

***ENVIRONMENTAL SPECIATION OF TIN AND LEAD BY  
HPLC-ICP-MS***

by

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## **ABSTRACT**

### ***ENVIRONMENTAL SPECIATION OF TIN AND LEAD BY HPLC-ICP-MS***

**Cristina Rivas Urraca**

New methodologies have been developed for the determination of organotin and organolead compounds in environmental samples.

Several high performance liquid chromatographic separations of organotin compounds have been tested and the best system (cation-exchange chromatography with methanol and a citrate buffer) employed for the determination of tributyltin (TBT), triphenyltin (TPhT), dibutyltin (DBT) and monobutyltin (MBT) in environmental samples. The coupling between high performance liquid chromatography (HPLC) and the inductively coupled plasma-mass spectrometer (ICP-MS) for this application has been modified to yield limits of detection of 0.44, 0.26, 1.4 and 0.23 ng.g<sup>-1</sup> as Sn for TBT, TPhT, DBT and MBT respectively. Different extraction procedures have been tested for the determination of organotin species in samples of environmental interest, such as sediments and biological materials. The values obtained for TBT, TPhT and DBT in the analysis of a mussel candidate reference material, CRM 477, have been incorporated in the certification campaign of this material.

A liquid chromatographic separation for trimethyllead (TML) and triethyllead (TEL) has also been developed. Artificial rain water has been analysed for TML. The system proved to be valid for the determination of TML in this sample, even in the presence of high amounts of inorganic lead.

Finally, isotope dilution analysis (IDA) was incorporated in the method. Tributyltin iodide (TBTI) and trimethyllead chloride (TMLCl), isotopically enriched in <sup>116</sup>Sn and <sup>206</sup>Pb, respectively, were synthesised. The mussel tissue CRM 477 was analysed with IDA-HPLC-ICP-MS for TBT. As for the analysis without isotope dilution, the result obtained was incorporated in the certification campaign. The analysis with this methodology gave a better precision in the overall determination than external calibration analysis. Artificial rain water, at two different concentration levels, was analysed for TML with IDA-HPLC-ICP-MS. Better precision and accuracy was obtained for the analysis of this material with this method than when external calibration procedures were employed.

IDA-HPLC-ICP-MS has proved to be a valid technique for the analysis of environmental samples. The technique simplifies the procedure, compensates for different sources of variability and, thus, the overall precision obtained in the analysis is improved compared to other calibration techniques.

## AUTHOR'S DECLARATION

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other U.K. University award.

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Signed: Cynthia Davis.....

Date: .....20/Dec/1996.....



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***PUBLICATIONS***

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## ABBREVIATIONS

AAS	Atomic absorption spectrometry
DCP	Direct current plasma
ETAAS	Electrothermal atomic absorption spectrometry
FAAS	Flame atomic absorption spectrometry
GFAAS	Graphite furnace atomic absorption spectrometry
HG-AAS	Hydride generation atomic absorption spectrometry
ICP-AES	Inductively coupled plasma-atomic absorption spectrometry
ICP-MS	Inductively coupled plasma-mass spectrometry
PAR	4-(2-Pyridylazo)resorcinol
MS	Mass spectrometry
TetraBT	Tetrabutyltin
TBT	Tributyltin
DBT	Dibutyltin
MBT	Monobutyltin
TPhT	Triphenyltin
DPhT	Diphenyltin
MPhT	Monophenyltin
TetraMT	Tetramethyltin
TMT	Trimethyltin
DMT	Dimethyltin
MMT	Monomethyltin
TetraET	Tetraethyltin
TET	Triethyltin
DET	Diethyltin
MET	Monoethyltin
TPrT	Tripropyltin
DPrT	Dipropyltin
DOcT	Diocetyl tin
CySn	Cyclohexyltin
TetraBL	Tetrabutyllead



<b>TetraML</b>	<b>Tetramethyllead</b>
<b>TML</b>	<b>Trimethyllead</b>
<b>DML</b>	<b>Dimethyllead</b>
<b>TetraEL</b>	<b>Tetraethyllead</b>
<b>TEL</b>	<b>Triethyllead</b>
<b>DEL</b>	<b>Diethyllead</b>
<b>TMEL</b>	<b>Trimethylethyllead</b>
<b>DEDML</b>	<b>Diethyldimethyllead</b>
<b>TEML</b>	<b>Triethylmethyllead</b>
<b>DMEL</b>	<b>Dimethylethyllead</b>
<b>MDEL</b>	<b>Methyldiethyllead</b>
<b>TMBL</b>	<b>Trimethylbutyllead</b>
<b>DMDBL</b>	<b>Dimethyldibutyllead</b>
<b>TEBL</b>	<b>Triethylbutyllead</b>
<b>DEDBL</b>	<b>Diethyldibutyllead</b>
<b>SPSA</b>	<b>Sodium 1-pentanesulfonic acid</b>
<b>NRCC</b>	<b>National Research Council of Canada</b>
<b>NIES</b>	<b>National Institute for Environmental Studies</b>
<b>NIST</b>	<b>National Institute of Standards and Technology</b>

## PRESENTATIONS

### ORAL

**Short Course on Plasma-Mass Spectrometry ICP-MS**, Department of Analytical Chemistry, Complutense University of Madrid, Spain, February 1996. *Coupling of ICP-MS to Chromatographic Techniques. Determination of Species.*

**Trace Environmental Analysis (Eurocourse)**, University of Plymouth, Plymouth, U.K., September 1995. *Coupled Techniques: HPLC-ICP-MS.*

**Meeting of Research Projects Funded by Standards, Measurements and Testing Programme (BCR)**, Brussels, Belgium, October 1994. *Speciation of Organotin and Organolead Species with High Performance Liquid Chromatography Coupled to Inductively Coupled Plasma Mass Spectrometry.*

### POSTER

**8th Biennial National Atomic Spectroscopy Symposium**, University of East Anglia, Norwich, U.K., July 1996. *Tin Speciation in Biological Samples Utilising Isotope Dilution-HPLC-ICP-MS.*

**Research and Development Topics**, University of Nottingham Trent, Nottingham, Nottinghamshire, U.K., July 1996. *A New Methodology for the Determination of Tin Species in Biological Samples: Isotope Dilution-HPLC-ICP-MS.*

**Research and Development Topics**, University of Hull, Hull, U.K., July 1995. *Speciation of Organotin and Organolead Compounds by Isotope Dilution Analysis-HPLC-ICP-MS.*

**1995 European Winter Conference on Plasma Spectrochemistry**, University of Cambridge, Cambridge, U.K., January 1995. *Isotope Dilution Analysis-High Performance Liquid Chromatography-Inductively Coupled Plasma-Mass Spectrometry (IDA-HPLC-ICP-MS): a New Analytical Method for the Determination of Organotin Species.*

**7th Biennial National Atomic Spectroscopy Symposium, University of Hull, Hull, U.K., July 1994. *The Application of Isotope Dilution-High Performance Liquid Chromatography-Inductively Coupled Plasma-Mass Spectrometry for the Determination of Organotin Compounds.***

**Research and Development Topics, University of Hertfordshire, Hatfield, U.K., July 1994. *Determination of Organotin Compounds Using Isotope Dilution-High Performance Liquid Chromatography-Inductively Coupled Plasma-Mass Spectrometry.***

**Research and Development Topics, University of Bradford, Bradford, U.K., July 1993. *Tin Speciation Using Isotope Dilution Inductively Coupled Plasma Mass Spectrometry.***

# ***1.INTRODUCTION***

# **1. INTRODUCTION**

## ***1.1. TRACE METAL SPECIATION***

The determination of trace metals has been of great concern in the last decades. A metal is considered as "trace" when its concentration is of 1 part per million (ppm) or lower<sup>(1)</sup>. Although trace metals are essential for human health, the exposure to high levels of these elements is associated with different diseases, such as cancer (arsenic, beryllium, chromium, nickel), lung disease (beryllium, cadmium), kidney failure (lead, cadmium) and neurological and reproductive disorders (lead and mercury)<sup>(2)</sup>. The main routes for trace metals intake for humans is through air respiration or ingestion of food and water.

Several studies have been conducted to assess the impact for human beings and for the environment of trace elements. Some of the data has been summarised elsewhere for different elements, such as arsenic, beryllium, cadmium, chromium, lead, mercury and nickel<sup>(3-9)</sup>.

In some cases the total concentration of the element may be sufficient to ascertain the toxicity of the sample. However, for some cases, the toxicity is highly dependant on the chemical form of the analyte. Thus, speciation studies have become of great importance in the last few years. Both terms (species and speciation) have their origin in biological sciences. In biology, a species is a group of organisms with hereditary traits that when interbreed conserve these traits<sup>(10)</sup>. Speciation refers to the

process that the different species are subject to during evolution<sup>(10)</sup>. Speciation in chemistry, on the other hand, involves the determination of the different species (or some of them) of the element (organic, inorganic, organometallic) in the sample<sup>(11)</sup>.

In general, the organic forms of an element are more toxic than the inorganic forms. Alkylmercury compounds, for example, are known to be more toxic than the inorganic forms of this element. Methylmercury is more rapidly absorbed in the human intestine than mercury(II) chloride, whilst  $\text{Hg}^0$  is not absorbed at all<sup>(12)</sup>. The alkyl species of this metal affect the central nervous system, inhibiting the enzymatic activity and the cell wall transport mechanisms.

The importance of speciation was shown in the 1950's in Minamata, Japan<sup>(13,14)</sup>. Inorganic mercury originating from a chemical plant was discharged into sea water. Here, it was methylated by sedimentary bacteria<sup>(15,16)</sup>. The methylmercury species was bioaccumulated by fish which were later consumed by the local population, resulting in a major intoxication problem.

One of the elements in which inorganic forms are more toxic than the organic ones is arsenic. Its compounds are used as herbicides, pesticides, as cotton desiccant and as wood preservatives. The inorganic forms arsenite ( $\text{AsO}_2^-$ ) and arsenate ( $\text{AsO}_4^{3-}$ ) are highly toxic to human beings (they are toxicants comparable to strychnine), while organic forms naturally occurring in fish, such as arsenocholine ( $(\text{CH}_3)_3\text{-As}^+(\text{CH}_2)_2\text{-OH}^-$ ) and arsenobetaine ( $(\text{CH}_3)_3\text{-As}^+\text{-CH}_2\text{-COO}^-$ ) could be considered non toxic ( $\text{LD}_{50}$  for both of them  $> 10000 \text{ mg.Kg}^{-1}$  of weight of rat)<sup>(17)</sup>.

Chromium and selenium speciation are also of environmental interest, due

to the different properties of each species. Cr(III) for example is essential for humans in glucose, lipids and metabolism, while Cr(VI) is highly toxic. The different occupational exposure limits (OEL) for water soluble compounds in indoor air is  $0.5 \text{ mg.m}^{-3}$  for Cr(III) and  $0.05 \text{ mg.m}^{-3}$  for Cr(VI), reflecting the different toxicity<sup>(18)</sup>.

### 1.1.1. Organotin compounds

Tin is a post-transition metal situated in the group 14 of the Periodic Table. Its atomic number is 50 and atomic mass is 118.69. It has ten natural isotopes, namely 112 (1.0%), 114 (0.65%), 115 (0.35%), 116 (14.4%), 117 (7.6%), 118 (24.1%), 119 (8.6%), 120 (32.8%), 122 (4.7%) and 124 (5.8%). The abundance of this element in the Earth's Crust is  $3 \text{ g.Ton}^{-1}$ <sup>(19)</sup>. The electronic structure is  $[\text{Kr}] 4d^{10} 5s^2 5p^2$ . The most frequent oxidation states in which it can be found are (II) and (IV), the latter being the most common one for organotin compounds.

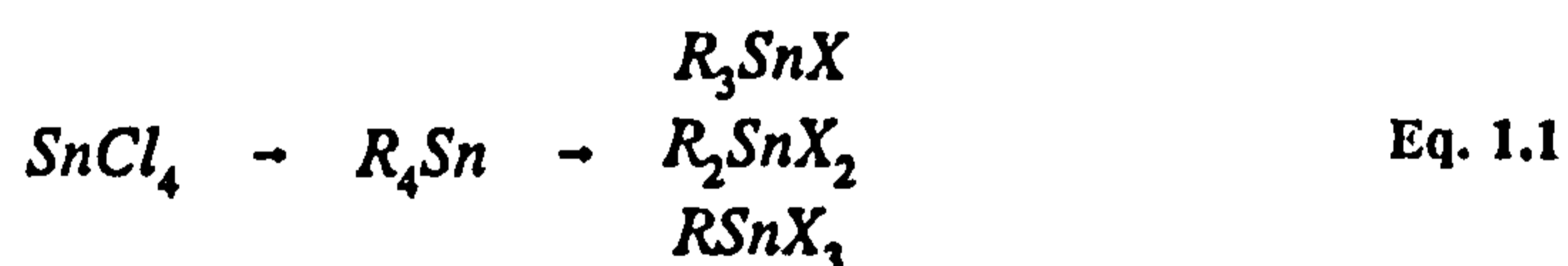
Löwig in 1852 prepared the first organotin compound but it was Sir Edward Frankland who initiated the first systematic study on these compounds. He prepared diethyltin diiodide in 1853 and tetraethyltin six years later<sup>(20,21)</sup>. Other compounds followed afterwards. It was not until a hundred years later that the first relevant application of these compounds was discovered. In the 1940's the plastics industry realised the importance of polyvinylchloride (PVC). This plastic suffered from being easily degradable with light or heat, becoming brittle and discoloured. Diorganotin compounds were found to stabilise PVC. They were introduced as stabilisers in United States of America in the late 1940's, in United Kingdom in 1951 and in the



mid 1950's in the rest of Europe and Japan<sup>(20,21)</sup>. In 1955 non-toxic octyltin compounds were approved to be used as stabilisers in food-contact PVC applications. This remains as the largest single application of organotin compounds to date.

The general formulation of organotin compounds is  $R_nSnX_{4-n}$ , where R represents an alkyl or aryl group, n being from 1 to 4 (thus becoming the four different types of organotin compounds, mono-, di-, tri- and tetraorganotin species), and X is an anion or equivalent group ( $F^-$ ,  $Cl^-$ ,  $OH^-$ , etc.). The properties of the organotin compounds depend greatly on the number of organic radicals and their nature. The X group does not have a marked effect on the properties, unless it is itself a group with biocidal or toxic properties of its own.

The most toxic organotin species correspond to  $n=3$  in the general formulation. Di- and monoorganotins follow. Tetraorganotins are of very low or no toxicity. The main use of the latter is as precursors in the synthesis of other organotin compounds from  $SnCl_4$ <sup>(20)</sup>:



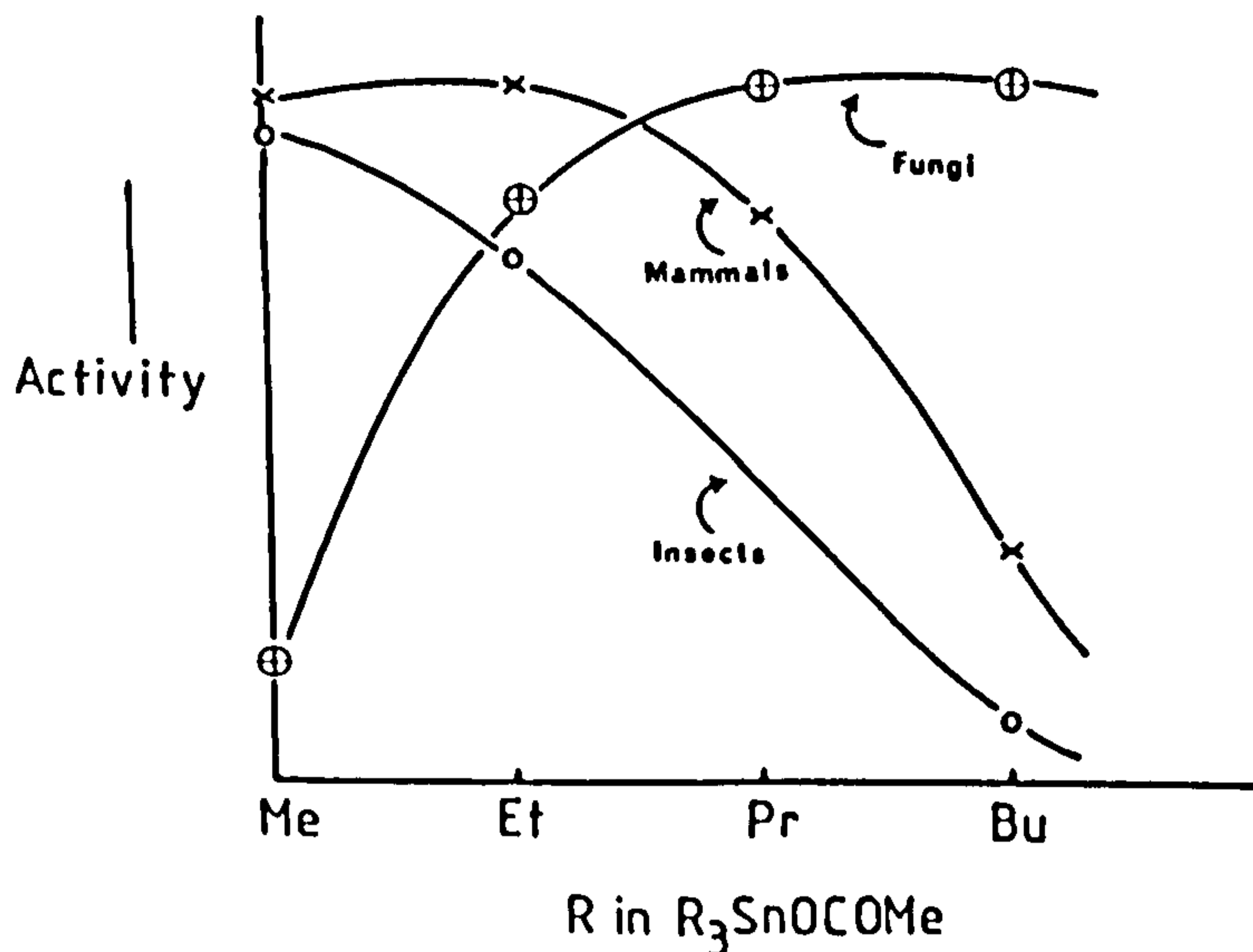
Tetraorganotins may have a delayed toxic effect associated with their degradation to lower chain organotin compounds (triorganotins in first instance). Both *in vivo* and *in vitro* studies have proved that this process occurs particularly in the liver<sup>(21-23)</sup>.



The main use of triorganotin compounds is as biocides in a large number of applications<sup>(20)</sup>, such as active ingredients in antifouling paints, in herbicides, fungicides and wood preservatives<sup>(23)</sup>. Diorganotin compounds are mainly used as polyvinylchloride (PVC) stabilisers; those containing Sn-S bonds are used as heat stabilisers, while when a bond Sn-O is present they are used as light stabilisers<sup>(23)</sup>. Monoorganotin compounds are widely employed in industry due to their low toxicity. They are used, together with diorganotin compounds, as PVC stabilisers, since the two groups act synergistically, improving the individual properties of each other<sup>(23)</sup>. Other applications involve their use as precursors of SnO<sub>2</sub> films on glass and as catalysts for homogeneous transesterification reactions.

The nature of the organic radical has a great effect on the toxicity of the compound. Their use depends mainly on the effect of this organic radical. As an example, in the trisubstituted group of organotin compounds (R<sub>3</sub>SnX), trimethyltin species show a higher toxicity against fungi and insects, triethyltin are the most toxic for mammals while tripropyl and tributyltin have a greater toxicity for fungi, molluscs, fish, bacteria and plants<sup>(19)</sup>. Figure 1.1 shows the different patterns. In the case of butyltin compounds, the difference between their effect against insects, fungi, fish, molluscs, etc. and mammals explains why they have been used so extensively as fungicides, wood preservatives and in antifouling paints.

Tetraalkyltin compounds exhibit low toxicity to mammals. The most toxic ones are the ethyltins. The longer the alkyl chain, the lower the toxicity. The danger regarding these compounds is their volatility and absence of odour, which makes them potentially hazardous.



**Figure 1.1.** *Effect of the alkyl group on the biological activity of tri-n-alkyltin acetate for different species (from C.J. Evans, S. Karpel. "Organotin Compounds in Modern Technology", Elsevier, Amsterdam, The Netherlands, 1985)*

The toxicity of triorganotin compounds is believed to be due to mitochondrial misfunctions. The processes involved have been summarised as follows<sup>(20,21,24,25)</sup>.

- 1/ Swelling and disruption is caused by the interaction with mitochondrial membranes.
- 2/ The organotin compounds can act as ionophores deranging mitochondrial functions by  $Cl^-/OH^-$  exchange across the lipid membrane.

3/ Finally, triorganotins inhibit energy conservation processes fundamental for the transformation of ADP into ATP.

The biological activity of triorganotin compounds is thought to be due to their ability to bind to certain proteins. The sites where this binding occurs are not well known. In general, the effects of triorganotin compounds both in humans and in animals has been found to be reversible<sup>(24)</sup>.

Diorganotin compounds appear to have a different mode of action to triorganotins. Some of the compounds with a low organic chain in the R group of the formulation ( $R_2SnX_2$ ) can inhibit the oxidation of  $\alpha$ -keto acids due to their combination with coenzymes with vicinal dithiol groups<sup>(20)</sup>. In this case the nature of the group X can affect the toxicity. As an example, dimethyltin dichloride is moderately toxic, while dimethyltin diisooctylthioglycolate  $[(CH_3)_2Sn(SCH_2CO_2-i-Oct)_2]$  is relatively non toxic since it already possesses two Sn-S bounds in the molecule<sup>(24,26)</sup>.

Both mono- and tetraorganotins have low mammalian biological activity. As mentioned above, there is a potential danger of toxicity with tetraorganotin compounds due to their possible degradation to more toxic species, especially trisubstituted ones. Inorganic tin is a non-toxic metal and its main use is in coating for food cans. At physiological pH the metal does not react and its oxides are insoluble.

The paths through which the organotins enter the environment depend on the use of the compound. Biocidal uses of organotin compounds only account for about

30% of the total use of these compounds, but their toxic effect will come primarily from this use of them. Organotin compounds are used in a wide number of industries. Figure 1.2 shows schematically the routes for organotin compounds entering the environment and the main areas affected.

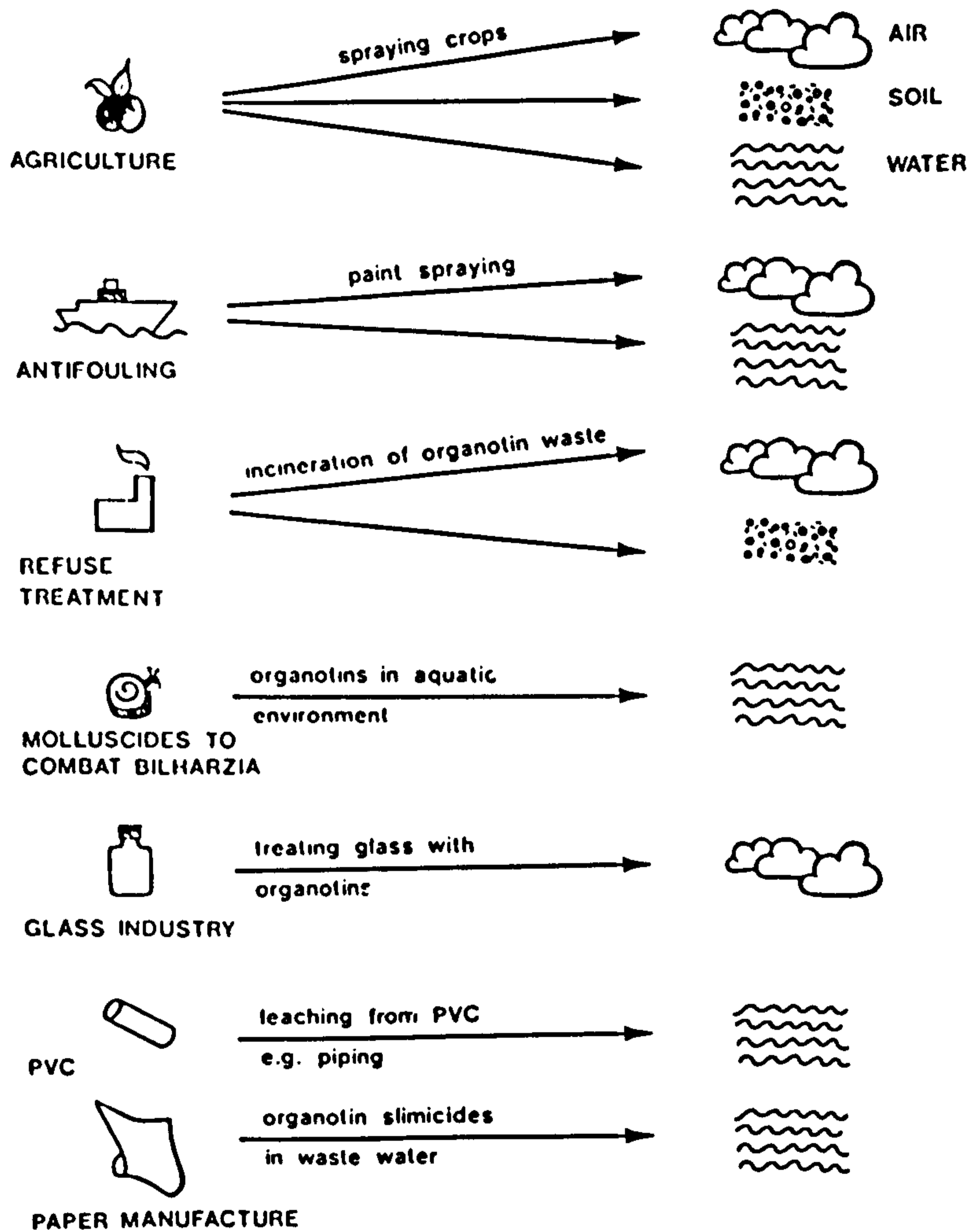


Figure 1.2. Routes for organotin compounds to enter the environment and areas affected (from C.J. Evans, S. Karpel. "Organotin Compounds in Modern Technology", Elsevier, Amsterdam, The Netherlands, 1985)

**PVC stabilisation:** This is the major use of organotin compounds and thus the most probable source of contamination into the environment. The organotins are likely contaminants of emissions during the fabrication process of PVC, leaching from PVC products or from the disposal of these products. Due to the safety standards followed during the manufacturing of PVC to stop any possible release of toxic vinylchloride monomer it is probably not a contamination during the manufacturing processes.

