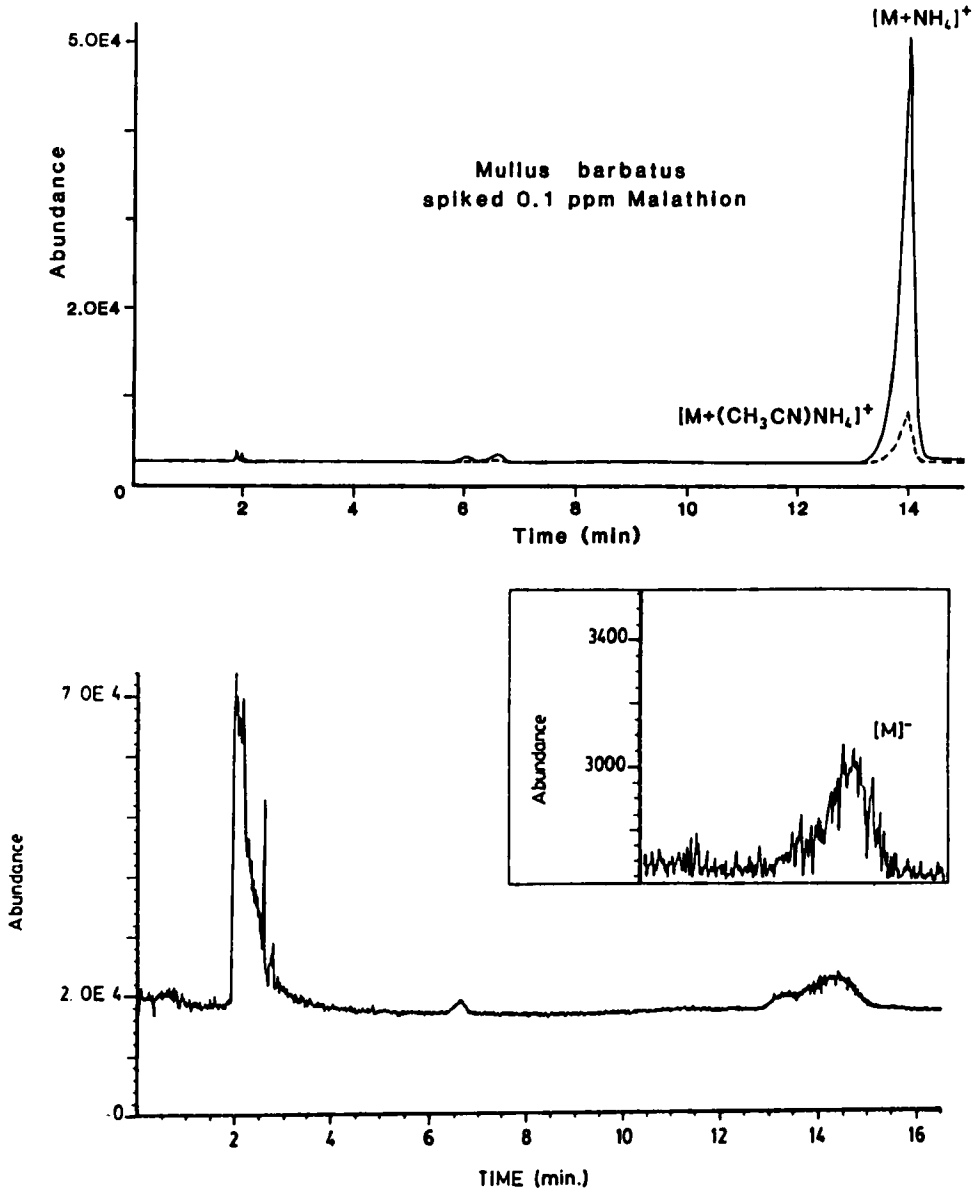


Figure 11 Thermospray LC-MS chromatograms of a fish sample spiked with 0.1 μg/g of malathion (5 ng injected). (A) positive ion mode (B) negative ion mode. From reference 17 with permission of J. Wiley & Sons Ltd., New York.

butyl isothiocyanate derivatization[16]. As can be seen, the direct determination yields a clean chromatogram whereas the chromatogram of the derivatized extract has many more peaks in it due to excess reagent and reaction products from other sample constituents. In addition, an extra cleanup step was required before the derivatization to eliminate the majority of the

Downloaded At: 15:25 18 January 2011

proteinaceous matter which would have prevented the reaction. These types of problems are not uncommon in carrying out derivatization reactions. Analysts should be aware of them.