The rates of leaching of organotins from PVC products have been reviewed elsewhere<sup>(27)</sup>. The rate depends on the length of the chain of the organic radical and on other factors, such as pH, concentration of analyte and type of solvent. Leaching rates of organotins from PVC containers into liquid food has been studied by Carr *et al.*<sup>(28)</sup>. The authors obtained values of leached tin after two months of storage at 30°C between 0 and 0.07 mg.Kg<sup>-1</sup>. The low levels of migration in any case suggest that this is not a significant contamination source.

One of the disposal procedures for PVC products is through incineration. This process should degrade the organotin compounds used as stabilisers into the non toxic inorganic tin. When the PVC is disposed of in a land fill, the leaching rate of organotins into the soil or subterranean water currents is very low, and so is the initial concentration of organotin from which it could leach.

Summarising, the PVC industry does not constitute a major source of organotins entering the environment.

**Wood preservation:** Organotins are applied to wood as preservatives as a



solution of 1-3% in an organic solvent, such as kerosene. Although brushing or spraying is used for remedial purposes, the main impregnation treatment is done in special enclosed chambers. Vacuum and pressure cycles are applied and once the solvent has evaporated the organotin is well impregnated in the wood. These closed systems are not likely to cause great contamination. On the other hand, the vapour pressure of organotin species is quite low, what makes timber products safe to use in-doors<sup>(29)</sup>.

**Agricultural uses:** The pesticides are normally air sprayed onto the crops. This means that the organotin compounds could contaminate soils, air and waters, due to this process.

There are five organotins commercially used as pesticides: triphenyltin acetate, triphenyltin hydroxide (both used as fungicides), tricyclohexyltin hydroxide, bis[tris(2-methyl-2-phenylpropyl)tin] oxide and 1-tricyclohexylstannyl-1,2,4-triazole (used as miticides)<sup>(21)</sup>. Bock<sup>(30)</sup> reviewed the physical, chemical and biological properties as well as toxicological analysis and environmental behaviour of triphenyltin compounds. The World Health Organisation (WHO) classed triphenyltin acetate and hydroxide as "safe agricultural chemicals" and indicated the waiting times between treatment and harvesting regarding each crop type<sup>(30)</sup>.

The concentration of the pesticides decreases rapidly due to the wind, the rain and the degradation because of light. Tricyclohexyltin hydroxide decreases its concentration in apples and pears to about 50% in 3 weeks due to photodegradation. A further 20-50% can be removed by thoroughly washing the fruit and, finally,

peeling removes most of the remaining pesticide. Thus, about 0.1 mg.Kg<sup>-1</sup> (as tin) may be expected in the flesh<sup>(21)</sup>.

An additional problem could be presented when animals are likely to eat leaves from recently sprayed crops. In this way, the organotin compounds could enter the food chain. In cows fed on sugar beet leaves containing 1 mg.Kg<sup>-1</sup> of triphenyltin acetate only 0.004 mg.Kg<sup>-1</sup> was found in milk<sup>(32)</sup>. No content above the blank level was found in meat. It could be concluded that even if the animal has been fed with contaminated vegetables, it is safe for humans to eat them. Any possible residue may be, anyway, degraded into less toxic organotin forms in the cooking process.

**Antifouling paints:** The detrimental effect of triorganotin compounds leached from antifouling paints in shellfish has been well known since the study carried out by Alzieu *et al.*<sup>(33)</sup> in 1980. Organotin compounds are used as active ingredients of antifouling paints; their method of action is by releasing toxic organotins in the surroundings of the boat to avoid the attachment of aquatic organisms to the hull of the vessel. These organisms (known as fouling) have a detrimental effect on the running costs of the vessels.

The major effect can be observed in harbours and marinas where the ships are stationary, due to a build up in the concentration of toxicants. Both waters and sediments are found to contain significant concentration of triorganotin compounds and their less toxic degradation products. The half life ranges from days in waters to months, even years, in sediments.

**Molluscicides:** Bis(tributyltin) oxide has been used to control the infection to humans of a parasite commonly found in Central and South America, Africa and Asia: Schistosome. As larvae, they enter some forms of aquatic snails, before being released into water as free swimming cercariae. It is at this stage when they present a danger for humans. The control method proposed consists in the use of small rubber pellets floating in the water, which release very low concentrations of organotin compounds, enough amount to control the Schistosome but low enough not to have any detrimental effect in the environment (fish and other organic aquatic life).

### 1.1.2. Organolead compounds

Lead is (as tin) situated in the group 14 of the Periodic Table. Its atomic number is 82 and the atomic mass 207.19. Lead has different natural isotopes (204, 206, 207, 208) with different abundances depending on what part of the Earth it is found. The abundance on the Earth's Crust is  $15 \text{ g.Ton}^{-1}$ <sup>(19)</sup>. The electronic structure is similar to the tin one, with the addition of another shell:  $[\text{Xe}] 4f^{14} 5d^{10} 6s^2 6p^2$ .

Although there are few organometallic compounds of divalent lead, the most common organometallic species of lead are of the form  $\text{R}_n\text{PbX}_{4-n}$  or  $\text{R}_6\text{Pb}_2$ <sup>(34)</sup>, where X is an anionic group and R an organic radical.

Lead is ubiquitous in the environment. The earliest use of this metal by humans dates back to 7000-5000 BC<sup>(35)</sup>. Some authors<sup>(36)</sup> have attributed the decay of the Roman Empire to the poisoning of food and drinks by this element from pipes and cooking utensils, in which it had been extensively used. Thereafter the use of



lead declined but, since the Middle Ages, it has again increased its use, particularly after the industrial revolution in the mid 18<sup>th</sup> century. The antiknock properties of lead compounds in gasolines were discovered in 1922<sup>(37)</sup>, giving way to a new industry. Nowadays, the lead battery industry accounts for 50% of the consumption of the total production of lead, being followed by the use of organolead compounds in the car industry. Lead chemicals and pigments contribute the rest of the consumption of this metal<sup>(24,38,39)</sup>.

The first preparation of an organometallic lead compound dates back to 1852 when Löwig reacted sodium-lead alloy with ethyl iodide to produce impure hexaethyldilead<sup>(40)</sup>, which on exposure to air lead to a white crystalline compound that proved to be triethyllead carbonate.

While there are a large number of organolead compounds, lead environmental contamination sources are dominated by a small number of tetraalkyllead compounds and their degradation products. This is due to the use of tetraalkyllead compounds in the gasolines (tetramethyllead, tetraethyllead and the three mixtures of both of them).

Considerable controversy was occasioned by the widespread use of lead in petrol and in 1975 the United States of America and Japan were the first countries to recommend using unleaded petrol in cars. The need to use catalytic converters to reduce air pollution from unburnt fuel, carbon monoxide and oxides of nitrogen, resulted in the widespread use of unleaded petrol (the lead being a very effective catalyst poison). The beneficial effects of these measures were soon clear. Thus,

Western Europe countries followed in decreasing the use of lead in petrol. In the United Kingdom the permissible level of refined lead in gasolines is 0.4 g.l<sup>-1</sup>, but the average level was already 0.15 g.l<sup>-1</sup> in 1985<sup>(24)</sup>.

Organolead compounds have been used for other purposes, such as wood and cotton preservatives, fungicides and antifouling agents. These uses represent only, though, a small proportion of the organolead industry.

Tetraorganolead compounds are fairly stable at room temperature while kept in the dark. They degrade to inorganic lead and organic radicals when irradiated with UV light<sup>(21)</sup>. Tri- and diorganolead compounds are much less stable than tetraorganolead species. They degrade, at room temperature, following a disproportionation reaction:



Unlike the first reaction, the second one is not reversible due to the high instability of the  $RPbX_3$  compounds<sup>(41)</sup>.

The reported toxicology of organolead compounds is related mainly to tetraethyllead since it has been used as an antiknock agent for many years now. Tetramethyllead started being used later and less information has been published about it. In general, the toxicity is related to animal laboratory experiments and

some cases of intoxication. Inorganic and tetraorganolead compounds are the most studied; there is less information available on other species.

Tetraethyllead is a liposoluble substance and thus is readily absorbed through the skin<sup>(21,24,42)</sup>. Its absorption will depend on many parameters such as skin thickness, concentration, time of exposure, etc. Tetraethyllead is less readily absorbed when diluted with petrol at concentrations around 0.1% (v/v). Tetramethyllead is less soluble through skin than the ethylated analogues.

The effect of tetraethyllead and tetramethyllead compounds absorbed through the lungs has been studied with a group of volunteers. They inhaled <sup>203</sup>Pb-labelled tetraethyllead and tetramethyllead. Approximately 50% of the vapour inhaled was exhaled again, but still between 30 and 40% was found in the body<sup>(43)</sup>.

When ingested, tetra derivatives can be degraded to the corresponding triorganolead species by the gastric hydrochloric acid<sup>(44)</sup>. The absorption rate of this species is not known, but it is expected to be high.

The main organ affected by trialkyllead species is the brain. Although this species may accumulate as well in liver and kidneys<sup>(38)</sup>, it is only in the brain that triethyllead is known to inhibit the glucose oxidation by brain slices. Glucose is the only substrate where the oxidation is affected.

The mechanism through which triethyllead and trimethyllead act as toxicants could be similar to that for organotin compounds, as reported by Cremer<sup>(45)</sup> and Aldrige<sup>(46)</sup>. These species may act directly on mitochondria and thus inhibit the

entrance of substances and the synthesis of ATP, probably through binding thiol groups. They may also increase the neuronal excitability by altering the  $\text{Cl}^-$  distribution across membranes, which produces an increase in the glucose utilization. This production affects the pyruvate oxidation and the ATP production, raising the amount of pyruvic and lactic acid. The result is a localised acidosis.

Finally, the lethal dose of trimethyllead and triethyllead is not known but could be extrapolated from the  $\text{LD}_{50}$  for rats. This has been found to be  $<36 \text{ mg Pb.Kg}^{-1}$  for trimethyllead chloride,  $80 \text{ mg Pb.Kg}^{-1}$  for tetramethyllead,  $20 \text{ mg Pb.Kg}^{-1}$  for triethyllead chloride and  $15 \text{ mg Pb.Kg}^{-1}$  for tetraethyllead when oral administration is used. These values indicate a lethal dose for adults of about  $0.25 \text{ g}$  of tetraethyllead and more than  $1 \text{ g}$  for tetramethyllead<sup>(44)</sup>.

The symptoms of alkyllead poisoning are non specific, such as headache, vomiting, fatigue, diarrhoea and particularly insomnia. At later stages the central nervous system starts showing effects like tremor and hypothermia and hypotonia.

## **1.2. METHODS FOR THE DETERMINATION OF CHEMICAL SPECIES**

### **1.2.1. Organotin compounds**

In order to achieve speciation, the different forms of an element have to be separated prior to their detection by atomic spectrometry since such techniques yield only total elemental information and, as mentioned above, the total content of the element may not give an accurate value of the toxicity. Although there have been

some attempts to differentiate between "soluble" and "non-soluble" tin (for example) in a particular solvent<sup>(47)</sup> or after a leaching procedure<sup>(48)</sup>, the most common way of separating the analytes is through chromatographic separations.

Gas chromatography (GC) provides a way of separating many species with very good resolution. There have been many methods published following this approach<sup>(49-72)</sup>. Unfortunately, organotin compounds are not volatile or thermally labile or stable enough to be separated with gas chromatography. They require a previous derivatisation step. This can be achieved via a Grignard reaction<sup>(52,53,55,56,64,68-72)</sup>, ethylation through the reaction with sodium tetraethylborate ( $\text{Et}_4\text{BNa}$ )<sup>(51,58,65,66)</sup> or the formation of the hydrides with sodium borohydride ( $\text{NaBH}_4$ )<sup>(49-51,57,60-64,67,72)</sup>. In some cases the volatile derivatives have been preconcentrated by cool trapping, in liquid nitrogen with or without GC packing material, which is heated afterwards in a controlled manner and thus, the species released depending on their boiling points<sup>(50,57,58,60-64)</sup>.

Liquid chromatography (LC), in particular high performance liquid chromatography (HPLC), offers the possibility of avoiding the time consuming step of derivatisation, minimising the number of processes involved in the determination, which makes the procedure less prone to contamination or loss of analyte. By avoiding derivatisation, the possibility of rearrangements and loss of analyte integrity is avoided. There are several methods published following this approach. A summary of them can be seen in Table 1.1.



Table 1.1. HPLC separation methods for organotin compounds

COLUMN	DETECTION SYSTEM	MOBILE PHASE	FLOW RATE (ml.min <sup>-1</sup> )	INJECTION VOLUME (μl)	ANALYTE SPECIES	REF
Nucleosil 5CN	GFAAS	Toluene + Tropolone (7.5 mg.l <sup>-1</sup> )	1.0	250	TBT	50
n.r.	MS	MeOH (50%) + H <sub>2</sub> O (50%) + NH <sub>4</sub> Ac (0.05 mol.l <sup>-1</sup> ) or Ammonium formate (0.05 mol.l <sup>-1</sup> )	1.0	20	TBT, TPhT	55
Lichrosorb C2, 10 μm	GFAAS	MeOH (100%)	1.5	20	TPhT, TBT, TPrT	73
Lichrosorb C18, 10 μm	GFAAS	MeOH (100%)	n.r.	20	TPhT	73
Waters Styragel 60A, <37 μm (500x7.5 mm) slurry packed in benzene	Refractive index detector	Benzene or Toluene or CCl <sub>4</sub> or CH <sub>3</sub> CN or Benzene + CCl <sub>4</sub>	1 or 2	25	TetraMT, TMT, DMT, MMT, Sn	74
Spherosil XOB 75 + Pyrocarbon, 20-31.5 μm (500x2.6 mm) slurry packed in bromobenzene	Refractive index detector	Benzene or Toluene or CCl <sub>4</sub> or CH <sub>3</sub> CN	n.r.	n.r.	TetraMT, TMT, DMT, MMT, Sn	74



Table 1.1. HPLC separation methods for organotin compounds (continued)

COLUMN	DETECTION SYSTEM	MOBILE PHASE	FLOW RATE (ml.min <sup>-1</sup> )	INJECTION VOLUME (μl)	ANALYTE SPECIES	REF
Styragel 100A, 10 μm (300x7.8 mm)	Refractive index detector	n.r.	3	n.r.	TetraMT, TMT, DMT, MMT, Sn	74
Styragel 500A, <37 μm (500x7.8 mm) slurry packed in benzene	Refractive index detector	n.r.	n.r.	n.r.	TetraMT, TMT, DMT, MMT, Sn	74
Styragel 100A, <37 μm (500x7.5 mm) slurry packed in benzene	Refractive index detector	n.r.	n.r.	n.r.	TetraMT, TMT, DMT, MMT, Sn	74
Waters μBondapak CN, 10 μm (300x3.9 mm)	Refractive index detector	n.r.	n.r.	n.r.	TetraMT, TMT, DMT, MMT, Sn	74
Perkin Elmer ODS-SIL-X-1, 13 μm (500x2.6 mm)	Refractive index detector	n.r.	n.r.	n.r.	TetraMT, TMT, DMT, MMT, Sn	74
Spherisorb ODS S5W (250x3.0 mm)	Refractometry	Acetone (70%) + Pentane (30%)	n.r.	50	TetraET, TET, DET, MET	75

Table 1.1. HPLC separation methods for organotin compounds (continued)

COLUMN	DETECTION SYSTEM	MOBILE PHASE	FLOW RATE (ml.min <sup>-1</sup> )	INJECTION VOLUME (μl)	ANALYTE SPECIES	REF
Spherisorb ODS S5W (250x3.0 mm)	Refractometry	Acetone (60 %) + Pentane (40%)	n.r.	50	TetraMT, TMT, DMT, MMT	75
Lichrosorb ODS, 10 μm (250xn.r. mm)	GFAAS	MeOH (97.5%) + H <sub>2</sub> O (2.5%)	0.1	20	TetraPhT, TetraBT, DBT	76
Spherisorb ODS S5W (250x3.0 mm)	AAS	Acetone (60%) + Pentane (40%)	1.0	50	TetraMT, TMT, DMT, MMT	77
Spherisorb ODS S5W (250x3.0 mm)	AAS	Acetone (70%) + Pentane (30%)	1.2	50	TetraET, TET, DET, MET	77
Partisil-10 SCX, 10μm (250x4.6 mm)	GFAAS	MeOH (70%) + H <sub>2</sub> O (30%) + NH <sub>4</sub> Ac (0.03 mol.l <sup>-1</sup> )	1.5-2.0	50-200	TPtT, TET, TMT	78
Partisil-10 SCX, 10μm (250x4.6 mm)	GFAAS	MeOH (70%) + H <sub>2</sub> O (30%) + NH <sub>4</sub> Ac (0.06 mol.l <sup>-1</sup> )	1.0	50-200	TPhT, TBT	78

Table 1.1. HPLC separation methods for organotin compounds (continued)

COLUMN	DETECTION SYSTEM	MOBILE PHASE	FLOW RATE (ml.min <sup>-1</sup> )	INJECTION VOLUME (μl)	ANALYTE SPECIES	REF
Whatman PXS cation exchange, 10 μm (250x4.6 mm)	Differential pulse detection	MeOH (60%) + H <sub>2</sub> O (40%) + NH <sub>4</sub> Ac (0.042 mol.l <sup>-1</sup> , pH=5.3)	1.0	n.r.	TBT, TET, TMT	79
Unisil QCN (cyanopropyl-bonded phase) (250x4.0 mm)	Fluorescence	Hexane (90%) + Ethyl acetate (5%) + HAC (5%)	1.2	n.r.	DOcT, DBT, DPpT, DET, DMT	80
Cyanopropyl-bonded silica 5 μm (250x4.6 mm)	Fluorescence	Toluene + HAC (3%) + EtOH (waterfree, 2%) + EtOH (water 4%, 1%) + Morin (0.0015%)	1.0	10	DOcT, DBT, DPpT, DPPhT, DET, DMT	81
Cyanopropyl-bonded silica 5 μm (100x4.6 mm, cartridge)	Fluorescence	Toluene + HAC (3%) + MeOH (2%) + Morin (0.0015%)	1.0	10	DOcT, DBT, DPpT, DPPhT, DET, DMT	81
MPLC™ Cartridge (cyano), 5 μm (100x4.6 mm)	Refractive index detector	Hexane + HAC (1%) + Ethyl acetate (1%)	1.0	10	TBT, TET, TMT, DBT	82

Table 1.1. HPLC separation methods for organotin compounds (continued)

COLUMN	DETECTION SYSTEM	MOBILE PHASE	FLOW RATE (ml.min <sup>-1</sup> )	INJECTION VOLUME (μl)	ANALYTE SPECIES	REF
MPLC™ Cartridge (cyano), 5 μm (100x4.6 mm)	Refractive index detector	Hexane (100%)	1.0	10	TBT, TPtT, TET, TMT, TPhT	82
MPLC™ Cartridge (cyano), 5 μm (100x4.6 mm)	Fluorescence	Toluene + HAC (5%) + MeOH (2%) + CH <sub>3</sub> CN (5%) + Morin (5 μmol.l <sup>-1</sup> )	1.0	10	MBT, MET, MMT	82
MPLC™ Cartridge (cyano), 5 μm (100x4.6 mm)	Fluorescence	Toluene + HAC (5%) + MeOH (2%) + CH <sub>3</sub> CN (8%) + Morin (5 μmol.l <sup>-1</sup> )	1.0	10	DBT, MBT, MMT	82
Partisil-10 SCX, 10 μm (250x4.6 mm)	FAAS	MeOH (70%) + H <sub>2</sub> O (30%) + NH <sub>4</sub> Ac (0.1 mol.l <sup>-1</sup> )	3.0	100	Sn(II), Sn(IV), TBT	83
Hamilton PRP-1, 10 μm (250x4.1 mm)	DCP	HAC (2.5%) + H <sub>2</sub> SO <sub>4</sub> (0.02 N) + KF (0.003 mol.l <sup>-1</sup> ) + 1-Hexanesulphonic acid (0.0003 mol.l <sup>-1</sup> )	2.5	200	TMT, DMT, MMT	84

Table 1.1. HPLC separation methods for organotin compounds (continued)

COLUMN	DETECTION SYSTEM	MOBILE PHASE	FLOW RATE (ml.min <sup>-1</sup> )	INJECTION VOLUME (μl)	ANALYTE SPECIES	REF
Polymethacrylate	Fluorescence	MeOH (70%) + H <sub>2</sub> O (30%) + Phosphate (10 mmol.l <sup>-1</sup> , pH=2.3)	n.r.	n.r.	DMT, DET, DPtT, DBT	86
SCX, 10 μm	GFAAS	MeOH (70%) + H <sub>2</sub> O (30%) + Ammonium citrate (0.08 mol.l <sup>-1</sup> )	0.5	20	TBT, DBT, TET	87
TSK gel ODS-8TM (250x4.6 mm)	AAS	THF (54%) + H <sub>2</sub> O (38%) + HAC (8%) + Tropolone (0.2%, m/v)	0.9	200	TPhT, DPhT, TBT, DBT, MBT, TMT, DMT, MMT	88
Partisil-10 SCX, 10μm (250x4.6 mm)	Laser enhanced ionization	MeOH (75%) + H <sub>2</sub> O (25%) + NH <sub>4</sub> Ac (0.05 mol.l <sup>-1</sup> , pH=5.1)	2.0	20	TBT, TET, TPtT	89
Partisil-10 SCX, 10μm (250x4.6 mm)	GFAAS	MeOH (70%) + H <sub>2</sub> O (30%) + Ammonium citrate	0.3	20	TBT, DBT	90
Partisil-10 SCX, 10μm (250x4.6 mm)	AAS	MeOH (70%) + H <sub>2</sub> O (30%) + NH <sub>4</sub> Ac (0.1 mol.l <sup>-1</sup> )	3.0	1.5	TBT, DBT	91



Table 1.1. HPLC separation methods for organotin compounds (continued)

COLUMN	DETECTION SYSTEM	MOBILE PHASE	FLOW RATE (ml.min <sup>-1</sup> )	INJECTION VOLUME (μl)	ANALYTE SPECIES	REF
Spherisorb ODS-2, 5 μm (250x4.6 mm)	ICP-AES,	MeOH (80%) + H <sub>2</sub> O (19%) + HAC (1%) + Sodium pentane sulphonate (0.004 mol.l <sup>-1</sup> )	1	n.r.	TMT, TPhT, TBT	92
	ICP-MS					
Adsorbosphere SCX, 5 μm (250x4.6 mm)	ICP-AES,	MeOH (85%) + H <sub>2</sub> O (15%) + Ammonium acetate (0.1 mol.l <sup>-1</sup> )	1	n.r.	TMT, TPhT, TBT	92
	ICP-MS					
Spherisorb ODS-2, 5 μm (50x4.6 mm)	ICP-AES	Sodium dodecyl sulphate (0.05 mol.l <sup>-1</sup> ) + HAC (3%) + Propanol (3%) + KF (5 mmol.l <sup>-1</sup> )	1	100	TMT, DMT, MMT	93
Spherisorb ODS-2, 5 μm (50x4.6 mm)	ICP-MS	Sodium dodecyl sulphate (0.1 mol.l <sup>-1</sup> ) + HAC (3%) + Propanol (3%)	1	100	TMT, TET, TPtT	93
Partisil-10 SCX, 10 μm (250x4.6 mm)	ECD (Reverse pulse amperometric detection)	MeOH (70%) + H <sub>2</sub> O (30%) + NH <sub>4</sub> Ac (0.1 mol.l <sup>-1</sup> , pH=5.5)	n.r.	n.r.	DOcT, DBT, DPtT, DET, DMT	94



Table 1.1. HPLC separation methods for organotin compounds (continued)

COLUMN	DETECTION SYSTEM	MOBILE PHASE	FLOW RATE (ml.min <sup>-1</sup> )	INJECTION VOLUME (μl)	ANALYTE SPECIES	REF
Partisil-10 SCX, 10 μm (250x4.6 mm)	FAAS	MeOH (70%) + H <sub>2</sub> O (30%) + NH <sub>4</sub> Ac (0.1 mol.l <sup>-1</sup> ) or Citrate	2.0	200	TBT, DBT	95
Partisil-10 SCX, 10 μm (250x4.6 mm)	ICP-MS	MeOH (60%) + H <sub>2</sub> O (40%) + Ammonium citrate (0.18 mol.l <sup>-1</sup> , pH=6)	1.0	200	TBT, DBT	96
Partisil-10 SCX, 10 μm (250x4.6 mm)	ICP-MS	MeOH (60%) + H <sub>2</sub> O (40%) + Ammonium citrate (0.3 mol.l <sup>-1</sup> ), pH gradient (6 to 3)	1.0	200	TBT, DBT, MBT	96
μBondapak, C18 (300x3.9 mm)	GFAAS	THF (2%) + Acetone (98% acetone + 2% HAc)	0.6	10	TBT, DBT, CyclohexylSn (CySn)	97
Spherisorb CN	UV	Hexane (90%) + THF (10%)	1.0	10	TBT, TET, TMT, TPhT, DPhT, DET, DMT	98

Table 1.1. HPLC separation methods for organotin compounds (continued)

COLUMN	DETECTION SYSTEM	MOBILE PHASE	FLOW RATE (ml.min <sup>-1</sup> )	INJECTION VOLUME (μl)	ANALYTE SPECIES	REF
RoSiL (CH <sub>3</sub> ) <sub>2</sub> CN	UV	Hexane (90%) + THF (10%)	1.0	10	TBT, TET, TMT, TPht, DBT, DPhT, DET, DMT	98
RoSiL CN	UV	Hexane (90%) + THF (10%)	1.0	10	TBT, TET, TMT, TPht, DBT, DPhT, DET, DMT	98
Deltabond CN	UV	Hexane (90%) + THF (10%)	1.0	10	TBT, TET, TMT, TPht, DBT, DPhT, DET, DMT	98
Nucleosil CN	UV	Hexane (90%) + THF (10%)	1.0	10	TBT, TET, TPht, DBT	98
Partisil-10 SCX, 10μm (250x4.6 mm)	HG-AAS	MeOH (80%) + H <sub>2</sub> O (20%) + NH <sub>4</sub> Ac (0.1 mol.l <sup>-1</sup> )	n.r.	100	Sn(IV), TBT	99

Table 1.1. HPLC separation methods for organotin compounds (continued)

COLUMN	DETECTION SYSTEM	MOBILE PHASE	FLOW RATE (ml.min <sup>-1</sup> )	INJECTION VOLUME (μl)	ANALYTE SPECIES	REF
Partisil-10 SCX, 10 μm (250x4.6 mm)	Laser excited atomic fluorescence	MeOH (80%) + H <sub>2</sub> O (20%) + NH <sub>4</sub> Ac (0.2 mol.l <sup>-1</sup> , pH=4.0)	4.0	20 or 200	TBT, TET, TMT	100
Nucleosil CN, 5 μm (150x4.6 mm)	ETAAS	Toluene + Tropolone (0.001%)	1.0	n.r.	TetraBT and TBT coelute, but are separated of DBT	101, 103
Partisil-10 SCX, 10 μm (250x4.6 mm)	Fluorescence	MeOH (80%) + H <sub>2</sub> O (20%) + NH <sub>4</sub> Ac (n.r.)	1.0	175	TBT	102
Inertsil ODS-2 (100x1 mm) (Scientific Glass Engineering Metal-free GLT μcol.)	ICP-MS	MeOH (25%) + H <sub>2</sub> O (75%) + Ammonium heptasulphonate (0.005 mol.l <sup>-1</sup> , pH=3.1)	0.03	0.5	MMT, DMT, DET, TMT	102
Partisil-10 SCX, 10 μm (250x4.6 mm)	ICP-AES	MeOH (60%) + H <sub>2</sub> O (40%) + NH <sub>4</sub> Ac (0.1 mol.l <sup>-1</sup> )	1.0	200	Sn(II), Sn(IV), TBT	104

Table 1.1. HPLC separation methods for organotin compounds (continued)

COLUMN	DETECTION SYSTEM	MOBILE PHASE	FLOW RATE (ml.min <sup>-1</sup> )	INJECTION VOLUME (μl)	ANALYTE SPECIES	REF
Zorbax-10 SCX (250x4.6 mm)	ICP-MS	MeOH (30%) + HAc (5%) + NH <sub>4</sub> Ac (0.05 mol.l <sup>-1</sup> )	n.r.	100	TBT, DBT, MBT	105
TSK gel ODS-80 TM (250x4.6 mm)	ICP-MS, UV	MeOH (80%) + H <sub>2</sub> O (14%) + HAc (6%) + Tropolone (0.1%, m/v)	0.9	100	TET, TBT, DBT, MBT	106
Hamilton PRP-1, 5 μm (150x4.1 mm)	ICP-MS	MeOH (94 or 99%) + H <sub>2</sub> O (5 or 0%) + Acetate (1%, pH=3-6) + Sodium pentanesulphonate (0.004 mol.l <sup>-1</sup> )	1	200	TMT, TBT, TPhT	107
Spherisorb ODS-2, 5 μm (250x4.6 mm)	ICP-MS	MeOH (80%) + H <sub>2</sub> O (19%) + Acetate (1%, pH=3) + Sodium pentanesulphonate (0.004 mol.l <sup>-1</sup> )	1	200	TMT, TBT, TPhT	107
TSK gel ODS 80-TM (250x4.6 mm)	ICP-MS	MeOH (80%) + H <sub>2</sub> O (14%) + HAc (6%) + Tropolone (0.1%, m/v)	0.9	100	TET, TBT, DBT, MBT	108

Table 1.1. HPLC separation methods for organotin compounds (continued)

COLUMN	DETECTION SYSTEM	MOBILE PHASE	FLOW RATE (ml.min <sup>-1</sup> )	INJECTION VOLUME (μl)	ANALYTE SPECIES	REF
Spherisorb SCX, 5 μm (250x4.6 mm)	ICP-MS	MeOH (60%) + Ammonium citrate (0.18 mol.l <sup>-1</sup> ) pH grad (3 to 6)	n.r.	100	TBT, DBT, MBT	109
Nucleosil CN, 5 μm (250x4 mm)	GFAAS	Toluene + Tropolone (0.0001%, m/v)	1	200	TBT, DBT	109
Whatman Partisil-10 SCX, 10 μm (250x4.6 mm)	Flourimetry	MeOH (80%) + H <sub>2</sub> O (20%) + Ammonium acetate (0.15 mol.l <sup>-1</sup> )	1	200	TPhT	110
Whatman Partisil-10 SCX, 10 μm (250x4.6 mm)	ICP-AES	MeOH (80%) + H <sub>2</sub> O (20%) + Ammonium acetate (0.1 mol.l <sup>-1</sup> ) + Tropolone (0.1%, m/v)	1	200	TBT, TPhT, DBT, MBT	111
Whatman Partisil-10 SCX, 10 μm (250x4.6 mm)	UV	MeOH (70%) + Acetate (0.1 mol.l <sup>-1</sup> ) + Benzyltrimethylammonium chloride (0.02 mol.l <sup>-1</sup> )	1	200	TBT, TPhT, DBT, MBT	112



Table 1.1. HPLC separation methods for organotin compounds (continued)

COLUMN	DETECTION SYSTEM	MOBILE PHASE	FLOW RATE (ml.min <sup>-1</sup> )	INJECTION VOLUME (μl)	ANALYTE SPECIES	REF
Whatman Partisil-10 SCX, 10 μm (250x4.6 mm)	ICP-MS	MeOH (60%) + H <sub>2</sub> O (40%) + Diammonium citrate (0.18 mol.l <sup>-1</sup> )	1	100	TBT, DBT	113
Excehpak SIL-C4A, 5 μm (50x4.6 mm)	ICP-MS	Tris(hydroxymethyl)aminomethane dodecylsulphate (0.05 mol.l <sup>-1</sup> ) + NH <sub>4</sub> NO <sub>3</sub> (3%, v/v) + HAc (3%, v/v) + EtOH (15%, v/v)	1	50	TMT, DMT, TPHT, DPHT, TBT, DBT	114
Partisil-10 SCX, 10 μm (250x4.0 mm)	ICP-MS	MeOH (80%) + H <sub>2</sub> O (20%) + NH <sub>4</sub> Ac (0.1 mol.l <sup>-1</sup> )	1.5	175	TBT	115
Partisil-10 SCX, 10 μm (2 cols 250x4.6 mm each)	ICP-MS	MeOH (70 and 85%) + H <sub>2</sub> O (30 and 15%) + Triammonium citrate (0.03 mol.l <sup>-1</sup> ) pH grad (5.8 to 3.4)	1.0	200	TBT, TPHT, DBT, MBT	This work

(n.r.: not reported)



The liquid chromatographic methods can be classified mainly into three categories:

- 1/ Ion-exchange chromatography,
- 2/ Reverse phase chromatography, and
- 3/ Normal phase chromatography.

The columns employed in cation exchange chromatography are normally packed with Partisil-10 SCX<sup>(78,79,83,87,89-91,94-96,100,102,104,110-113,115)</sup>. Other packing materials used are Spherisorb SCX<sup>(75,77,92,98,107,109)</sup>, Adsorbosphere<sup>(92)</sup> or Zorbax<sup>(105)</sup>. The mobile phases are in all the cases a mixture of methanol and water, varying the percentage of the first component between 60 and 85%. The buffers employed are always ammonium salts of either citrate or acetate, in different concentrations. Only one case uses a second salt in the mobile phase. The detectors employed range between non element specific ones (UV detectors<sup>(112)</sup>, differential pulse detection systems<sup>(79)</sup> or reverse pulse amperometric detectors<sup>(94)</sup>) to element specific ones, such as atomic absorption (graphite furnace or flame)<sup>(78,83,87,90,91,95,99)</sup>, laser enhanced ionization<sup>(89)</sup>, laser excited atomic fluorescence<sup>(100)</sup>, fluorescence<sup>(102,110)</sup>, inductively coupled plasma-atomic emission spectrometry (ICP-AES)<sup>(92,104,111)</sup> or inductively coupled plasma-mass spectrometry (ICP-MS)<sup>(92,96,105,109,113,115)</sup>. Different approaches have been employed for the coupling of the chromatographic system to the detector in order to improve the sensitivity of the overall system (hydride generation (HG)<sup>(99)</sup>) or to make both parts compatible to each other (bleeding oxygen with the nebuliser gas in ICP-MS to avoid the deposition of carbon on the surface of the cones). The

elution is isocratic in all the cases but two, both of which are based on the elution of monobutyltin (MBT) with a pH gradient from 6 to 3<sup>(96,109)</sup>.

Reverse phase chromatography has also been widely employed<sup>(73-77,84,88,92,97,102,106-108)</sup> although the methods published normally report on the separation of calibrants. The separation of the analytes in real samples is in many cases problematic and not so straight forward. The advantage of employing reverse phase chromatography is the possibility of using complexing agents, such as tropolone (2-hydroxy-2,4,6-cycloheptatrienona), in the extraction procedure of the analytes from the samples, thus helping the leaching of the species from the matrix. The most successful separation was obtained on a TSK gel ODS 80-TM column<sup>(88)</sup> with a mobile phase made of tetrahydrofuran (THF, 54%), water (38%), acetic acid (8%) and tropolone (0.2%, m/v). The authors report the separation of eight species, namely tributyltin (TBT), dibutyltin (DBT), monobutyltin (MBT), triphenyltin (TPhT), diphenyltin (DPhT), trimethyltin (TMT), dimethyltin (DMT) and monomethyltin (MMT). This approach appears to be the closest to a gas chromatographic separation method regarding the number of analytes which can be separated in the same run. This mobile phase has been modified by other authors<sup>(106)</sup> in order to make it compatible with ICP-MS (MeOH-80%, H<sub>2</sub>O-14%, HAc-6%, tropolone-0.1%, m/v), although in this case the number of analytes reported as separated was only four (TET, TBT, DBT and MBT).

The mobile phases employed with reverse phase chromatography use other organic solvents as well as methanol, such as tetrahydrofuran, acetone, hexane or pentane.

The last approach used is normal phase chromatography<sup>(50,74,80-82,98,101,103,109)</sup>. This type of separation has been achieved with cyano phases in all the cases and rarely applied to real samples. The mobile phases are based on methanol, toluene or hexane, with a second component which could be tetrahydrofuran, hexane, acetonitrile, acetic acid, ethylacetate or ethanol. This type of separation is also compatible with the use of complexing agents in the extraction procedure.

Organotin compounds (except phenyl derivatives) do not have chromophores or fluorophores to enable their direct determination with ultraviolet-visible or fluorimetric techniques. The determination of these analytes with such detection systems can be achieved either indirectly or via a post-column reaction. The first approach is most commonly used with UV-VIS detectors, while the second has been largely used in the case of fluorimetry. Morin or hydroxyflavone are two commonly employed reagents; they can also be included as part of the mobile phase<sup>(81,82)</sup>.

The separation of species with varying number of substituent groups (*e.g.*  $R_4Sn$ ,  $R_3SnX$ ,  $R_2SnX_2$ ,  $R_1SnX_3$ ) or the separation of species with the same number of substituent groups but where the group varies (*e.g.*  $Me_3SnX$ ,  $Bu_3SnX$ ,  $Ph_3SnX$ ) is easier to obtain than the separation of a mixture of the two above analyte types.

Another type of chromatography, which is much less frequently employed, is liquid micellar chromatography. The columns employed have been Spherisorb (C-18 type)<sup>(93)</sup> or a silica based column with butyl groups attached, Excelpak SIL-C4A<sup>(114)</sup>. The high concentration of sodium dodecylsulphate (SDS), used to form the micelles, employed by Suyani *et al.*<sup>(93)</sup> induced clogging of the injector of the ICP

torch. This problem was overcome by Inoue *et al.*<sup>(114)</sup> by means of changing to tris(hydroxymethyl)aminomethane dodecylsulphate (TDS) and the use of a tapered injector. This method allowed the separation of six organotin compounds (TBT, DBT, TPhT, DPhT, TMT, DMT) in about 20 min. It is worth mentioning the short length of the column employed (5 cm). The method has not been tested with real samples.

Supercritical fluid chromatography fills the gap between liquid and gas chromatography due to the properties (density, viscosity and diffusion coefficient) of the supercritical fluid being between those of a gas and of a liquid. This type of separation is especially suitable for the more substituted organotin compounds, such as tetra- and trisubstitutes. Shen *et al.*<sup>(116)</sup> reported the use of carbon dioxide (CO<sub>2</sub>) as the supercritical fluid for this purpose. The mobile phase was, thus, non polar and under this conditions the elution of TBT or DBT was not possible. The authors suggest that a modifier (such as methanol) could be used to increase the solvent strength. Tetrabutyltin (TetraBT) and tetraphenyltin (TetraPhT) were separated within 3 min in a capillary column SB-Octyl-50, 2.5 m length, 50 μm i.d., coated with a 0.25 μm thickness film. The detection limits reported were lower than for an HPLC system due to the higher transport efficiency (100%) of the sample to the plasma and the better ionization of gaseous samples in the plasma.

Vela *et al.*<sup>(117)</sup> improved this separation by employing a SB-Biphenyl-30 capillary column, 0.25 μm film thickness, 50 μm i.d., with different lengths (2, 5 and 10 m) using again CO<sub>2</sub> as mobile phase. TetraBT, TBT, TetraPhT, and TPhT were separated within 10 min after controlling the temperature of the interface as

well as the pressure ramp for CO<sub>2</sub>. ICP-MS was the detector employed for these two methods of supercritical fluid chromatography. Blake *et al.*<sup>(118)</sup> have published a study on a new interface between supercritical fluid chromatography and ICP-MS using the same column as Vela and coworkers<sup>(117)</sup> (in this case 2 m long) for the separation of TetraBT and TBT.

Dachs *et al.*<sup>(119)</sup> used a SE-52 capillary column, 10 m length, 0.1 mm i.d., 0.4 μm film thickness, with CO<sub>2</sub> as supercritical fluid and a flame photometric detector to separate six organotin species (tripropyltin (TPrT), TBT, TPhT, TetraBT, DBT and DPhT) within 35 min. This was the first publication of the separation of disubstituted derivatives (DBT, DPhT) by this chromatographic method.

A relatively novel approach was the separation of the organotin species with capillary electrophoresis. Poboży *et al.*<sup>(112)</sup> have reported a separation of TMT, TET, TBT and TPhT following this technique. The capillary employed was a quartz one, 75 μm inner diameter, 100 cm length and 60 cm length to the detector. A split-vent tubing sample introduction system was employed. Indirect UV detection (λ=210 nm) with benzyltrimethylammonium chloride (BTMA) in the electrolyte was used. The final composition of this was 20 mmol.l<sup>-1</sup> tartaric acid (used as a complexing agent to modify the apparent mobility factors of organotin compounds), 20% MeOH and 4 mmol.l<sup>-1</sup> BTMA, at pH=2.6. The four analytes were separated within 20 min; the detection limits reported were better than for HPLC approaches.



### 1.2.2. Organolead compounds

The most common way of separating the organic species of lead prior to their detection has been through gas chromatography<sup>(120-156)</sup>. This approach has the disadvantage of requiring a derivatization step for most of the organolead species. Only the tetrasubstituted species are directly suitable for gas chromatography. The derivatization procedure used normally is a Grignard reaction, either with *n*-butylmagnesium bromide (BuMgBr)<sup>(120,124,125,128,129,132,134,136,139-142,144-147,149,152,157)</sup> or *n*-propylmagnesium bromide (PrMgBr)<sup>(125,126,130,131,133,136,140,147,154)</sup>. The derivatisation requires at least three additional steps in the total analytical procedure (extraction into an organic solvent, Grignard reaction and elimination of excess of reagents and water). Ethylation using sodium tetraethylborate (NaEt<sub>4</sub>B)<sup>(122,138,143,148-150)</sup> simplifies the procedure since the derivatization can be carried out in aqueous media and there is no need for elimination of excess of reagent. When an extraction into a solvent is employed, complexing agents such as sodium diethyldithiocarbamate (NaDDTC) can be used to help the extraction efficiency.

Several types of detectors have been used, such as atomic absorption<sup>(120,124,125,131,133-135,143-147,150,152,153,157)</sup> and emission<sup>(130,141,148,151)</sup> spectrometry, plasma sources (microwave induced plasma (MIP)<sup>(122,126,128,129,132,136,140,147,149,154)</sup>, inductively coupled plasma (ICP)<sup>(137,138)</sup> and alternating current plasma<sup>(156)</sup>) and mass spectrometers<sup>(123,139,142,143,155)</sup>. Organolead species have been determined in many different types of matrices, ranging from soils, sediments, wines, polar snow and waters to biological samples.



Liquid chromatography, although it does not require the derivatization of the analytes, has not been so widely used for the determination of organolead species. A summary of the methods published can be seen in Table 1.2.

Almost all the methods employ a reverse phase column. In some of the cases an ion-pairing reagent, such as ammonium tetramethylenedithiocarbamate<sup>(162)</sup> or amino or sodium pentanesulphonate<sup>(164,165,167)</sup>, was added to the mobile phase. The main advantage of this type of chromatography (reverse phase or ion-pairing) is that a complexing agent can be used to help the extraction efficiency of the organolead compounds from the samples.

Ion-exchange chromatography suffers from the high affinity of inorganic lead for the stationary phase, which requires high concentrations of acid or organic solvent in the mobile phase to elute it. Some attempts have, however, been made to utilise this approach. Thus, Blaszkewicz *et al.*<sup>(161)</sup> determined trimethyllead (TML), dimethyllead (DML), triethyllead (TEL) and diethyllead (DEL) in urine samples with solid phase enrichment on silica gel, followed by an ion-exchange column (Partisil-10 SCX). Al-Rashdan *et al.*<sup>(164)</sup> also tried ion-exchange chromatography; the column was Adsorbosphere SCX, 5  $\mu\text{m}$  particle size. The authors could not obtain a good separation between the analytes, especially between triphenyllead (TPhL) and TEL, although they investigated different mobile phase compositions, pH, buffers and buffer strength.