LC-mass spectrometry

By far the most exciting research in environmental analysis by LC is in the area of LC-mass spectrometry. Unequivocal identification of contaminants by LC at present is very difficult. Mass spectrometry is the best means of structural identification for confirmation of environmental contaminants. It is easily interfaced with GC and is now routinely used in many labs around the world. However mass spectrometry is much more difficult to interface with LC due to the presence of the liquid mobile phase. There has been a great amount of work published in the literature in recent years on developing useful interfaces. Several include thermospray, direct liquid introduction, moving-belt, electrospray (ion spray), and plasmaspray interfaces. For environmental contaminants, the thermospray interface is most commonly used. Figure 11 shows how LC-thermospray mass spectrometry can be used for the detection of malathron in a fish sample (red mullet) at 0.1 μg/g with selected ion monitoring in both positive and negative ion modes[17]. In this particular case the positive ion mode is much more sensitive than the negative ion mode. Another example is shown in figure 12 for an extract of mussel contaminated with 74 mg/g of the marin toxin, domoic acid[18]. Here LC with ion spray mass spectrometry was used in the positive ion mode. It can be seen that in addition to the main domoic acid peak, other peaks are observed with selected ion monitoring of m/z 312. These are domoic acid isomers. Sub-nanogram amounts of the compounds can be detected.

Unfortunately with LC-mass spectrometry the sensitivity is very compound dependent and is also very much affected by the composition of the mobile phase. More research is required on the technique for it to be generally applicable to the routine confirmation of results obtained by LC.

Downloaded At: 15:25 18 January 2011

CONCLUSION

LC continues to play an ever-increased role in the determination of environmental contaminants, particularly in foods. Technological advances in equipment have already resulted in improved capabilities for detecting these substances at trace levels. Improved sample purification methods have also led to better detection limits. Finally, important studies are being made toward refining LC-mass spectrometry as a routine confirmation technique suitable for a wide range of chemical contaminants at μg/g levels or less in environmental samples.