Table 1.2. HPLC separation methods for organolead compounds

COLUMN	DETECTION SYSTEM	MOBILE PHASE	FLOW RATE (ml.min <sup>-1</sup> )	INJECTION VOLUME (μl)	ANALYTE SPECIES	REF
Lichrosorb C18 ODS 10 μm (250x4.6 mm)	GFAAS	MeOH (80% to 100%) + H <sub>2</sub> O (20 to 0%)	0.5	20	TetraML, TetraEL, TMEL, DEDML, TEML	76
C18 10 μm (250x4.6 mm)	GFAAS	MeOH (98%) + H <sub>2</sub> O (2%)	0.2	20	TetraEL, TEML, DEDML, TMEL, TetraML	87
Nucleosil 5-C18 Macherey-Nagel & Co., D-5160 Düren (200x6x4 mm)	UV (post column reaction with PAR)	MeOH (20% to 90%) + Acetate buffer (80% to 10%, 0.1 mol.l <sup>-1</sup> , pH=4.6)	1	20	TML, TEL, TetraML, TetraEL, Pb(II)	158
Lichrosorb 10-C18 Merck, D-6100 Darmstadt (300x2.0 mm)	UV (post column reaction with PAR)	MeOH (80%) + Acetate (20%, 0.1 mol.l <sup>-1</sup> , pH=4.6)	1	20	TML, TetraML, TetraEL	158

Table 1.2. HPLC separation methods for organolead compounds (continued)

COLUMN	DETECTION SYSTEM	MOBILE PHASE	FLOW RATE (ml.min <sup>-1</sup> )	INJECTION VOLUME (μl)	ANALYTE SPECIES	REF
Waters Resolve C18, 5 μm (150x3.9 mm)	UV-VIS (post column liquid-liquid extraction)	MeOH (20%) + NH <sub>4</sub> Ac (0.1 mol.l <sup>-1</sup> ) + HAC (pH=4.6)	1	200	TML, TEL, DPhL	159
Whatman Partisil ODS3, 5 μm (250x4.6 mm)	UV (post column reaction with PAR)	MeOH (20%) + Acetate buffer (0.1 mol.l <sup>-1</sup> ) and step grad to MeOH (40%) + Acetate buffer (0.1 mol.l <sup>-1</sup> )	1	n.r.	TML, DMEL, MDEL, TEL	160, 166
Partisil SCX-10, 10 μm (250x4.6 mm)	UV (post column reaction with PAR)	MeOH (70%) + H <sub>2</sub> O (30%) + NH <sub>4</sub> Ac/HAC	1	20	TML, DML, TEL, DEL	161
Partisil ODS-III, 5 μm (250x4.6 mm)	UV (post column reaction with PAR)	MeOH (20%) + H <sub>2</sub> O (80%) + NaAc/HAC	1	1000	TML	161

**Table 1.2.** HPLC separation methods for organolead compounds (continued)

COLUMN	DETECTION SYSTEM	MOBILE PHASE	FLOW RATE (ml.min <sup>-1</sup> )	INJECTION VOLUME (μl)	ANALYTE SPECIES	REF
Lichrosorb C18, 7 μm (200x4 mm)	MS	MeOH (20%) + H <sub>2</sub> O (80%) + NH <sub>4</sub> Ac/HAc (pH=4.5)	1	20	TML	161
Nucleosil C18 Macherey-Nagel & Co. (150x4.6 mm)	AAS	MeOH (75%) + H <sub>2</sub> O (25%) + Ammonium tetramethylene-dithiocarbamate (600 μg.ml <sup>-1</sup> )	1	50	TML, TEL, DML, DEL, Pb(II)	162
Hypersil ODS	AAS	MeOH (85%) + H <sub>2</sub> O (5%) + 1,4-dioxane (10%, v/v) + dithizone (300 μg.ml <sup>-1</sup> ) with grad elution to MeOH (80%) + H <sub>2</sub> O (5%) + 1,4-dioxane (10%, v/v) + HAc (5%) + Sodium dimethyldithiocarbamate (300 μg.ml <sup>-1</sup> )	1	50	TMBL, DMDBL, TEBL, DEDBL, TetraBL, TML, DML, Pb(II)	162

Table 1.2. HPLC separation methods for organolead compounds (continued)

COLUMN	DETECTION SYSTEM	MOBILE PHASE	FLOW RATE (ml.min <sup>-1</sup> )	INJECTION VOLUME (μl)	ANALYTE SPECIES	REF
Nucleosil C18 Macherey-Nagel & Co. (150x4.6 mm)	AAS	MeOH (75%) + H <sub>2</sub> O (15%) + 1,4-dioxane (10%, v/v) + dithizone (300 μg.ml <sup>-1</sup> ) with grad elution to MeOH (80%) + H <sub>2</sub> O (10%) + 1,4-dioxane (10%, v/v) + dithizone (300 μg.ml <sup>-1</sup> )	1	50	TML, TEL, DML, DEL, Pb(II)	162
Lichrosphere 60 Merck Select 5, 5 μm (150x4 mm)	Pulse amperometric detector	MeOH (90%) + LiClO <sub>4</sub> (10%, 0.1 mol.l <sup>-1</sup> , pH=7.0)	1.5	20	TML, TEL	163
Lichrosphere 60 Merck Select 5, 5 μm (150x4 mm)	Pulse amperometric detector	MeOH (75%) + CHCl <sub>3</sub> (10%) + LiClO <sub>4</sub> (15%, 0.1 mol.l <sup>-1</sup> )	1.5	20	TML, TEL	163
Lichrosphere 60 Merck Select 5, 5 μm (150x4 mm)	Pulse amperometric detector	CH <sub>3</sub> CN (90%) + LiClO <sub>4</sub> (10%, 0.1 mol.l <sup>-1</sup> , pH=7.0)	1.5	20	TML, TEL	163



Table 1.2. HPLC separation methods for organolead compounds (continued)

COLUMN	DETECTION SYSTEM	MOBILE PHASE	FLOW RATE (ml.min <sup>-1</sup> )	INJECTION VOLUME (μl)	ANALYTE SPECIES	REF
Adsorbosphere SCX Alltech Associates, 5 μm (250x4.6 mm)	ICP-AES	MeOH (70%) + H <sub>2</sub> O (30%) + NH <sub>4</sub> Ac (0.1 mol.l <sup>-1</sup> , pH=7)	1	100	Not enough resolution	164
Econosphere C18 Alltech Associated, 5 μm (250x4.6 mm)	ICP-AES, ICP-MS	MeOH (10% to 70%) + H <sub>2</sub> O (90% to 30%) + NH <sub>4</sub> Ac (0.1 mol.l <sup>-1</sup> , pH=4.6)	1	100	TML, TEL, Pb(II)	164
Spherisorb Phase Sep ODS-2 C18, 5 μm (250x4.6 mm)	ICP-AES, ICP-MS	MeOH (70%) + H <sub>2</sub> O (30%) + Sodium pentanesulphonate (4 mmol.l <sup>-1</sup> , pH=3.0)	1	100	TML, TEL, TPhI, Pb(II)	164
Nucleosil C18, 5 μm (250x4.6 mm)	ICP-MS	MeOH (30% to 90%, grad) + H <sub>2</sub> O (70% to 10%) + Sodium pentane- sulphonate (8 mmol.l <sup>-1</sup> , pH=3.0)	1	50	TEL, TPhL, TetraEL, Pb(II)	165
CETAC Technology C18 Peek column (50x1.6 mm)	ICP-MS	CH <sub>3</sub> CN (20%) + H <sub>2</sub> O (80%) + Ammonium pentanesulphonate (5 mmol.l <sup>-1</sup> , pH=3.4)	0.1	2	TML, TEL, Pb(II)	167

Table 1.2. HPLC separation methods for organolead compounds (continued)

COLUMN	DETECTION SYSTEM	MOBILE PHASE	FLOW RATE (ml.min <sup>-1</sup> )	INJECTION VOLUME (μl)	ANALYTE SPECIES	REF
Nucleosil C18, 120-5 (250x4 mm)	UV	MeOH (20% to 55%) + HSCH <sub>2</sub> CH <sub>2</sub> OH (0.02%, v/v) + Citric acid buffer (0.2 mol.l <sup>-1</sup> , pH=6.7)	1	20	DML, DEL, TML, TEL	168
		MeOH (40%) + Methylthioglycolate (0.02%, v/v) + Citric acid buffer (0.1 mol.l <sup>-1</sup> , pH=5.8)			TML, DML, TEL, DEL	168
Zorbax ODS	Laser enhanced ionization spectrometry	MeOH (90%) + H <sub>2</sub> O (10%) + NH <sub>4</sub> Ac (0.01 mol.l <sup>-1</sup> )	1	20	TetraEL, TEL, TetraML, TML	169
Perkin Elmer C8 3 μm (30x3 mm)	ICP-MS	MeOH (8%) + H <sub>2</sub> O (92%) + HAC (0.1 mol.l <sup>-1</sup> , pH=4.7)	1.0	100	TML, TEL, Pb(II)	170

**Table 1.2.** HPLC separation methods for organolead compounds (continued)

COLUMN	DETECTION SYSTEM	MOBILE PHASE	FLOW RATE (ml.min <sup>-1</sup> )	INJECTION VOLUME (μl)	ANALYTE SPECIES	REF
TSK G 3000 SW, Altex, (300x7 mm)	ICP-MS	Tris(hydroxymethyl)aminomethane (Tris) in HCl (0.1 mol.l <sup>-1</sup> , pH = 7.2)	0.5	100	Lead associated to different molecular weights fractions of blood	171

(n.r.: not reported)

Methanol is commonly used in the mobile phases at very different concentrations, from 8%<sup>(170)</sup> to 100%<sup>(76)</sup>. Acetonitrile is the other organic solvent employed. Robecke and Cammann<sup>(163)</sup> reported that the separation with methanol can be equally achieved with acetonitrile. Gradient elution has been adopted in some of the methods<sup>(76,158,162,164,165,168)</sup> to speed up the elution and improve the peak shape. Acetate buffer, giving a pH between 4.5 and 5.0 is the most frequently used. Citric acid buffer has also been employed when there is a need for a higher pH in the mobile phase<sup>(168)</sup>.

Several types of detectors have been employed. UV detection after post column reaction of the analytes, converting them into coloured complexes with 4-(2-pyridylazo)resorcinol (PAR) after their decomposition into dialkyllead species with iodine, has been coupled to several types of columns<sup>(158,159,161,168)</sup>. Commonly used element specific detection systems are atomic absorption spectrometry<sup>(76,87,162)</sup>, ICP-AES<sup>(164)</sup> and ICP-MS<sup>(164,165,167,170)</sup>. Finally, mass spectrometry<sup>(161)</sup>, electrochemical systems<sup>(163)</sup> and laser enhanced ionization spectrometry<sup>(169)</sup> have also been employed. The combination HPLC-ICP-MS is practically the only one capable of detecting the contents of organolead species in environmental samples.

Gercken and Barnes<sup>(171)</sup> used size exclusion chromatography coupled to ICP-MS to speciate lead associated with fractions of different molecular weight in blood. ICP-MS was capable of differentiating lead, using time resolved acquisition software, from other metals also present in the same fraction.

Carey *et al.*<sup>(172)</sup> proposed a SFC-ICP-MS method for the determination of

tetrabutyllead (TetraBL) and tributyllead (TBL) and diethylmercury (DEM) using a SB-Biphenyl-30 capillary column (2.5 m x 50  $\mu$ m, 0.25  $\mu$ m film thickness) with CO<sub>2</sub> as supercritical fluid. The separation achieved was not ideal and has not been applied to real samples.

Mikac and Branica<sup>(173,174)</sup> have developed a method to speciate between inorganic lead, di- and trialkyllead species. The method was based on a selective extraction procedure followed by electrochemical detection. Water samples were extracted into hexane in the form of diethyldithiocarbamate complexes. The ionic forms of lead were then backextracted into acidified water (pH = 1.5 with nitric acid) and determined by differential-pulse anodic stripping voltammetry. The determination was carried out in the presence of the precipitate from inorganic lead after addition of barium sulphate, in order to eliminate Pb(II) from the solution. Di- and trisubstituted compounds were deposited at -0.1 V, while at -0.7 V only the dialkylspecies were deposited. The concentration of the trialkyllead compounds can then be calculated from the difference between both measurements.

Differential pulse polarography has also been employed by Al-Allaf *et al.*<sup>(175)</sup> for the determination of alkyllead compounds in dimethylsulphoxide (DMSO). While PbCl<sub>2</sub> or Pb(OOCH<sub>3</sub>)<sub>2</sub> give only one reduction peak at -0.51 V vs Ag/AgCl electrode, disubstituted lead chlorides (RR'PbCl<sub>2</sub>, R=R'=Et or Ph, or R=Me and R'=Ph) show three and trisubstituted lead chloride or acetate (Et<sub>3</sub>PbAc, R<sub>2</sub>R'PbCl, R=R'=Et or Ph, or R=Ph and R'=Me) only present two peaks. Following this approach is thus possible to speciate some mixtures of organolead compounds, such as Ph<sub>3</sub>PbCl + Et<sub>3</sub>PbCl, Ph<sub>3</sub>PbCl + Et<sub>3</sub>PbAc, Ph<sub>3</sub>PbCl + Et<sub>2</sub>PbCl<sub>2</sub> and



$\text{Ph}_3\text{PbCl} + \text{PhMePbCl}_2$ , and organolead species from other organometallic compounds ( $\text{Ph}_3\text{PbCl} + \text{Ph}_3\text{SiCl}$  or  $\text{Ph}_3\text{PbCl} + \text{Ph}_3\text{SnCl}$ ).

Finally, Ou, Thomas and Jing<sup>(176)</sup> speciated between tetraethyllead and the rest of the ethyllead compounds on the basis of their different hydrophobicity. They extracted soil samples into a mixture of n-hexane and disodium ethylenediaminetetraacetic salt in water, pH=9.0. After shaking and centrifuging the two phases were separated. Tetraethyllead is highly hydrophobic and remained in the organic phase, while ionic ethyllead species are highly soluble in water, but not in organic solvents. They used this extraction procedure to study the biological and chemical degradation rates of tetraethyllead in soils. To achieve this, soils (autoclaved and non-autoclaved) were spiked with  $^{14}\text{C}$ -labelled tetraethyllead and the  $^{14}\text{C}$  activity was counted by liquid scintillation at regular intervals, both as tetraalkyllead and the other alkyllead species.

### ***1.3. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)***

The International Union of Pure and Applied Chemistry (IUPAC) defined chromatography (literally "colour-writing" from Greek) as<sup>(177)</sup>:

"...a method used primarily for the separation of components of a sample, in which the components are distributed between two phases, one of which is stationary while the other moves. The stationary phase may be a solid, or a liquid supported on a solid, or

a gel. The stationary phase may be packed in a column, spread as a layer, or distributed as a film, etc. In these definitions, "chromatographic bed" is used as a general term, to denote any of the different forms in which the stationary phase may be used. The mobile phase may be gaseous or liquid."

When the mobile phase consist of a liquid, the chromatography is then liquid chromatography. In the case of solid stationary phases, these can be spread over glass, plastic, aluminium or paper, or packed in columns of different diameters. If these column are of relatively narrow bore, typically 4.6 mm of inner diameter or less, and the liquid mobile phase is pumped through the column at high flow rates (1 to 5 ml.min<sup>-1</sup>) the separation obtainable is very efficient and leads to relatively short analysis time. This type of liquid chromatography is known as high performance liquid chromatography (HPLC)<sup>(177)</sup>.

There are several types of columns in HPLC. They differ in the type and particle size of the stationary phase, length, i.d., etc. Besides, differences between two columns of the same characteristics occur. The efficiency of a column can be evaluated (and thus the column compared with other columns) through the column plate number (N). This is the most important characteristic of a column and describes the ability of a column to produce sharp and narrow peaks and achieve good resolution between peaks. It gives an idea of the theoretical number of sorption-desorption exchanges of the analyte between the mobile and the stationary phase, depending on the distribution coefficient between both phases. Each section of the column where sorption-desorption processes occur is called a theoretical plate

and  $N$  is the total number of theoretical plates in the column.  $N$  is affected by stationary and mobile phases, temperature, analyte, column length, particle size and flow rate.  $N$  is directly proportional to column length and inversely proportional to particle size. High flow rates or increased viscosity of the solvent in the mobile phase also lead to lower  $N$  values.

For a given set of conditions,  $N$  can be calculated from any of the following equations<sup>(178)</sup>:

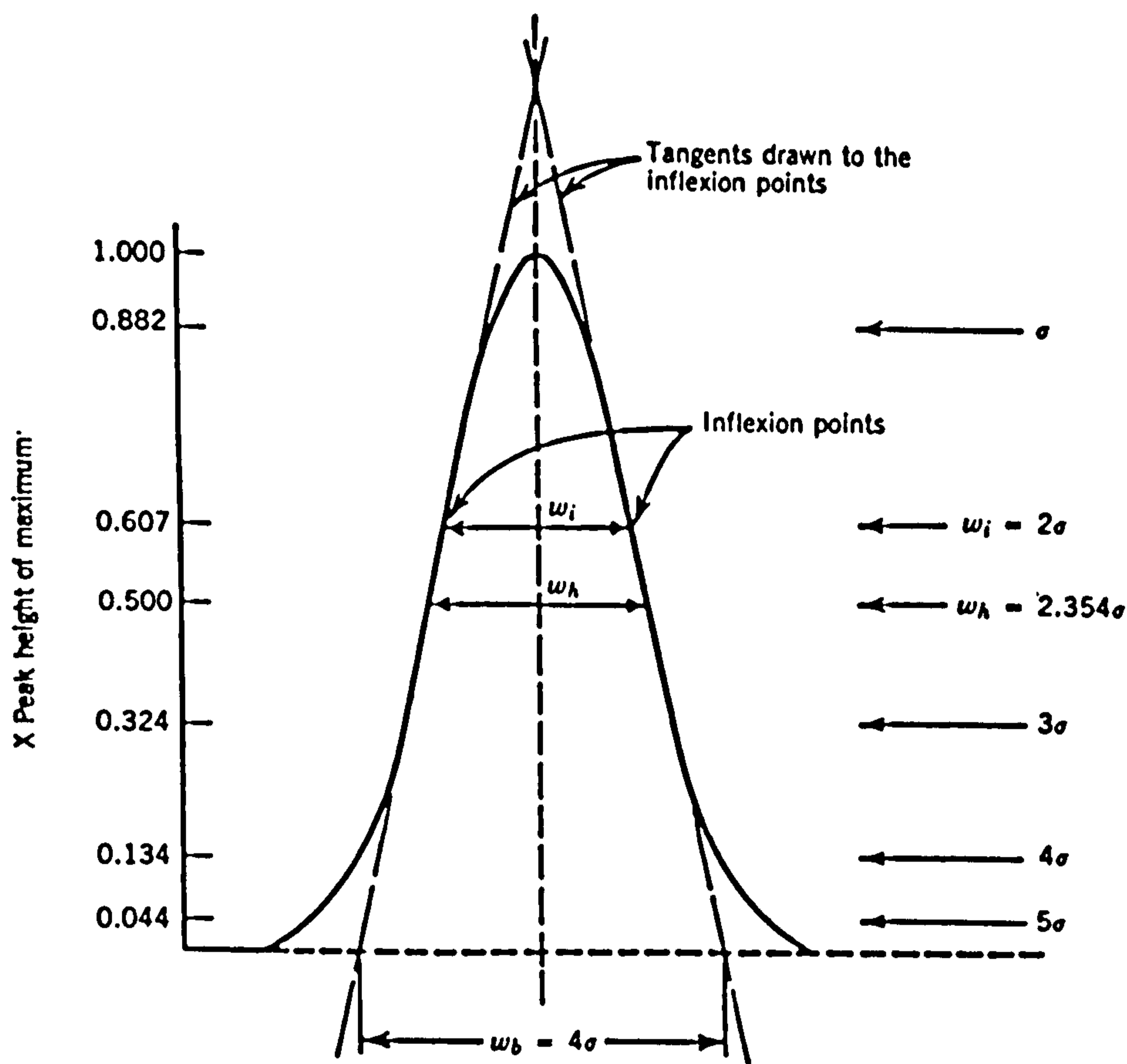
$$N = 5.54 \left( \frac{t_R}{w_h} \right)^2 \quad \text{Eq. 1.4}$$

$$N = \left( \frac{t_R}{\sigma} \right)^2 \quad \text{Eq. 1.5}$$

$$N = 16 \left( \frac{t_R}{w_b} \right)^2 \quad \text{Eq. 1.6}$$

where  $t_R$  is the uncorrected retention time of the analyte (Figure 1.3).

The measure of  $\sigma$  or  $w_b$  depends on the accuracy of drawing tangents to the inflexion points of the peaks. Thus, the easiest way to calculate  $N$  is through Eq. 1.4.



**Figure 1.3.** Representation of an ideal chromatographic peak and different bandwidth measurements:  $w_h$  is the width of the peak at half height in time units,  $\sigma$  is the peak width at 0.882 fold the height of the peak ( $h$ ) and  $w_b$  is the peak width in time units at peak base (from L.R. Snyder, J.L. Glajch, J.K. Kirland. "Practical HPLC Method Development", John Wiley & Sons, New York, U.S.A., 1988)

The efficiency can also be defined as the height of column equivalent to a theoretical plate, H. Both N and H are related through the length of the column, L<sup>(177)</sup>:

$$N = \frac{L}{H} \quad \text{Eq. 1.7}$$

The retention time at which species not retained in the column elute is known as the "dead time" ( $t_0$ ). The efficiency of the column can be calculated taking this volume into account. This is the effective plate number,  $N_{eff}$ , defined after Eq. 1.4 as<sup>(177)</sup>:

$$N_{eff} = 5.54 \left( \frac{t_R - t_0}{w_h} \right)^2 \quad \text{Eq. 1.8}$$

Equivalent expressions can be derived from Eq. 1.5 and 1.6.

The quality of the separation between any two peaks in a chromatogram is given by the resolution,  $R_s$ . This can be calculated through<sup>(178)</sup>:

$$R_s = 2 \frac{t_R^2 - t_R^1}{w_b^1 + w_b^2} \quad \text{Eq. 1.9}$$

or:

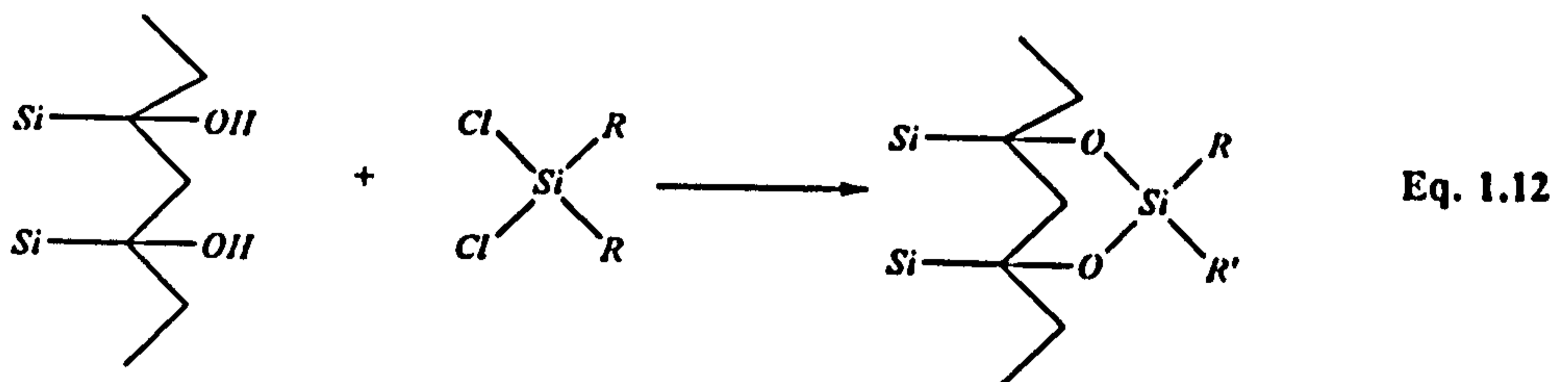
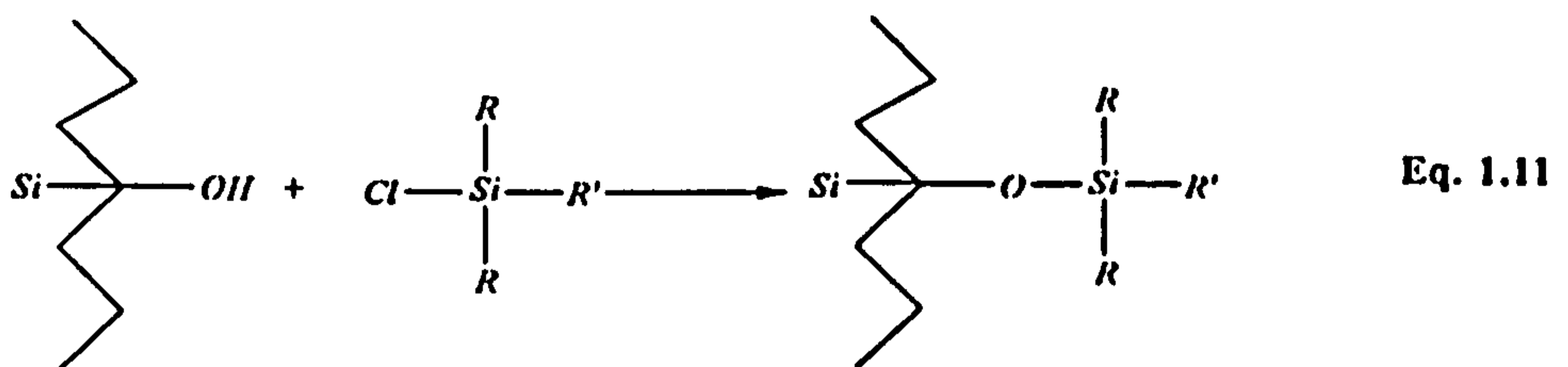


$$R_s = 1.18 \frac{t_R^2 - t_R^1}{w_h^1 + w_h^2} \quad \text{Eq. 1.10}$$

where  $t_R$  is the retention time,  $w_b$  is the width of the peak at the base and  $w_h$  is the width at half height, for analytes 1 and 2 (these are indicated by the superscripts).  $R_s$  is small for overlapping bands, while peaks with base line resolution show a value of  $R_s \geq 1.5$ .

There are several classifications of liquid chromatography. One of them is based on the sorption mechanism of the analyte between the mobile and the stationary phase. This is the most common classification and a summary can be seen in Table 1.3.

Bonded phases can be prepared by the reaction of the silanol groups in the surface of the silica to chlorosilanol, either mono- or difunctional<sup>(177)</sup>:



**Table 1.3.** *Modes of separation in liquid chromatography based on sorption mechanisms*

<b>TYPE</b>	<b>STATIONARY PHASE</b>	<b>INTERACTION TYPE</b>	<b>SPECIES</b>
Adsorption	Solid (polar or apolar)	Polarity effects, intermolecular forces	Species from different functional group families
Bonded phase	Liquid chemically bonded on a solid support	Non specific interactions	Organic compounds, etc.
Ion-exchange	Silica or polymer with ion-exchange groups chemically bonded	Exchange of counter ion with mobile phase	Ions or ionisable molecules
Ion-pairing	Liquid chemically bonded on a solid support	Ionic interactions	Ionic or ionisable molecules
Affinity	Ligand covalently bonded to a gel matrix	Reversible biological specificity of a ligand for a certain protein (key-lock system)	Proteins, compounds with specific affinity for the ligands
Exclusion	Silica or polymer gel	Physical space	Large molecules

R are normally -CH<sub>3</sub> groups, R' is a hydrocarbon chain (C6, C8, C18). This case gives an apolar stationary phase. R can be a polar functional group (such as -C≡N or -NH<sub>2</sub>) in which case the stationary phase is polar. The first case (stationary phase less polar than the mobile phase) is known as reverse phase chromatography. The second case (stationary phase more polar than the mobile phase) is normal phase chromatography. Reverse phase chromatography has been widely used due to the fact that almost all organic molecules have hydrophobic parts in their structure able to interact with the non polar stationary phase.

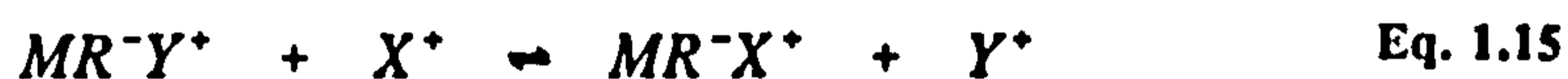
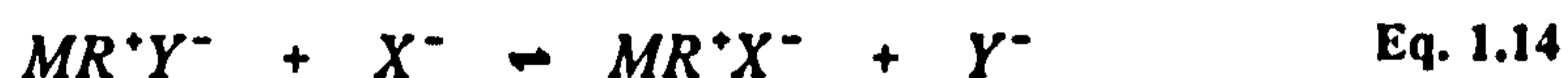
Since the stationary phases in ion-pairing chromatography are the same for bonded phase chromatography, ion-pairing chromatography can be either normal or reverse phase, the latter being the most common type. Solvents for reverse phase chromatography are generally inexpensive compared to normal phase chromatography.

Ion-pairing chromatography is based on the addition to the mobile phase of, normally, a big organic molecule (ion-pairing reagent) with opposite charge to that of the analyte. Both species form the ion pair, uncharged, and the hydrophobic region of the ion-pairing reagent interacts with the stationary phase, thus separating the analytes.



Another approach to explain the mechanism of ion-pairing chromatography is that the ion-pairing reagent binds to the stationary phase and the analytes interact with it in an ion-exchange mechanism. Probably, the true mechanism is the result of both effects.

The stationary phase in ion-exchange chromatography is a rigid solid (M) with charged sites (R), either positive or negative. Counter ions in the stationary phase (Y) can be exchanged with ions in the analyte (X)<sup>(177)</sup>:



Eq. 1.14 represents anion-exchange chromatography, while Eq. 1.15 represents cation-exchange chromatography. Ionic strength, pH and complexing agents have a marked effect in the elution of the analytes.

#### **1.4. INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICP-MS)**

At the end of the 1960s, after the initial studies of Greenfield *et al.*<sup>(179)</sup> and Wendt and Fassel<sup>(180)</sup>, inductively coupled plasma source atomic emission spectrometry was established as a new technique with a great potential in multielemental analysis at trace levels. The technique was useful for the analysis of samples in solution. It proved problematic for geological samples due to high

backgrounds from the main components of these samples. One of the potential applications of ICP source atomic emission spectrometry was the mining industry and this pushed the development of a new technique which could offer ease of sample introduction, speed of operation and low detection limits. Gray studied the feasibility of different detection systems to be coupled to an ICP, since the potential of the plasma as an ionisation source was obvious: an ICP supplied enough ionisation energy for most elements to be detected by AES using ion lines. Some of the detection systems studied were atomic fluorescence spectrometry (AFS), instrumental neutron activation analysis (INAA), atomic absorption spectrometry (AAS) and dispersive and non-dispersive X-ray fluorescence (XRF). He concluded that mass spectrometry was the best option to obtain a simple spectra, good resolution and detection limits and offered a sensitivity relatively uniform along the Periodic Table<sup>(181)</sup>.

Spark source mass spectrometers were the instruments more commonly available at the time. The detection limits obtainable were in the order of 1 in  $10^6$  in the solid or below and gave simple spectra with only a few polyatomic ion peaks. Matrix effects were minimal. The main problem with this detection system is the few samples per day that can be analysed and the skilled sample preparation required. These problems arise from the type of ion source employed. Initial studies for the coupling between plasmas and mass spectrometers were carried out with direct current plasmas (DCP). The detection limits obtained were quite poor. This could be attributed to the lower temperature of the DCP ( $\approx 3000$ - $5000$  K) vs that of the ICP ( $\approx 7000$ - $10000$  K), and this lead to the use of ICP as an ion source for mass



spectrometry, giving much lower detection limits than DCP<sup>(182)</sup>.

The coupling involved the use of a robust aperture which would stand both high temperature and the difference in pressure from atmospheric pressure in the plasma to the vacuum required by the mass spectrometer. Additional pumping stages were incorporated in the system, after the plasma, to gradually reduce the pressure. In 1981, Gray<sup>(182)</sup> obtained a spectrum with a performance such as the one to be expected from an ICP.

Commercial instruments were available in the market at the beginning of 1983, one being the PlasmaQuad from VG Isotopes, Ltd. (U.K.), the second being the Sciex system in Canada. The first instruments in customer laboratories were installed in 1984<sup>(182)</sup>.

An schematic diagram of an ICP-MS instrument can be seen in Figure 1.4.

#### 1.4.1. Nebulisers

Although gas and solid sample introduction is also possible, the most common way of sample introduction for ICP-MS is as a liquid. However, the sample has to be in the form of gas, vapour or aerosol of fine droplets or solid particles to enter the plasma. This can be achieved by forming an aerosol of the liquid sample, via a nebulisation system. These devices are essentially the same as for ICP-AES, since the sample introduction requirements are approximately the same for both techniques.

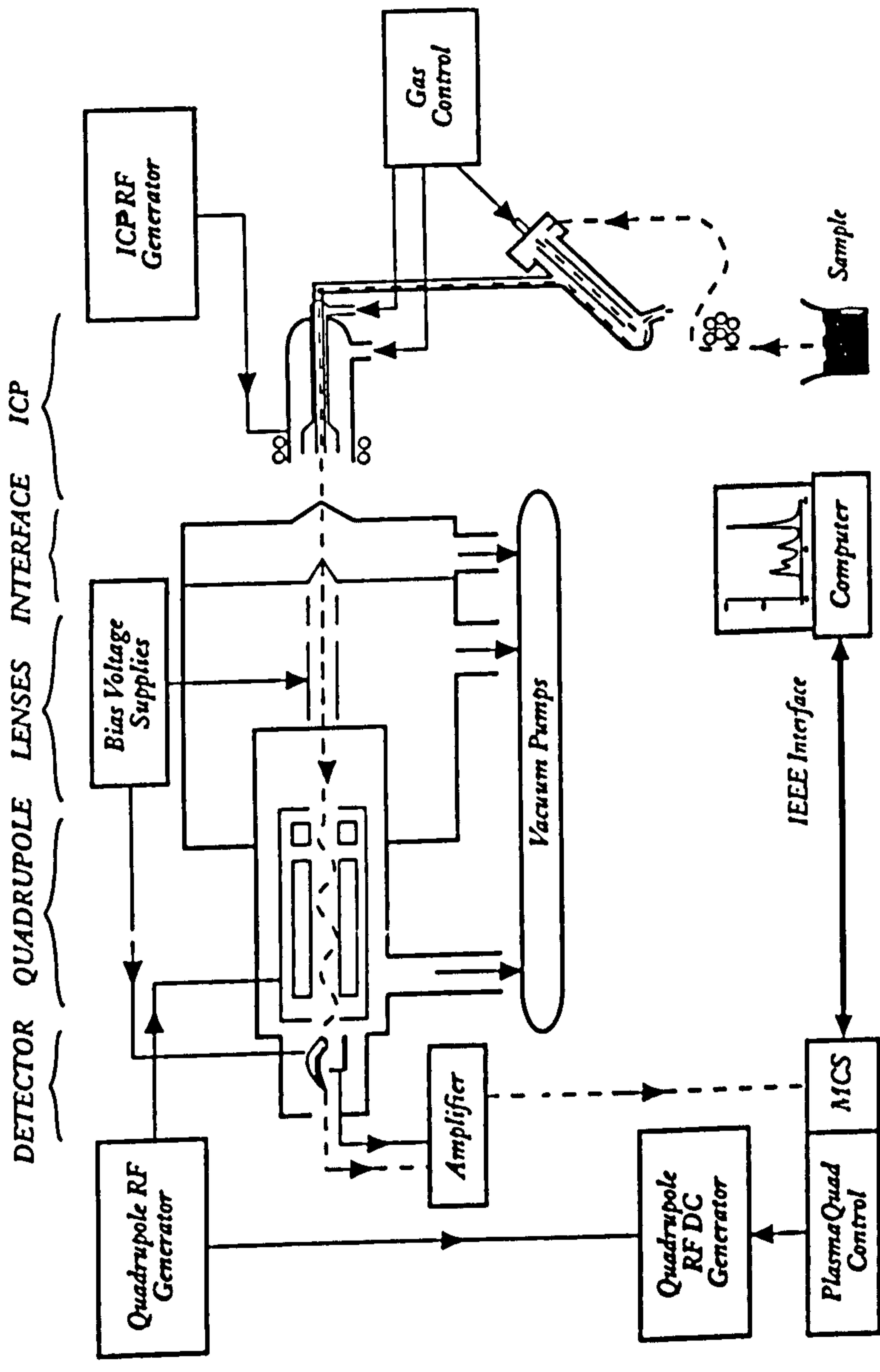
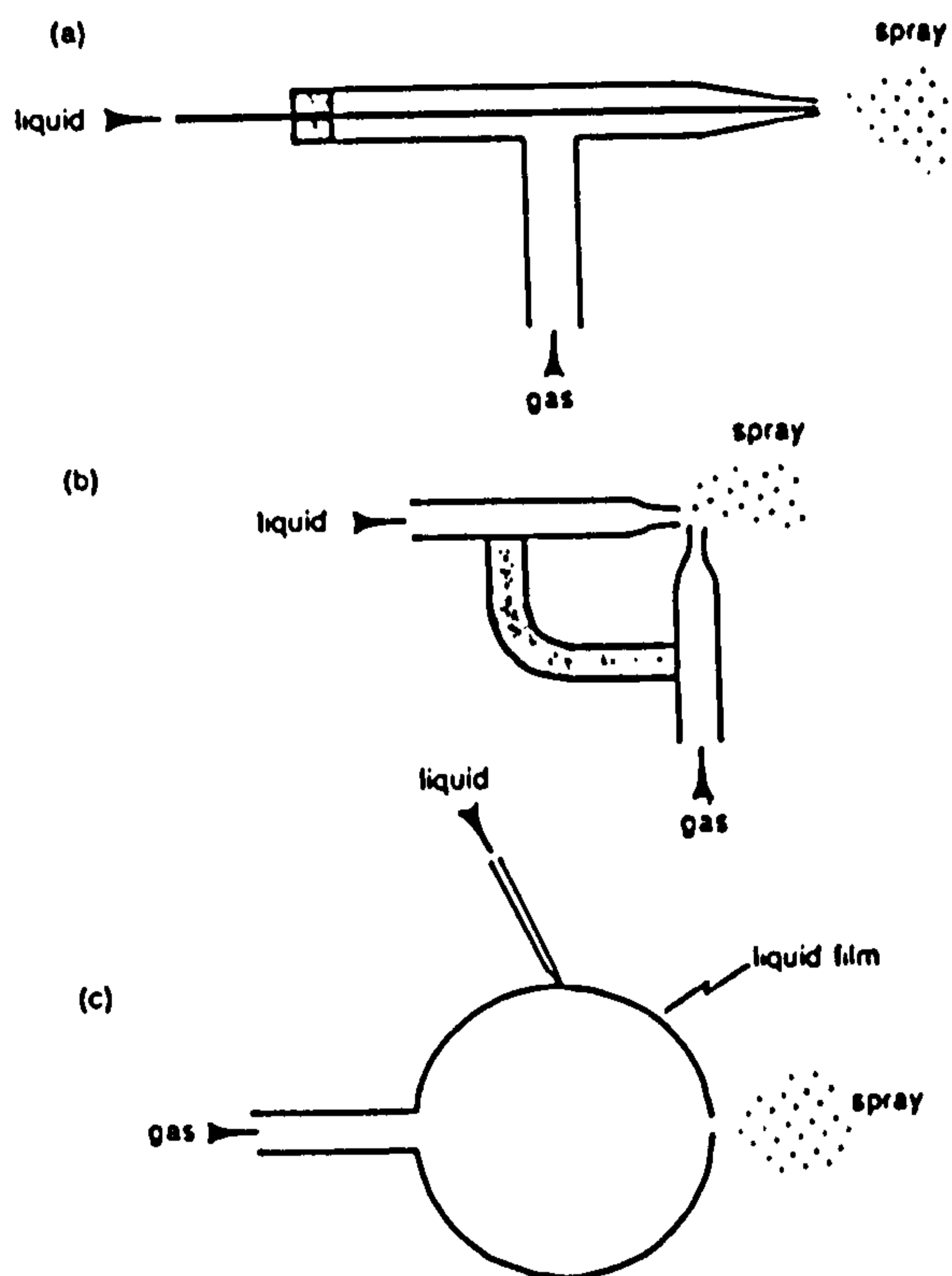


Figure 1.4. Schematic diagram on an inductively coupled plasma-mass spectrometer instrument (from VG Instruments PlasmaQuad II Manual, 1994)

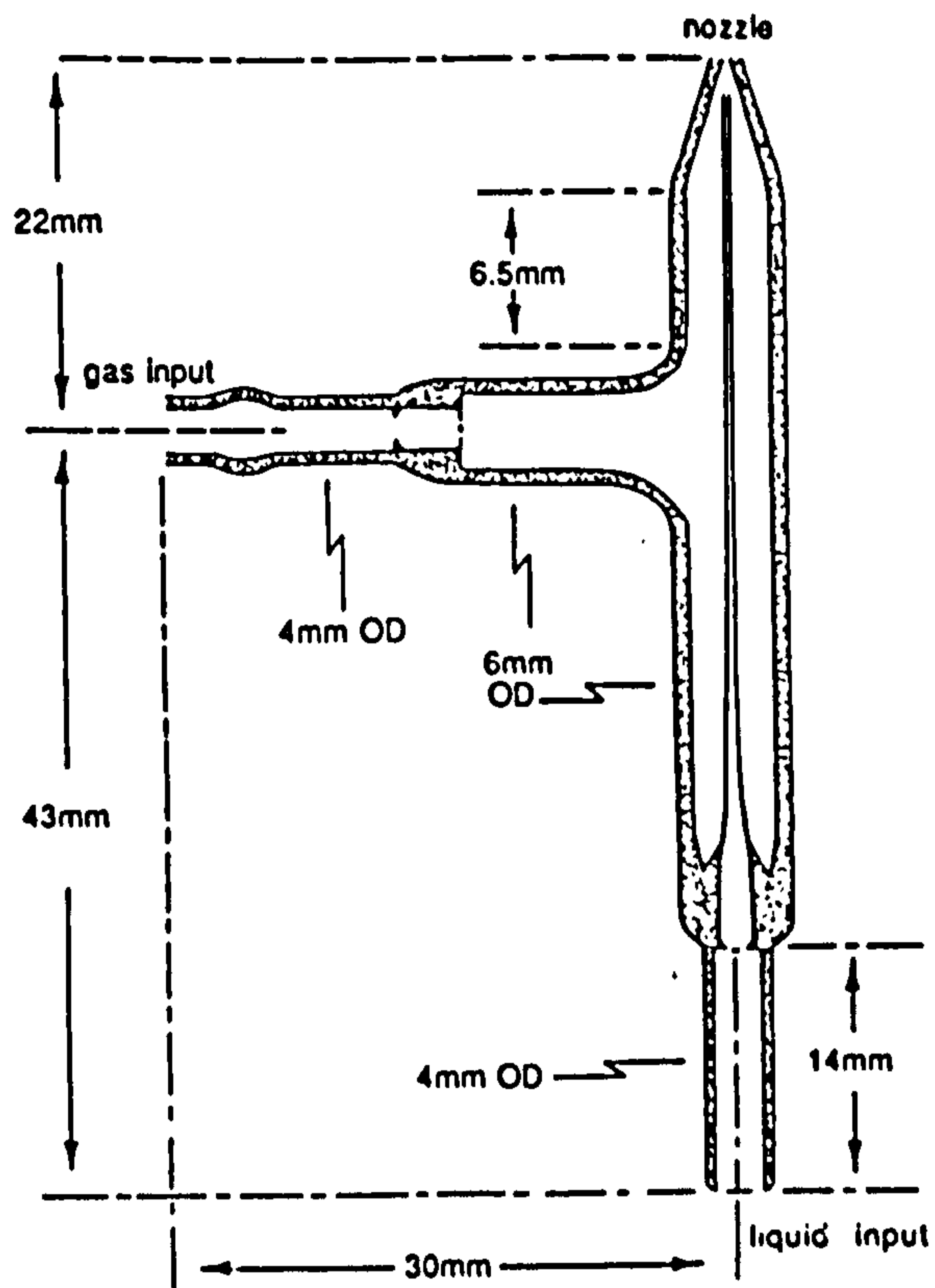
Nebulisers can be divided into two main categories: pneumatic and ultrasonic nebulisers. The first are based on the formation of an aerosol due to a high speed jet obtained after passing the sample and/or nebuliser gas through a small orifice. The second type requires an ultrasonic transducer<sup>(183)</sup>. There are other types, much less used than these two, such as a thermospray<sup>(184)</sup> or jet impaction<sup>(185)</sup>.

There are three main types of pneumatic nebulisers, which can be seen in Figure 1.5. These are:

- 1/ *Concentric flow*. The most widely used (Figure 1.6), it is based on a capillary (through which the sample runs) placed inside glass tubing. Between the capillary and the external tubing, the nebuliser gas flows. The tip of the nebuliser is of smaller diameter than the rest, which produces an aerosol of the sample. They can be obtained with limited or so called "high" salt tolerance and for low or regular (1 ml.min<sup>-1</sup>) nebuliser gas flows. Concentric flow nebulisers are "free running", *i.e.* there is no need to pump the sample, although this possibility offers some advantages, some of which are that viscosity effects are reduced, liquid intake rate and nebuliser gas flow rate can be optimised individually, faster pumping rates can be applied between samples to speed up the washing time and, finally, the stabilising effect on the plasma<sup>(182)</sup>. Narrow bore tubing and higher pump rates give a better precision (lower noise) than wider bore tubing and lower pumping speed<sup>(182)</sup>.



**Figure 1.5.** *Schematic diagram of the three main types of nebulisers. (a) concentric, (b) cross flow, (c) Babington type (from K.E. Jarvis, A.L. Gray, R.S. Houk. "Handbook of Inductively Coupled Plasma Mass Spectrometry", Blackie & Sons Ltd., Glasgow, U.K., 1992)*

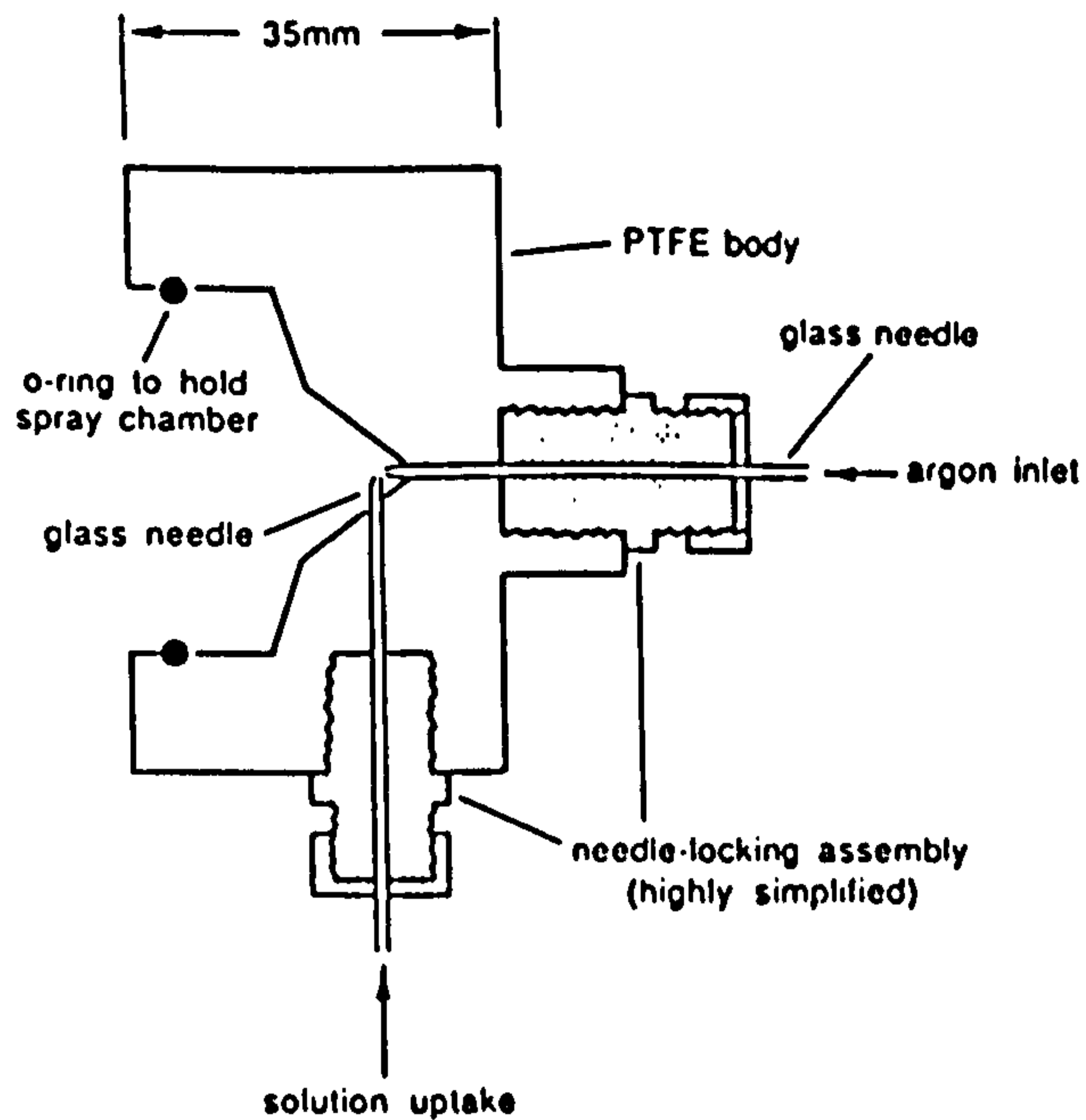


**Figure 1.6.** *Detail of a Meinhard concentric nebuliser (from K.E. Jarvis, A.L. Gray, R.S. Houk. "Handbook of Inductively Coupled Plasma Mass Spectrometry", Blackie & Sons Ltd., Glasgow, U.K., 1992)*

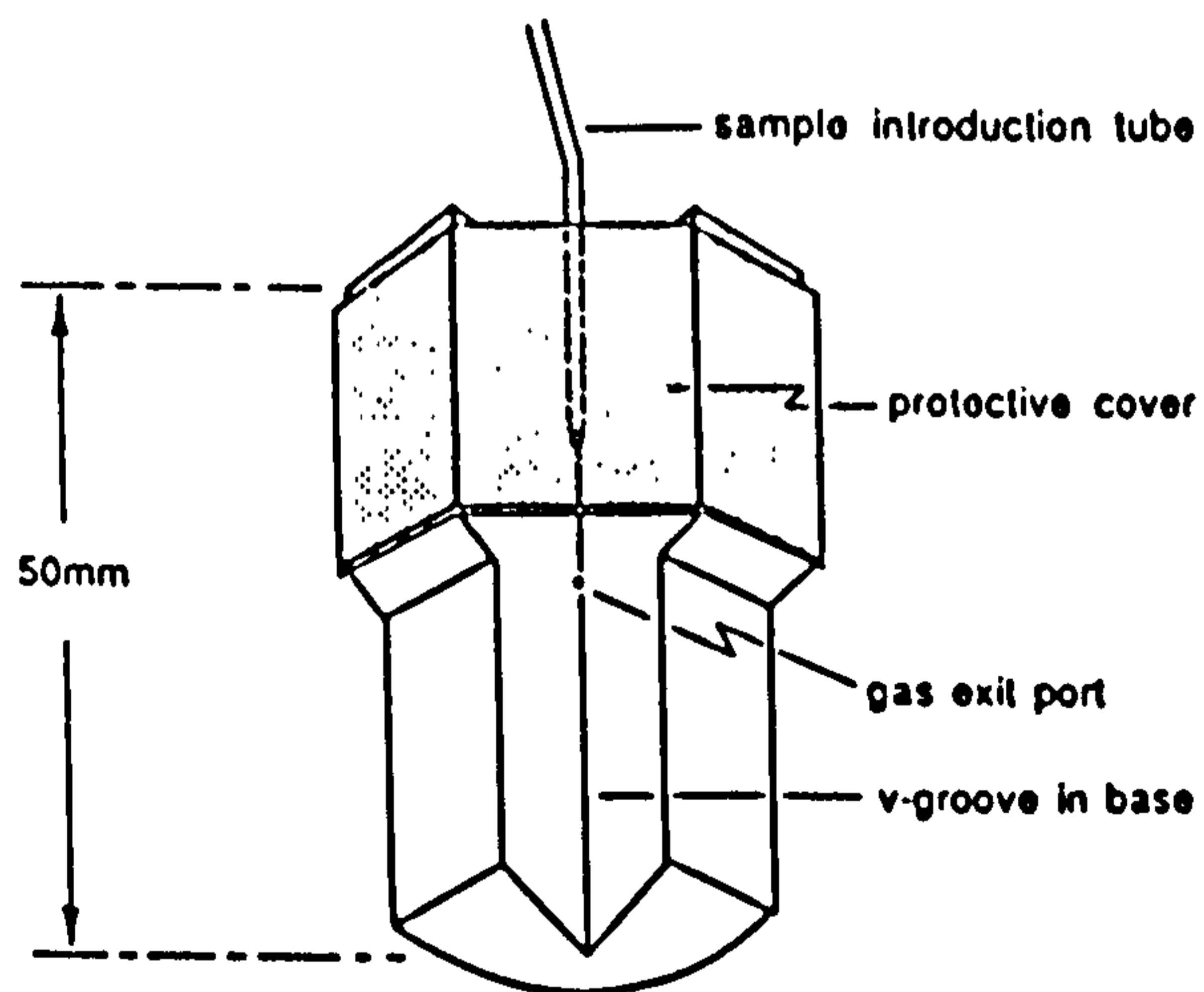


- 2/ *Cross-flow nebulisers.* Figure 1.7 shows a simplified cross-flow nebuliser. The aerosol is formed by the nebuliser gas jet formed at the tip of the gas capillary, which lifts up the liquid. They are less prone to blocking than concentric flow nebulisers. They, however, need a peristaltic pump to pump the sample through.
- 3/ *Babington type nebuliser.* The liquid is left to flow over a sphere, forming a film over it. There is an orifice in the sphere through which the nebuliser gas flows. When the liquid passes over this orifice, an aerosol is formed. This type of nebuliser is less prone to blockage and, thus, has a greater tolerance to high dissolved solid samples. The liquid has to be pumped into the system. Since the sphere gets completely wet, this type of nebuliser has a large memory effect. Suddendorf and Boyer<sup>(186)</sup> described in 1978 a Babington type nebuliser in which the liquid is constrained in a V-groove (Figure 1.8), which reduces significantly the memory effect. There are now several commercially available V-groove type nebulisers, two main types are the de Galan type<sup>(187)</sup> and Ebdon type<sup>(188)</sup>.