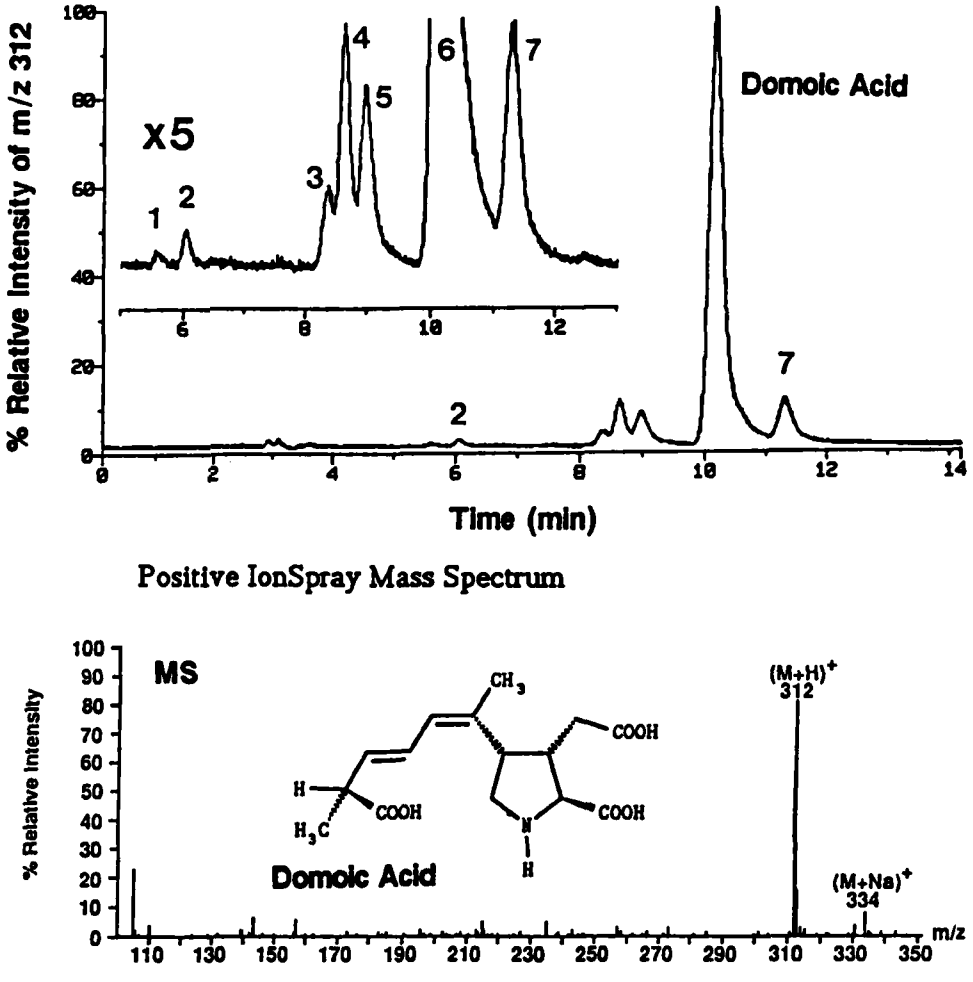


Figure 12 Top: Ion-spray LC-MS chromatograms of domoic acid in a toxic mussel extract. Positive ion mode, selected ion monitoring of m/z 312. Peaks 1-7 are domoic acid isomers. Peak 6 is domoic acid (equivalent to 74 ng injected). Bottom: Ion spray mass spectrum of 70 ng domoic acid in the positive ion mode. From reference 18 with permission from Heydon and Son Ltd., London.

References

1. J.F. Lawrence, editor *Liquid Chromatography in Environmental Analysis*, (Humana Press Inc., Clifton, USA, (1984).
2. J.F. Lawrence, *Anal. Chem.* **52**, 1122A–1135A (1980).
3. J.F. Lawrence, *Organic Trace Analysis by Liquid Chromatography*, (Academic Press Inc., New York, USA (1981).
4. J.F. Lawrence, *Chromatographia* **24**, 45–50 (1987).
5. J.F. Lawrence, L.G. Panopio, *J. Assoc. Off. Anal. Chem.* **63**, 1300–1303 (1980).
6. J.F. Lawrence, L.G. Panopio and H.A. McLeod, *J. Agric. Food Chem.* **28**, 1019–1022 (1980).
7. J.F. Lawrence and C. Ménard, *J. Chromatogr.* **466**, 421–426 (1989).
8. J.F. Lawrence and C. Ménard, *Fres. J. Anal. Chem.* **339**, 494–498 (1991).

Downloaded At: 15:25 18 January 2011

9. J.F. Lawrence and B.S. Das, *Int. J. Environ. Anal. Chem.* **24**, 113–131 (1986).
10. J.F. Lawrence, R.K. Chadha and C. Ménard, *J. Assoc. Off. Anal. Chem.* **73**, 77–79 (1990).
11. J.F. Lawrence, *J. Chromatogr. Sci.* **23**, 484–487 (1985).
12. J.F. Lawrence, *J. Chromatogr. Sci.* **25**, 444–452 (1987).
13. J.F. Lawrence and C. Ménard, *J. Assoc. Off. Anal. Chem.* **74**, 1006-1012 (1991).
14. J.J. Sullivan and M.M. Wekell in *Seafood Quality Determination, Developments in Food Science, Volume 15*, (D.E. Kramer and J. Liston, editors, Elsevier, New York, USA, (1987) pp 357–371.
15. Y. Oshima, K. Sugino and T. Yasumoto, in *Mycotoxins and Phycotoxins '88*, (S. Natori, K. Hashimoto and Y. Ueno, editors, Elsevier, New York, USA, (1989) pp 311–318.
16. J.F. Lawrence and C. Ménard, *J. Chromatogr.* **550**, 595–601 (1991).
17. D. Barceló, *Biomed. Environ. Mass. Spec.* **17**, 363–369 (1988).
18. M.A. Quilliam, B.A. Thomson, G.J. Scott and K.W.M. Siu, *Rapid Commun. Mass. Spec.* **3**, 145–150 (1989).

Downloaded At: 15:25 18 January 2011