Based on the design of Babington, a frit type nebuliser was described by Layman and Lichte in 1982<sup>(189)</sup>. This nebuliser produces droplets with a mean size of 1  $\mu\text{m}$ , more appropriate to the size of drop that should reach the plasma, thus, being a more efficient nebulisation system. Instead of a V-groove, the nebuliser has a fine glass frit. The main problems of this nebuliser are a long wash out time and problems with samples with high dissolved solids.

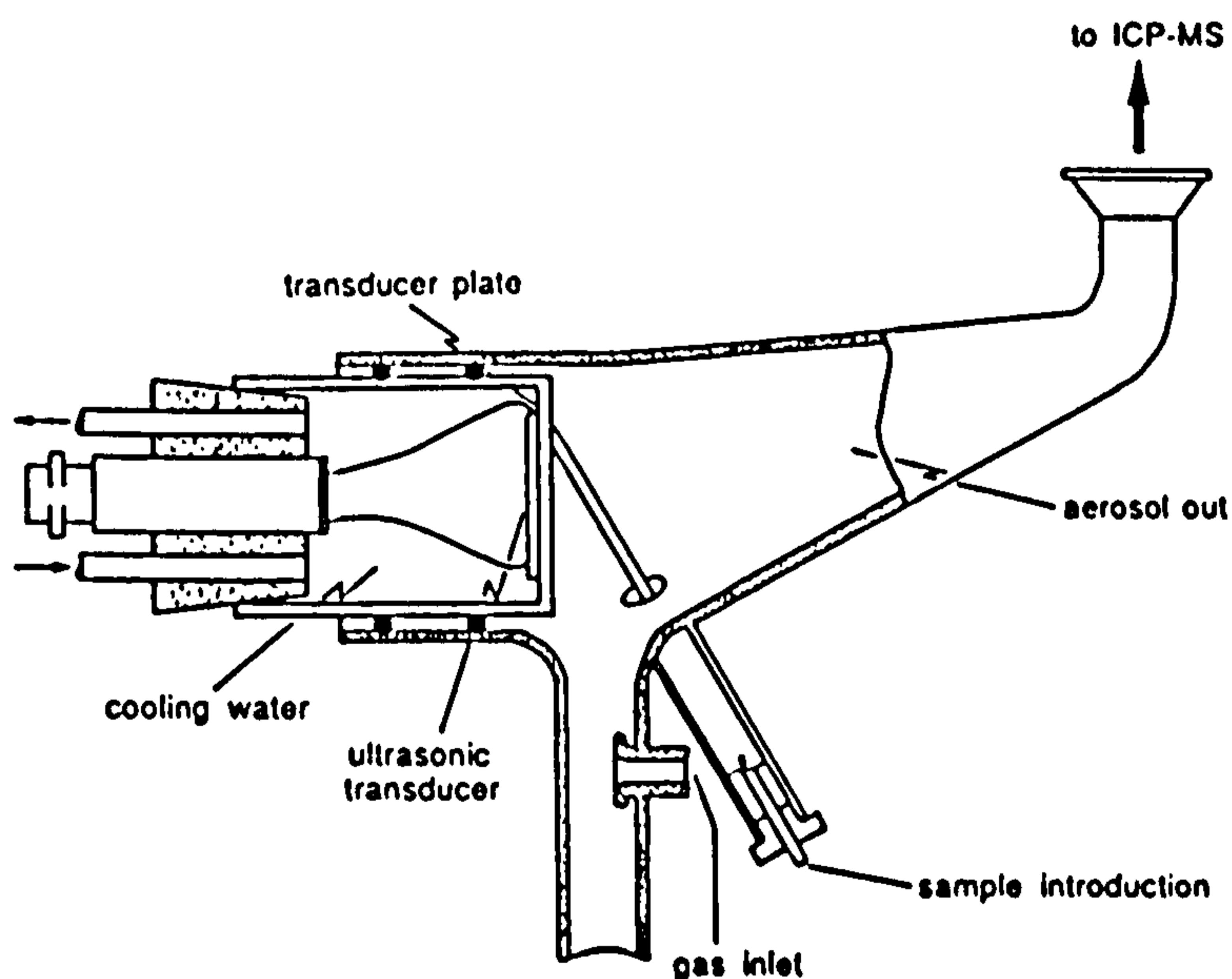


**Figure 1.7.** *Simplified glass capillary cross flow nebuliser (from K.E. Jarvis, A.L. Gray, R.S. Houk. "Handbook of Inductively Coupled Plasma Mass Spectrometry", Blackie & Sons Ltd., Glasgow, U.K., 1992)*



**Figure 1.8.** *V-groove Babington type nebuliser (from K.E. Jarvis, A.L. Gray, R.S. Houk. "Handbook of Inductively Coupled Plasma Mass Spectrometry", Blackie & Sons Ltd., Glasgow, U.K., 1992)*

The liquid sample in an ultrasonic nebuliser is fed to the surface of a piezoelectric transducer operated at a frequency of between 0.2 and 10 MHz. The ultrasonic wave produces pressure waves over the surface of the transducer, which generates the aerosol. The efficiency of this form of nebulisation is higher than with other devices, but the sensitivity obtainable is not as good as might be expected due to the amount of water that enters the plasma, increasing for example the number of polyatomic interferences. In order to overcome this problem, the aerosol is normally desolvated, by first heating it and condensing the water vapour formed. These nebulisers have longer wash out times than most other types. They are expensive since they require a separate radio-frequency generator. An example of an ultrasonic nebuliser is shown in Figure 1.9.



*Figure 1.9. Schematic diagram of an ultrasonic nebuliser (from K.E. Jarvis, A.L. Gray, R.S. Houk. "Handbook of Inductively Coupled Plasma Mass Spectrometry", Blackie & Sons Ltd., Glasgow, U.K., 1992)*

### **1.4.2. Spray chambers**

The aerosol droplets produced by a nebuliser should be of a diameter of less than 10  $\mu\text{m}$  so that the desolvation, volatilisation and atomization of the analyte in the plasma is as rapid and efficient as possible. The commonly used pneumatic nebulisers produce a wide distribution of droplet size, usually up to  $10^{-4}$  m<sup>(191)</sup>. The main use of a spray chamber is thus to separate the larger droplets from the smallest, allowing the latter to reach the plasma. Unfortunately, 98 to 99% of the sample is lost in this process when employing commonly used nebuliser/spray chamber designs<sup>(188,190,191)</sup>. The separation of the droplets occurs through a variety of processes which eliminate the larger drops according to the different trajectories inside the spray chamber and collisions with the walls or a bead placed inside the chamber. The larger drops go to waste and the smaller droplets are carried into the torch and subsequently the plasma. Most common designs of spray chambers employ flow reversal, cyclones or impact beads. The two first types cause changes in the flow direction as well as impaction on the walls of the chamber. Those employing impact beads operate by placing a device which intercepts the flow of the aerosol and provides an in-line impaction site.

The most common spray chamber is the Scott-type double-pass spray chamber<sup>(190)</sup>. It is based on flow reversal of the aerosol and it is normally cooled at a certain temperature to stabilise the plasma.

There are some parameters that can be used to evaluate the performance of a spray chamber:

- 1/ *Transport efficiency.* The silica gel trap method<sup>(188,193)</sup> can be employed to obtain the transport efficiency. Four U-tubes filled with silica gel should be connected to the exit of the spray chamber. These U-tubes are weighed before and after the passage of a certain amount of water (also weighed) through the nebuliser/spray chamber system. The efficiency of the system can be calculated as the difference in weight of the U-tubes dried and wet. Cyclone spray chambers give better transport efficiency, possibly as a result of allowing bigger drops to pass through the system
- 2/ *Washout time.* The wash-out time of a sample introduction system is the time required to clean the system and is most commonly defined as the time required for the signal to return to 1% of the original maximum. In ICP-MS studies, this time can be calculated by monitoring the decrease in the signal of a solution of 100 ng.mt<sup>-1</sup> of <sup>115</sup>In after being replaced with a solution without In.

The amount of water that goes into the plasma affects drastically the number of polyatomic interferences produced. The amount of water vapour that reaches the plasma can be minimised (and thus improving the sensitivity) by cooling the spray chamber and condensing part of the water vapour produced from the aerosol. It is normal to keep the temperature of the spray chamber at 8°C with a water jacket.

The effect of cooling the spray chamber is more beneficial when samples with a high content of organic solvents are being analysed. The spray chamber



should be cooled at lower temperatures than when passing aqueous solutions, since plasmas only tolerate a certain amount of organic solvents. In these cases the spray chamber is often cooled at  $-10^{\circ}\text{C}$ .

### 1.4.3. Torch and plasma

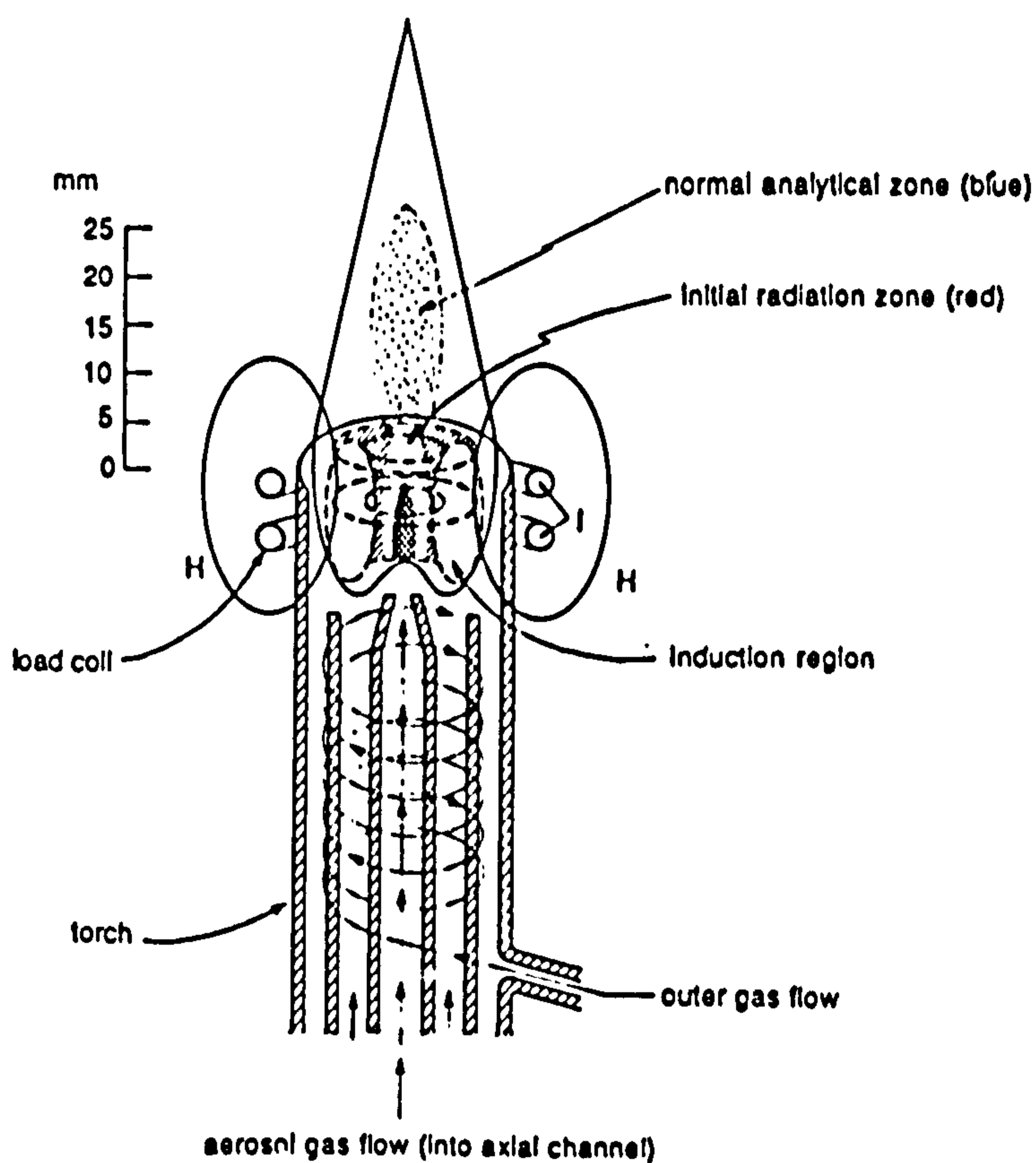
Greenfield *et al.*<sup>(179)</sup> in United Kingdom and Wendt and Fassel<sup>(180)</sup> one year later in U.S.A. published the first papers on “plasma spectroscopy”. Since then, many text books have been published and studies on plasma properties reported<sup>(192-196)</sup>.

A plasma is an electrodeless discharge in a gas at atmospheric pressure, maintained by energy coupled to it<sup>(182)</sup>. Inductively coupled plasmas are sustained from a RF generator via a coupling coil. The gas normally used is argon, although other gases (nitrogen, helium) and mixtures of them (with methane, oxygen, ethane, etc.) can be used.

The plasma is generated within and at the end of a series of quartz tubes, known as a torch. A schematic of an ICP torch can be seen in Figure 1.10. The torch is formed from three tubes. The external one is the main plasma support (coolant gas) and protects the tubes of the torch. The gas flow is normally  $10-15 \text{ l.min}^{-1}$ . The second tube carries through the auxiliary gas (flow rate between 0 and  $1.5 \text{ l.min}^{-1}$ ) and it is mainly used to keep the hot plasma ( $8000-10000 \text{ K}$ ) out of the end of the capillary inner tube tip, to avoid this one melting. Finally, the sample is carried into the plasma via the nebuliser gas through the inner tube, called the injector, at about  $1.0 \text{ l.min}^{-1}$ . This flow produces a high speed jet at the end of the

injector, which punches a hole through the plasma.

The accuracy and quality of the quartz employed in the construction of a torch is important to give stability to the plasma. Injector tubes are normally of 1.5 mm, although smaller (down to 0.8 mm) and bigger bore (up to 3 mm) can be used, depending on the sample type (organic sample-narrower injector; slurry or high dissolved solid samples-wider diameters).



**Figure 1.10.** *Schematic diagram of an ICP torch and the induced magnetic field. The shaded zones are induced when a solution of Y is introduced in the system (from K.E. Jarvis, A.L. Gray, R.S. Houk. "Handbook of Inductively Coupled Plasma Mass Spectrometry", Blackie & Sons Ltd., Glasgow, U.K., 1992)*

There are different types of injectors used, which are mainly the tapered injector tip, capillary injector tip and complete capillary injector tip. The torch can be constructed in one piece or in several, the most common design among the latter having the injector separate. This design allows easy interchange of the injector of the torch (different inner diameter injectors, to replace an old one, or of different materials). Torches suitable for low flows, for mixed gas plasmas or coupled to sheathing devices can be obtained. Mixed gas plasmas are reported to enhance the sensitivity for certain elements due to the change in the characteristics of the plasmas<sup>(197,198)</sup>. The main use of mixed gases plasmas is the reduction of polyatomic interferences (see "Spectral interferences"). Mixed gas plasmas are also used to avoid depositions of solid on the cones of the interface, especially when analysing organic based solutions<sup>(199-203)</sup>. Sheathing devices can be coupled just before the torch without any modification to it.

The torch is placed horizontally, centered with the orifices of the cones in the interface. Approximately 25 mm of coolant tube should be placed inside the load coil, with a distance of 3 to 5 mm from the coil to the auxiliary tube. A distance of between 10 and 15 mm should be between the torch and the cones.

The ultimate aim of the sample introduction system is to produce ions that can be conducted to the entrance of the mass spectrometer. These ions are produced by volatilisation, atomisation and ionisation of the sample in the plasma. There are different parts in the plasma where these processes occur. The different areas of the plasma may be seen passing a solution of yttrium through<sup>(182)</sup>. Just after the injector tip a red area can be observed due to the YO band emission. This is called the initial

radiation zone(IRZ). After this area, the normal analytical zone (NAZ) can be seen in blue, due to the emission of yttrium ions. The sample may contain some water when it exits the injector tube. This water is rapidly dried, vapourised and dissociated. The outer region of the plasma is reported to be at about 10000 K<sup>(181,182)</sup>. By thermal conduction, energy is transferred to the inner channel, where the sample is. During its transit through the torch, the analyte follows different stages, represented in Table 1.4. At the outside of the torch, the temperature of the plasma (and of the central channel) begins to fall. The plasma also begins to mix with the ions, atoms, residual molecular fragments and unvolatilised particulates that came out of the torch. The temperature of this mixture when it reaches the cones has been reduced to  $\approx 6000$  K<sup>(181)</sup>.

**Table 1.4.** *Analyte solution history in the ICP*

<b>Location</b>	<b>State</b>	<b>Event</b>
Sample vessel	Solution	Aspiration or pumping
Nebuliser	Aerosol droplets	
Spray chamber	Selected droplets	Dia. > 10 $\mu$ m rejected
Torch capillary	Droplets	Transport to the plasma
Plasma central channel	Droplets	Desolvation
Plasma central channel	Salt particles	Volatilisation
Plasma central channel	Molecular vapour	Dissociation
Plasma central channel	Atoms	Excitation and ionisation
Free space	Atoms and ions	Exit from torch

Although early publications on ICP-MS claimed that it could be a technique free from interferences<sup>(204-206)</sup> many studies have been conducted since to ascertain the type of interferences and ways to overcome them. Interferences in ICP-MS can be subdivided into two categories<sup>(181,182,207-209)</sup>:

- 1/ *Spectral interferences*. Also called isobaric interferences. These are caused by species with the same mass-to-charge ratio as the analyte. They can be derived from another element with an isotope at the same  $m/z$  of the analyte, from polyatomic species formed in the plasma, double charged ions, hydroxides and oxides of refractive elements carried from the plasma. Some examples are:  $^{54}\text{Fe}^+$  on  $^{54}\text{Cr}^+$ ,  $^{40}\text{Ar}^{16}\text{O}^+$  on  $^{56}\text{Fe}^+$ ,  $^{86}\text{Sr}^{16}\text{O}^{1}\text{H}^+$  on  $^{103}\text{Rh}^+$ ,  $^{37}\text{Cl}^{18}\text{O}^+$  on  $^{55}\text{Mn}^+$ . Fortunately, almost all the elements have an isotope free from isobaric interferences. Overlapping interferences are well studied and documented and, thus, are the easiest to overcome. Polyatomic interferences are difficult to quantify and so to solve. Several attempts have been made. The most common way to overcome isobaric interferences is with mathematical corrections based on the abundance of the interferent calculated from another isotope of the interferent free of interference. Mixed gas plasmas have also been used. The gas added may be a molecular gas, such as nitrogen or methane, or a noble gas, such as helium or xenon. The thermodynamic properties of the mixed gas plasma are different than those of Ar-only plasmas. For example, helium has been added to improve detection limits since



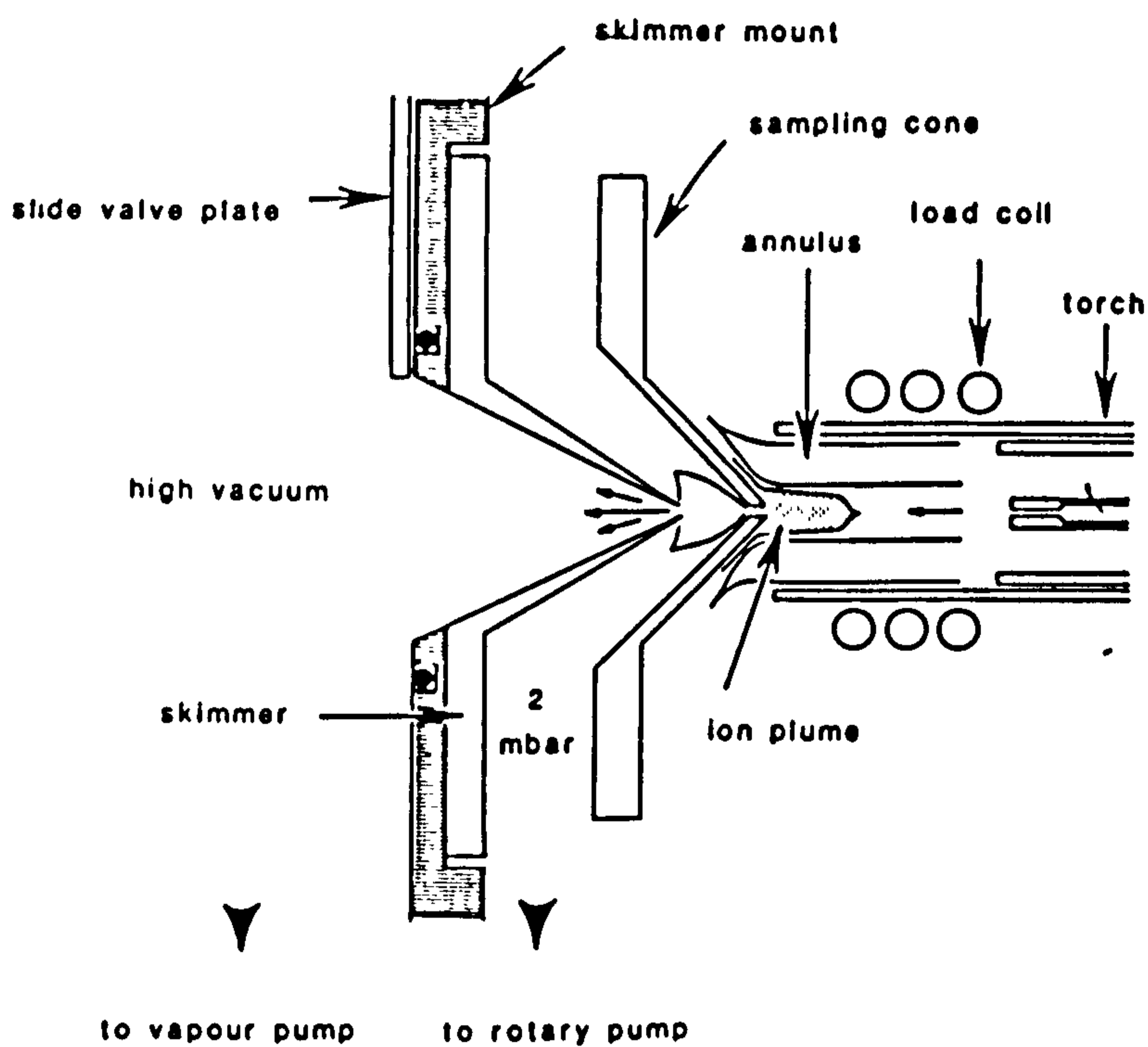
it forms a more ionising and conductive plasma and thus reduces the existence of refractive element oxides.

2/ *Non-spectral interferences.* These are matrix dependant. Normally they cause a decrease in the analyte signal. They can be due to physical effects or due to analyte enhancement or suppression of the signal from species concomitant in the sample. Physical effects can be due to the high content of solids or organic solvents in the sample, which may induce the deposition of substances on the cones and thus cause a drift in sensitivity. Nitrogen can cause a great reduction in the interference of  $^{40}\text{Ar}^{35}\text{Cl}^+$  and  $^{40}\text{Ar}^{37}\text{Cl}^+$ , making it possible analyse  $^{75}\text{As}^+$  or  $^{77}\text{Se}^+$  in samples with high content of chloride<sup>(209)</sup>. Oxygen is necessary when analysing high organic content samples to oxidise the carbon and stop its deposition on the cones. Easily ionisation elements present in the sample, such as Na, K, Ca, etc., produce complex effects in the plasma which are not yet fully understood but in the NAZ a decrease in the population of ions may be observed.

#### 1.4.4. ICP-MS interface (cones)

An schematic diagram of the ICP-MS interface can be seen in Figure 1.11. Sampling and skimmer cones are an essential part of the interface. The interface should be able to extract the ions from the plasma. The design of the cones to obtain the most representative sample from the plasma is an area of interest for instrument manufacturers. The cones are made of aluminium, nickel, copper or platinum. Nickel

is most commonly used, although Pt-tip ones are often used when analysing organic solutions to avoid the oxidation of the Ni cones. The cones are normally screwed to a front plate which is water cooled. The orifice of the sampler varies between 0.7 to 1.1 mm, while the skimmer oscillates between 0.7 and 1.0 mm<sup>(209)</sup>. The distance between the cones is normally 6-7 mm. The skimmer tip shape is very important for the sensitivity and the formation of some polyatomic species which could cause interference. The normal pressure between both cones (expansion chamber) is about 1-3 mbar.

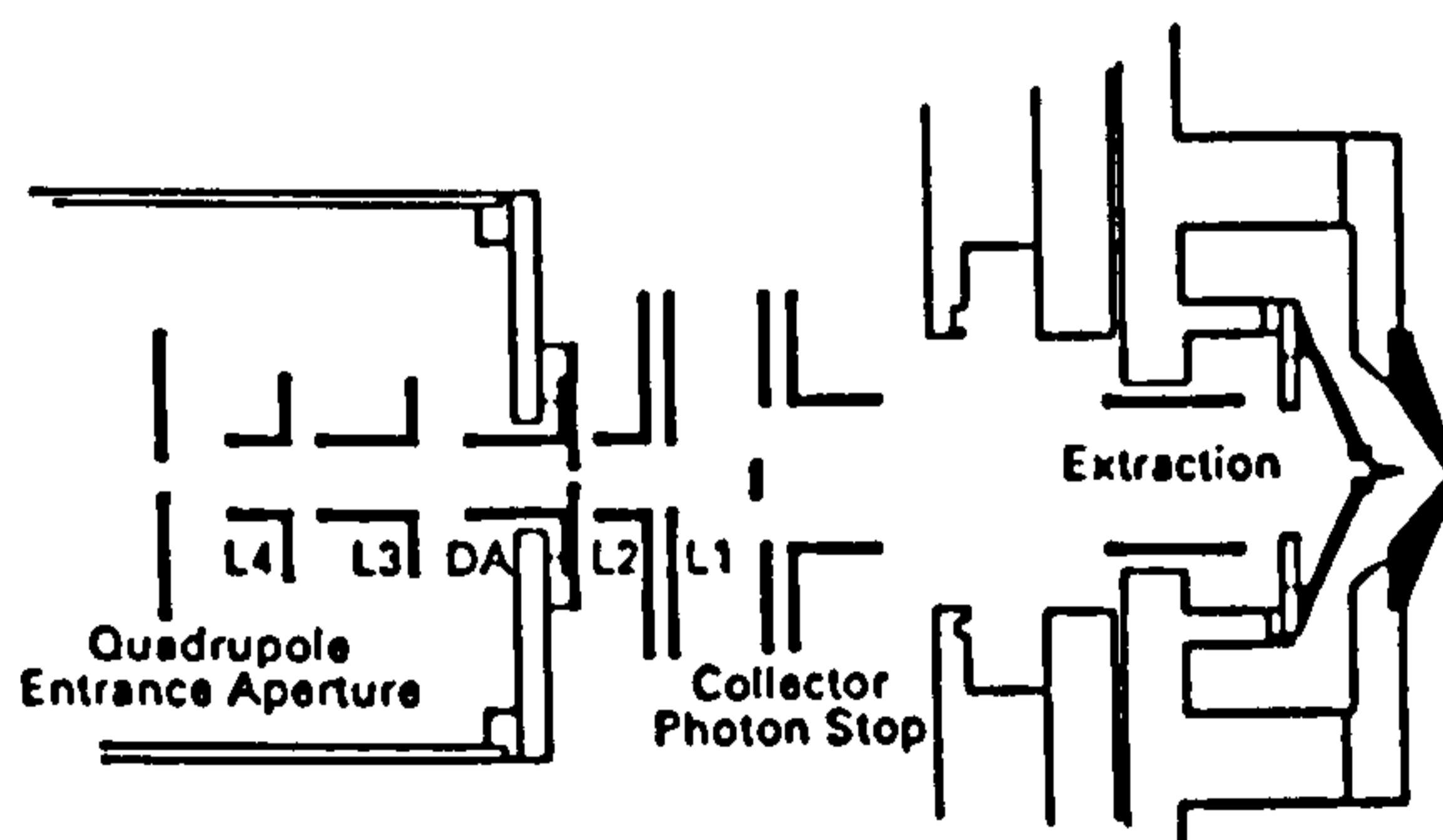


**Figure 1.11.** Schematic diagram of the ICP-MS interface (from A.R. Date, A.L. Gray. "Applications of Inductively Coupled Plasma Mass Spectrometry", Blackie & Sons Ltd., Glasgow, U.K., 1989)

### 1.4.5. Ion lenses

The ions that have passed through the skimmer orifice have now to be directed into the mass analyser. This is the mission of the ion lenses. A typical ion lens system can be seen in Figure 1.12. Each ion lens has a central disc to prevent photons passing through and thus contributing to the background. Also, the photon stop implies the loss of 50-80% of the ions. To minimise this problem, some manufacturers have offset the ion path from the entrance to the mass analyser. The ions have different paths along the ion lens, depending on their mass, since they have different kinetic energy. This translates into differences in selectivity for different elements. Normally, as a compromise, the voltages in the lenses are selected to optimise the path of an ion in the middle of the mass range.

The mass analyser operates at high vacuum pressures. In order to achieve this, two vacuum stages are operated behind the skimmer. Both stages are separated between them by a differential pumping aperture, and from the expansion chamber by a slide valve.



*Figure 1.12. Typical ion lenses system for ICP-MS (from K.E. Jarvis, A.L. Gray, R.S. Houk. "Handbook of Inductively Coupled Plasma Mass Spectrometry", Blackie & Sons Ltd., Glasgow, U.K., 1992)*

#### 1.4.6. Mass analyser

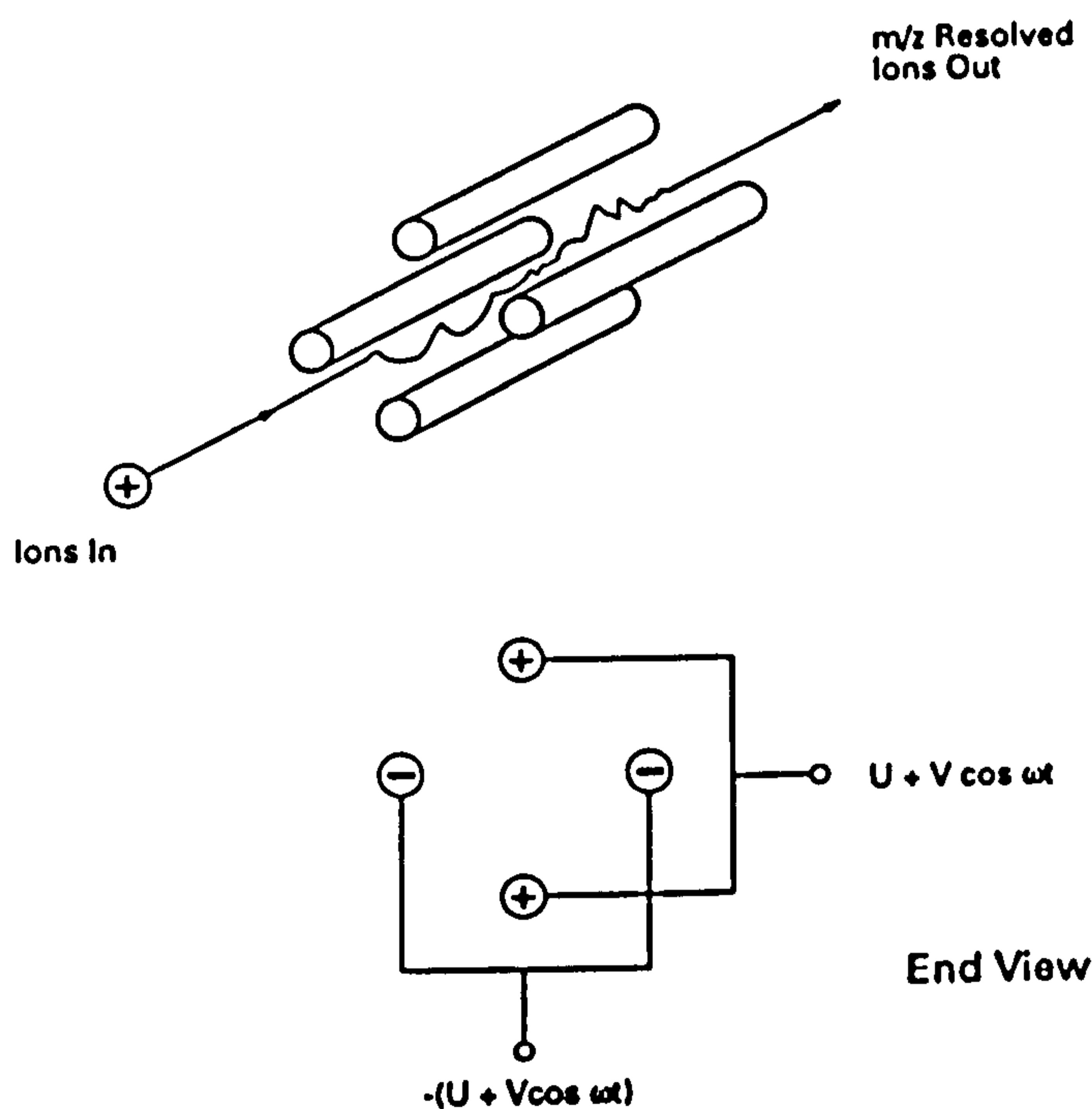
Most instruments use a quadrupole mass spectrometer, although there are instruments based on magnetic sectors, which are capable of much better resolution.

A quadrupole mass analyser acts just as an ion filter, along which axis a stable ion path exists for ions of a particular mass. A quadrupole consists of four metal or metallised surface rods, suspended in parallel, equidistant from the axis. The rods are normally of 12-18 mm of diameter and about 200 mm long<sup>(181)</sup>. Ideally, the rods should have a hyperbolic shape, although round rods that approximate a hyperbola are used. A schematic diagram can be seen in Figure 1.13. The four rods are connected two by two to the rods opposite. Each pair have DC and RF voltages applied to them. The DC voltage is positive in one pair, negative in the other; the RF are of same amplitude, but opposite in sign (180° out of phase). The mass that will be transmitted through the quadrupole can be selected by modifying the DC and RF voltages. The mass can be changed at a rate of up to 3000 mass units per second. The resolution obtainable is usually of 1 mass unit.

If more resolution is required (to resolve polyatomic interferences from the analyte mass) magnetic sectors can be used. These can achieve a resolution of up to 30000<sup>(209)</sup> (defined as  $M/\Delta M$ <sup>(182)</sup>, being  $\Delta M$  the width of the peak at mass  $M$  at 5% of the peak height).

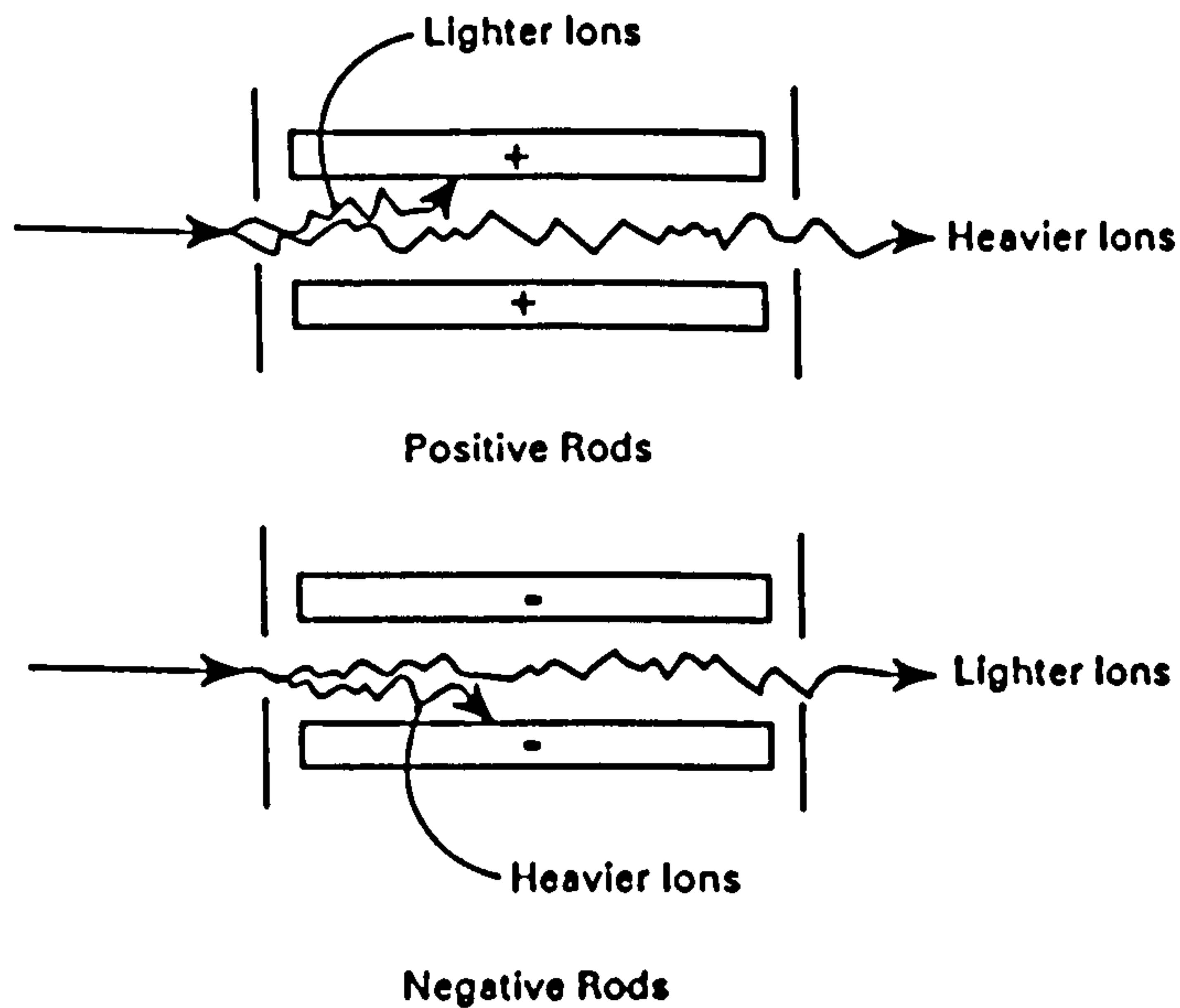
A double focusing magnetic sector mass spectrometer can be seen in Figure 1.14. The ICP is not altered, but both cones in the interface are isolated electrically from the vacuum chamber and biased at a high positive potential. When the ions

pass through the cones, they form an ion beam. Ion lenses are used to change the shape of the beam, from a circular cross-section to slit-like section. The beam passes through an electrostatic energy analyser and ions within a kinetic energy region are selected. These ions are then passed through a magnetic sector, separated depending on their  $m/z$  ratio, and detected. Each of these processes has its own pumping stage and are separated from each other with relatively small apertures. More information on principles of magnetic sector mass spectrometers was published by Roboz<sup>(210)</sup> in 1968.

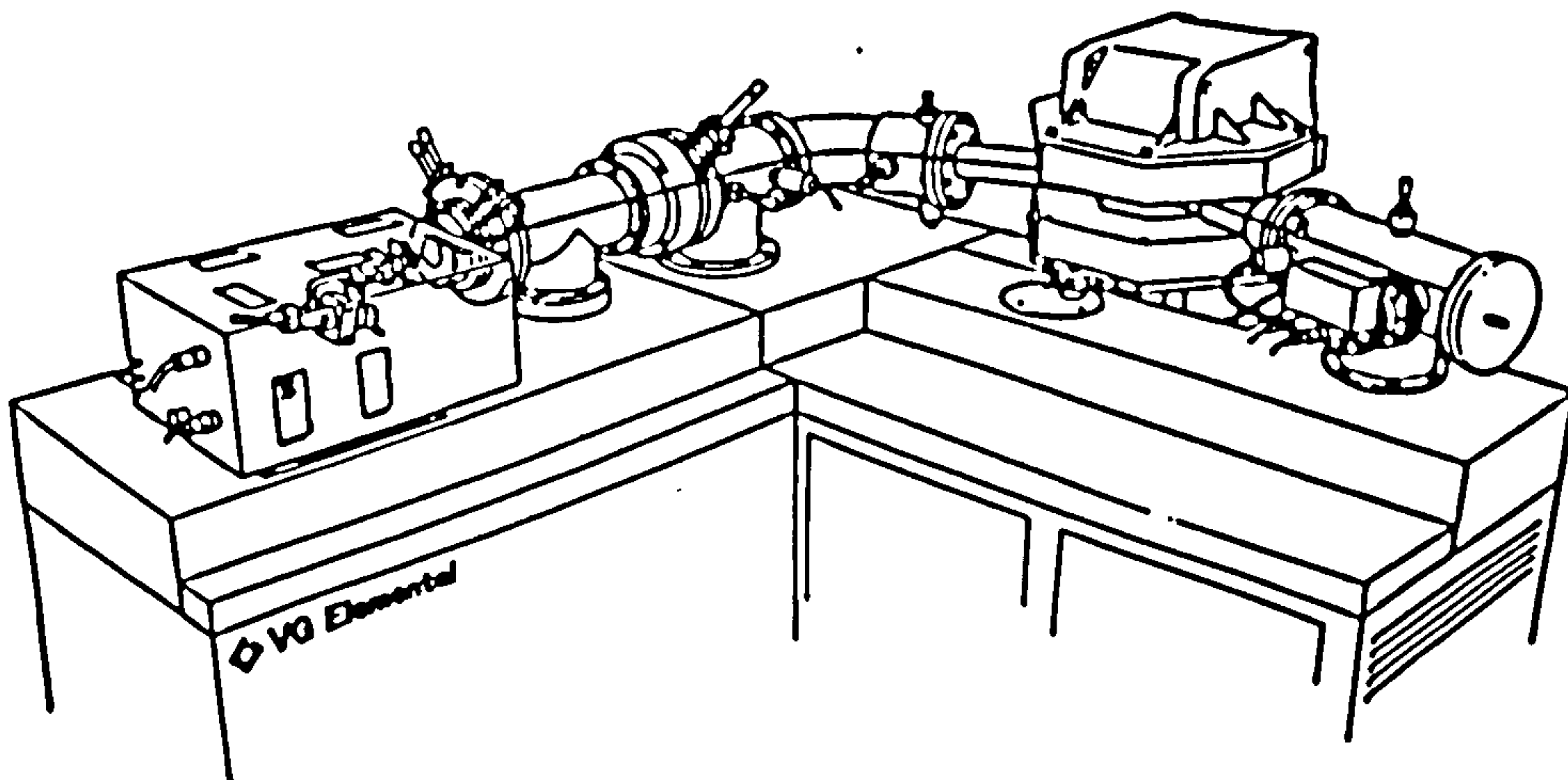


**Figure 1.13.(a)** Schematic diagram of a quadrupole showing ion trajectory and applied voltages (from K.E. Jarvis, A.L. Gray, R.S. Houk. "Handbook of Inductively Coupled Plasma Mass Spectrometry", Blackie & Sons Ltd., Glasgow, U.K., 1992)





**Figure 1.13(b).** Side views of the ion separation processes in the two rod planes of a quadrupole (from K.E. Jarvis, A.L. Gray, R.S. Houk. "Handbook of Inductively Coupled Plasma Mass Spectrometry", Blackie & Sons Ltd., Glasgow, U.K., 1992)



**Figure 1.14.** Double focussing magnetic sector mass spectrometer from VG (from K.E. Jarvis, A.L. Gray, R.S. Houk. "Handbook of Inductively Coupled Plasma Mass Spectrometry", Blackie & Sons Ltd., Glasgow, U.K., 1992)

#### 1.4.7. Ion detection systems

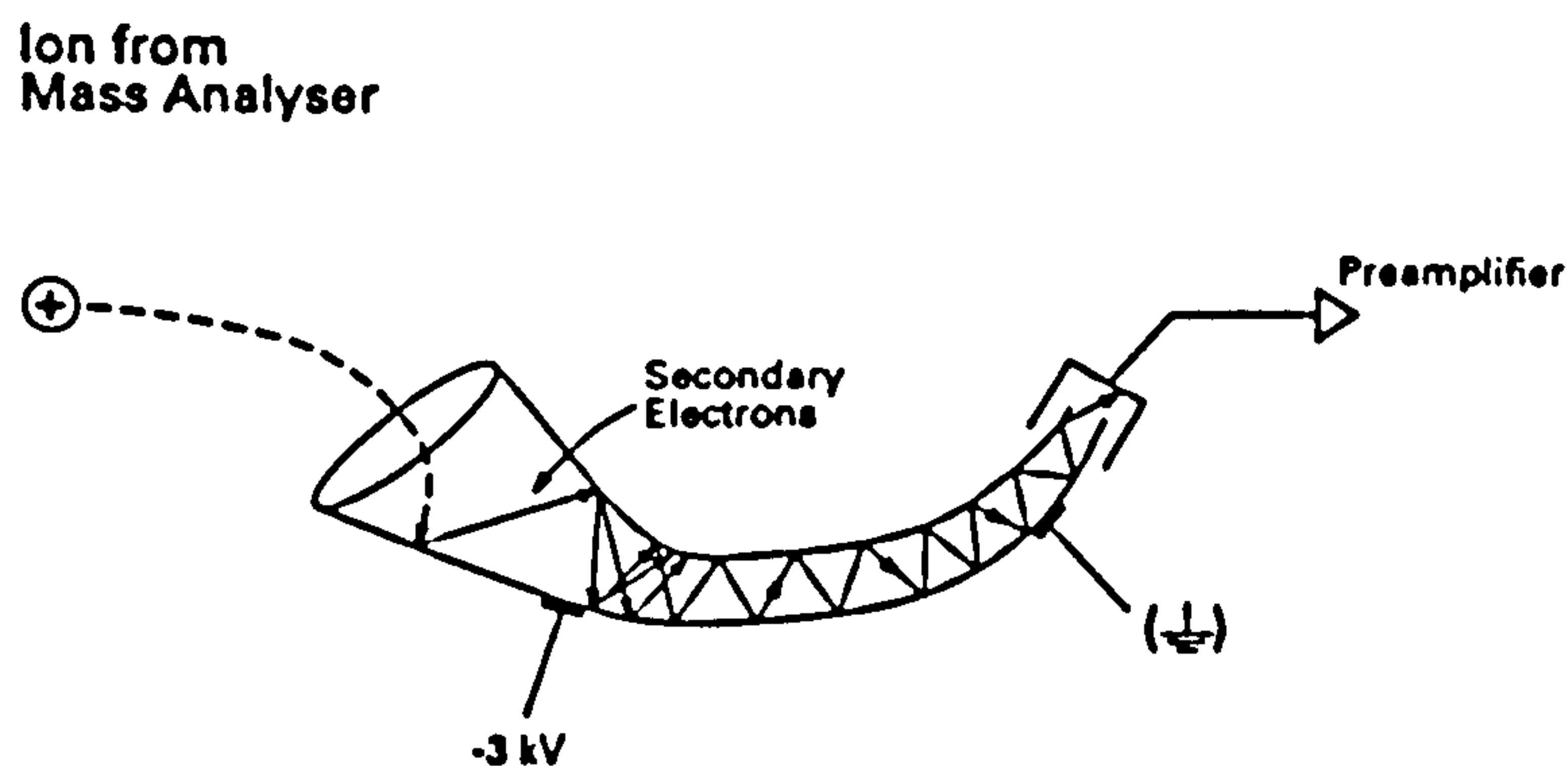
ICP-MS is used to analyse samples at very low concentrations, below  $1 \mu\text{g}\cdot\text{ml}^{-1}$  in many cases. Taking this into account, the number of ions that reach the detection system (after loss in nebulisation, extraction from the plasma and losses in the ion lenses and mass analysers) is very small. These can give typically currents of  $10^{-13} \text{ A}^{(18)}$ . The background is also very small, and this is the reason for the high sensitivity of the technique.

The most common ion detection system is based on chaneltron electron multipliers (Figure 1.15). These consist of an open glass tube with a cone at one end. The inside of the tube is coated with a film of lead oxide semiconducting material. The tube is connected to a power supply and a high negative voltage is applied to the entrance (normally -3 kV), while the outer extreme is grounded. Any positive ion that leaves the mass analyser is attracted to the electron multiplier and crashes against the tube. One or more secondary electrons are released, which will be attracted to the exit of the chaneltron due to the different potential at the end of the tube. Along its way, each electron hits the surface and releases more electrons that follow the same process. The result is that a single ion gives a pulse of up to  $10^{18}$  electrons<sup>(18)</sup> at the collector. The voltage applied to the electron multiplier can be increased in order to attract more ions, which is necessary at the end of the life of the chaneltron.

The signal can be measured by pulse counting. The pulse of  $\approx 10^{18}$  electrons given from an ion is sensed by the collector and amplified. This pulse goes to a

counting circuit, which is set to be able to discriminate above a certain threshold.

An A/D converter can be fitted after the detector for mean current detection for the analysis of more concentrated samples.



*Figure 1.15. Channeltron electron multiplier (from K.E. Jarvis, A.L. Gray, R.S. Houk. "Handbook of Inductively Coupled Plasma Mass Spectrometry", Blackie & Sons Ltd., Glasgow, U.K., 1992)*

### **1.5. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETRY (HPLC-ICP-MS)**

The combination of the speciation capabilities of HPLC with ICP-MS as a very sensitive detector, which can also be used in an element specific mode, results in a very powerful technique for environmental analysis. There have been several reviews on the topic, dealing with both the practical aspects of the coupling and the types of samples analysed<sup>(211-219)</sup>.

The coupling is quite straightforward. It just requires a piece of tubing that connects the outlet of the HPLC column with the nebuliser of the ICP-MS. The flow rates of HPLC are compatible with those of sample intake in ICP-MS. The coupling HPLC-ICP-AES has also been achieved<sup>(211)</sup> and the connection is similar for ICP-MS. There are a few considerations that should be taken into account in this coupling. It should tend to minimise the dead volume of the system and, thus, maintain the separation obtained in the HPLC column. One of the important features of the coupling is therefore the length and internal diameter of the tubing: it should be as short and of as smaller diameter as possible. Teflon tubing of 0.3 mm i.d. and of not more than 30-40 cm length is used<sup>(201-203)</sup>. The spray chamber internal volume and its position in the coupling line are important factors to be taken into consideration. The internal volume of the spray chamber may induce the mixing of the resolved and separated peaks from the HPLC column. A small internal volume spray chamber with small dead volume is then desired. The spray chamber could be placed close to the exit of the HPLC system (thus, outside the torch box), allowing a short liquid transport time and longer aerosol transport to the plasma, or in its normal position in the torch box of the ICP-MS. This assembly involves a longer liquid transport and shorter aerosol transport. The best configuration is the one with longer aerosol transport since it reduces the mixing of the separated analytes.

Some chromatographic separations involve aqueous mobile phases with high salt content. These salts may induce the clogging of the injector capillary or the cone orifices. This problem may be overcome by using a wider bore injector or a tapered tip one or running HNO<sub>3</sub> between HPLC injections to dissolve the salts. If the

surface of the cone is being damaged, a different material cone could be used<sup>(212)</sup>.

Another problem arising from the nature of the eluent can be the content of organic solvents in the mobile phase. The temperature of the cooling of the spray chamber should be reduced to minimise the amount of organic solvent that reaches the plasma. To maintain a stable plasma, higher RF powers can be applied, normally up to 1800 W. These requirements are the same as those for running organic-based samples in the normal operating ICP-MS mode. An addition of oxygen with the nebuliser to avoid carbon deposits on the surface of the cones is also necessary. The high organic content of certain mobile phases, also carrying salts as buffers, may induce the clogging of the injector tube. A sheath gas device can be added to the system<sup>(203)</sup> to avoid this process; the use of a tapered tip injector also helps avoid this problem<sup>(203)</sup>.

More efficient desolvation of the aerosol may be required, especially when the mobile phases have high percentages of highly volatile organic solvents, such as acetonitrile. A semipermeable membrane, with an external counter flow of argon, can be employed to decrease the amount of methanol or acetonitrile (the most common solvents used in HPLC) vapour in the aerosol<sup>(220)</sup>. This desolvation system still only facilitates the use of mobile phases of up to 20% acetonitrile<sup>(221)</sup>. A more efficient method is to heat the spray chamber, thus increasing the formation of aerosol, passing the aerosol through a membrane and afterwards through a cooling condenser<sup>(222-224)</sup>. This system allows the use of mobile phases of up to 95% acetonitrile in water<sup>(223,224)</sup>.



A unique feature of HPLC-ICP-MS is the capability of the system to perform isotope ratio measurements and, consequently, isotope dilution analysis, due to the mass specificity of the detection system<sup>(182,212)</sup>. Rottmann and Heumann<sup>(225)</sup>, Heumann *et al.*<sup>(226)</sup> and García-Alonso *et al.*<sup>(227)</sup> have published methods involving isotope dilution analysis and HPLC-ICP-MS. A more detailed discussion of this coupling can be seen in section 1.7.

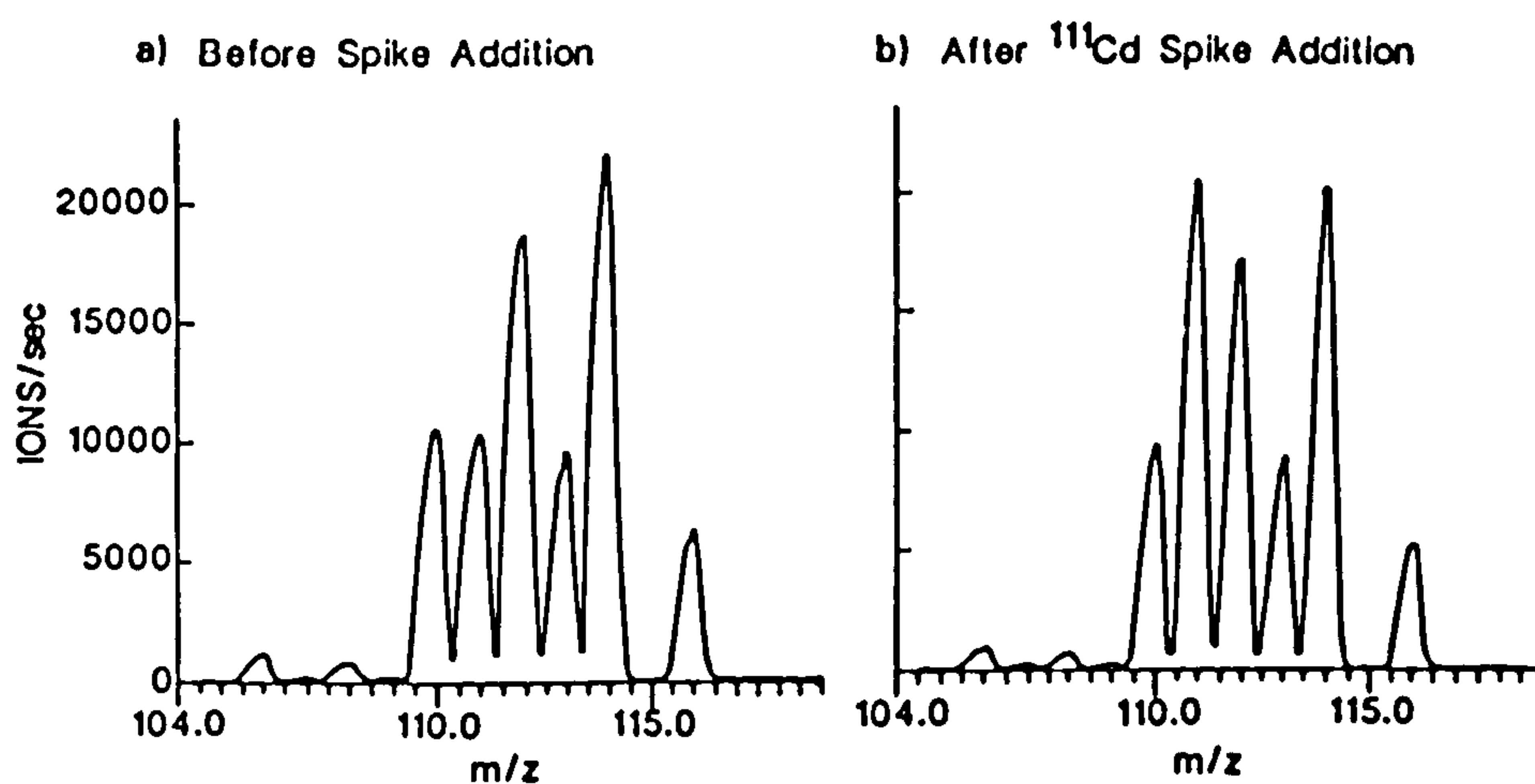
### **1.6. ISOTOPE DILUTION ANALYSIS (IDA)**

Isotope dilution analysis is a calibration procedure. It is based on the isotope ratio between two isotopes of an element in the sample after spiking this with one of the less abundant isotopes of the analyte. This less abundant isotope spiked acts as an internal calibrant. The technique is considered the best way of calibrating, since the internal calibrant employed will be affected the same as the analyte, as the instrument drifts with time (both analyte and calibrant are detected at the same time) or by interferences or chemical processes. If the sample is spiked and perfectly homogenised before any sample treatment, this calibration strategy also compensates for any losses that may occur during the sample preparation since these will affect equally both isotopes and their ratio will remain unchanged.

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The method can be applied to the analysis of any element with at least two stable isotopes or even monoisotopic elements with a radioisotope of long enough half life (*e.g.* Mn).

In Figure 1.16 isotope dilution analysis is shown “graphically”. Figure 1.16(a) represents the natural abundances of Cd in a sample. Figure 1.16(b) shows the abundances of the different isotopes of Cd after spiking the sample with a solution enriched in  $^{111}\text{Cd}$ . The ratio between  $^{114}\text{Cd}/^{111}\text{Cd}$  in the spiked sample is employed to find out the concentration of Cd in the original sample.



**Figure 1.16.** *“Graphical determination of Cd by isotope dilution analysis: (a) natural abundances of Cd, (b) abundances after spiking (from K.E. Jarvis, A.L. Gray, R.S. Houk. “Handbook of Inductively Coupled Plasma Mass Spectrometry”, Blackie & Sons Ltd., Glasgow, U.K., 1992)*

The concentration of the element in the sample can be calculated<sup>(228)</sup>:

$$C_x = \frac{C_s W_s}{W_x} \frac{A_s - RB_s}{RB_x - A_x} \quad \text{Eq. 1.16}$$

where:

$C_x$  is the concentration of the analyte in the sample (in  $\mu\text{g}\cdot\text{g}^{-1}$  or  $\text{ng}\cdot\text{g}^{-1}$ , normally),

$C_s$  is the concentration of the analyte in the spike solution,

$W_s$  is the weight of spike,

$W_x$  is the weight of sample,

$A_s$  is the abundance of the reference isotope in the spike,

$B_s$  is the abundance of the spike isotope in the spike,

$A_x$  is the abundance of the reference isotope in the sample,

$B_x$  is the abundance of the spike isotope in the sample, and

$R$  is the ratio between reference and spike isotopes in the sample after spiking.

There are two main sources of errors in IDA. The first is derived from incomplete mixing of the spike solution with the sample. Ideally the sample should be in liquid state to allow the homogenisation. If the spike does not mix properly with the sample, the measured isotope ratio is incorrect since the isotopes are selectively lost. Any contamination will induce an error in the measured isotope ratio and, thus, it should also be avoided.

The second source of error is due to the mass analyser itself. The two isotopes selected for IDA should be free of isobaric interferences. If the interference is due to another element and this one has an isotope free of interferences, then the interference can be corrected for. If the interference is due to polyatomic ions it is more difficult to correct for. The mass spectrometer also is biased towards certain

masses. Different isotopes are transmitted and detected with different efficiencies. This can be corrected for measuring the isotope ratio of a standard of known isotopic composition and calculating the mass bias or mass discrimination factor,  $K$ , which can be considered constant for a limited mass range<sup>(227)</sup>:

$$K \Delta M = \frac{R_{\text{exp}} - R_{\text{theo}}}{R_{\text{theo}}} \quad \text{Eq. 1.17}$$

where:

$R_{\text{exp}}$  is the measured isotope ratio,

$R_{\text{theo}}$  is the expected isotope ratio, and

$\Delta M$  is the mass difference between the measured isotopes.

The measured isotope ratio can then be corrected<sup>(227)</sup>:

$$R_{\text{corr}} = \frac{R_{\text{exp}}}{1 + K \Delta M} \quad \text{Eq. 1.18}$$

It is this  $R_{\text{corr}}$  that should be incorporated into Eq. 1.16 to calculate the concentration of analyte in the sample.

If a pulse counting detection system is employed, there is a time before and after each pulse in which the detector is "dead", it cannot detect any ions. This dead time of the detector is due to the electronics of the system. The effect of the detector dead time ( $\tau$ ) on the count rate (counts.s<sup>-1</sup>) of an isotope can be expressed as<sup>(227)</sup>:

$$I_{real} = \frac{I_{meas}}{1 - \tau I_{meas}} \quad \text{Eq. 1.19}$$

where:

$I_{real}$  is the count rate in the ideal case where there were no detector dead time effect, and

$I_{meas}$  is the count rate measured.

The detector dead time can be calculated by measuring the isotope ratio of two isotopes at different concentrations at which the effect of detector dead time will be minimal (*i.e.* at low count rates, but not so low as to give bad counting statistics)<sup>(229)</sup>:

$$I_m - R_{m/M} = \tau I_m I_M (1 - R_{m/M}) \quad \text{Eq. 1.20}$$

where:

$I_m$  is the count rate for the minor isotope,

$I_M$  is the count rate for the major isotope,

$R_{m/M}$  is the isotope ratio, and

$\tau$  is the detector dead time.

The slope of the graph of  $I_m - R_{m/M} I_M$  vs  $I_m I_M (1 - R_{m/M})$  will give an empirical value for  $\tau$  in seconds. The isotope ratio should be blank subtracted.

The ratio between the reference isotope and the spike isotope after spiking and homogenisation of the sample should be optimised to give the best accuracy.

The best precision in the isotope ratio is obtained when the isotope ratio is close to



$1^{(228)}$ , and so the amount of spike should be calculated to obtain a ratio close to 1. Heuzen *et al.*<sup>(230)</sup>, García-Alonso<sup>(227,231)</sup> and Longerich<sup>(232)</sup> have published more detailed studies on the effect of over and underspiking in precision and accuracy of isotope ratios.

Finally, a previous study of the concentration of the spike solution is normally required, unless this solution is a standard solution. This is done by reverse isotope dilution analysis (R-IDA). The spike solution is spiked with a known amount of natural isotopic abundance materials which have to be standards.

IDA has some disadvantages. One is the initial cost of buying the enriched isotopic material. This cost is afterwards spread through various years of use since only a few ng are necessary for each analysis. The technique is not normally used for many routine applications. The main problem may be to find the enriched isotopic material required for a particular analysis, although more standards are being made available as the technique is being more widely used.

### ***1.7. ISOTOPE DILUTION ANALYSIS-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETRY (IDA-HPLC-ICP-MS)***

IDA has been mostly employed with thermal ionization mass spectrometry (TIMS) with which better accuracy in the isotope ratio can be obtained. ICP-MS offers the advantage of higher sampling rate, with detection limits adequate for

environmental analysis. The accuracy of the analysis is limited by the inhomogeneity of the sample and noise generated by the sample introduction process and not because of the isotope ratio values. The coupling HPLC-ICP-MS has been used in conjunction with IDA to obtain highly precise and sensitive analysis of different types of samples. Rottmann and Heumann<sup>(225)</sup> proposed on-line IDA-HPLC-ICP-MS. They suggested two possible configurations. The first one involved the on-line mixing of the sample with the spike solution before the separation in the analytical column takes place. The spike solution must contain the species in the same form as all of these that are going to be separated. The second approach involved mixing the sample carrier with the spike solution after the HPLC column. In this case, the spike solution does not have the different species in the same form as the species of interest. This approach was useful in the case of humic substances, for example, for which the equivalent enriched isotope compound is not available. Heumann *et al.*<sup>(226)</sup> used the first configuration for the determination of iodide and iodate in mineral water with ion-exchange chromatography. Braverman<sup>(233)</sup> determined rare earth elements (REEs) with HPLC-ICP-MS, comparing the results obtained with IDA and external calibration for Nd. Fission products and actinides in spent nuclear fuels have also been analysed by IDA-HPLC-ICP-MS<sup>(227)</sup>. The different REEs were separated in an ion-exchange column prior to their introduction into a glove-box ICP-MS.

## **1.8. AIMS OF THE STUDY**

There are few certified reference materials (CRMs) for speciation studies on organotin or organolead compounds. The National Research Council of Canada (NRC) produced PACS-1, a harbour marine sediment certified for TBT, DBT, and MBT at the ppm level. The Standards, Measurements and Testing (BCR) Programme has two more sediments CRMs, numbers 462 (a coastal sediment certified for TBT and DBT) and 424 (harbour sediment with an indicative value for TBT) at the ppb level. Phenyltin compounds are not certified in any of these cases. It is clear from the widespread of the results from the certification campaigns that great problems occur when trying to analyse organotin species in these materials. Only one CRM biological material is available (NIES-11, fish tissue), certified for TBT and with an indicative value for TPhT. The case is similar for organolead compounds, since there are no CRMs for organolead species.

The Standards, Measurements and Testing Programme, in an attempt to produce a number of CRMs, both for organotin and organolead compounds, started a programme to improve analytical methodologies for butyl- and phenyltin and trimethyllead compounds.

The first step should be the improvement of the separation of the different species. Liquid chromatography offer some advantages (as mentioned above) over gas chromatography. Few separation methods exist for the analysis of butyl- and phenyltin compounds in real samples and, thus, the separation of as many species as possible should be attempted.

CRMs of biological materials are scarce and the preparation of a mussel tissue, certified for butyl- and phenyltin species was started. An extraction method for the speciation of organotin compounds in this sample should be developed.

The separation of organolead compounds is normally affected by the high content of inorganic lead present in environmental samples. A separation of this species from trimethyllead should be attempted.

In order to improve the accuracy, isotope dilution could be incorporated in the analytical methodology. This will compensate for certain errors derived from the long and tedious extraction procedures. ICP-MS is a very sensitive detection system (which can be used in element selective mode) capable of isotope ratio measurements, which will enable isotope dilution analysis (IDA)-HPLC-ICP-MS.

Therefore the aims of this work were: to improve and develop new methodologies for the determination of organotin and organolead species in a variety of samples by coupled HPLC-ICP-MS; to validate these methods so that they could be used in certification campaigns; to improve the accuracy of these methods by the use of IDA-HPLC-ICP-MS.

## ***2. INSTRUMENTATION***



## **2. INSTRUMENTATION**

### **2.1. CHEMICALS AND REAGENTS**

#### **2.1.1. Organotin speciation**

The tributyltin chloride (TBT, 96% pure), dibutyltin chloride (DBT, 96%), monobutyltin chloride (MBT, 95%), triphenyltin chloride (TPhT, 95%), diphenyltin chloride (DPhT, 96%), monophenyltin chloride (MPhT, 98%), triethyltin bromide (TET, 97%), and dimethyltin chloride (DMT, 97%) were obtained from Aldrich Chemical Co. Ltd., Dorset, U.K. Trimethyltin chloride (TMT) was purchased from Alfa, Karlsruhe, Germany. Inorganic tin calibrants were prepared from an inorganic tin calibrant solution (ICP-DCP grade),  $9990 \mu\text{g}\cdot\text{ml}^{-1}$ , in HCl, obtained from Aldrich Chemical Co. Ltd., Dorset, U.K. Stock solutions ( $1000 \mu\text{g}\cdot\text{g}^{-1}$ ) were prepared in HPLC-grade methanol (Rathburn Chemicals Ltd., Peebleshire, U.K.) and stored in the dark at  $4^{\circ}\text{C}$ .

HPLC-grade methanol was also used to prepare the mobile phases for separation studies. The triammonium citrate, citric acid and ammonium acetate used as buffers in this work were purchased from Fisons Analytical Reagent, Loughborough, U.K. The tropolone was obtained from Aldrich Chemical Co. Ltd., Dorset, U.K.

Glacial acetic acid, obtained from BDH, Poole, U.K., and toluene (HPLC

grade), obtained from Rathburn Chemicals Ltd., Peebleshire, U.K., were used for the extraction of the organotin compounds from the sediments.

Lipase (type VII), protease (type XIV), trypsin (type III) and pancreatin enzymes employed in the enzymatic extraction of organotin species from biological tissue were obtained from Aldrich Chemical Co. Ltd., Dorset, U.K. Ethanol was obtained from Rathburn Chemicals Ltd., Peebleshire, U.K. Sodium dihydrogenphosphate buffer was BDH AristaR grade (BDH, Poole, U.K.). Citric acid as above. Ammonium bicarbonate was AnalaR grade purchased from BDH, Poole, U.K.

Dichloromethane was AristaR grade obtained from BDH (BDH, Poole, U.K.). Hexane (HPLC grade) and chloroform (HPLC grade) were supplied by Rathburn Chemicals Ltd., Peebleshire, U.K. Sodium chloride, AristaR grade, was purchased from BDH (BDH, Poole, U.K.).

Tetramethylammonium hydroxide (TMAH), 20% in water, was obtained from Aldrich Chemical Co. Ltd., Dorset, U.K.

Hydrochloric acid, AristaR grade, was obtained from BDH (BDH, Poole, U.K.).

The sediment certified reference material PACS-1 was purchased from the National Research Council of Canada (Ottawa, Canada). Fish tissue NIES-11 was obtained from the National Institute for Environmental Studies of Japan, Onogawa, Japan. Mussel tissue candidate reference material CRM 477 was obtained from the

Standards, Measurements & Testing Programme (BCR), Brussels, Belgium.

Milli-Q deionised water (Millipore, Massachusetts, U.S.A.) or equivalent was used throughout the study.

### **2.1.2. Organolead speciation**

Trimethyllead chloride (TML) and triethyllead chloride (TEL) were obtained from Alfa, Karlsruhe, Germany. Inorganic lead solution, Spectrosol grade, was obtained from BDH, Poole, U.K. Stock solutions ( $1000 \mu\text{g.g}^{-1}$ ) were prepared in HPLC-grade methanol (Rathburn Chemicals Ltd., Peebleshire, U.K.) and stored in the dark at  $4^{\circ}\text{C}$ .

Sodium acetate used as buffer was purchased from Fisons Analytical Reagent, Loughborough, U.K. Glacial acetic acid, as above, was obtained from BDH, Poole, U.K. Sodium 1-pentanesulphonic acid (SPSA) was purchased from Aldrich Chemical Co. Ltd., Fluka brand, Dorset, U.K.

Standard reference materials NIST 981 (common lead isotopic standard), NIST 982 (equal-atom lead isotopic standard) and NIST 983 (radiogenic lead isotopic standard enriched in  $^{206}\text{Pb}$ ) were obtained from the National Institute of Standards and Technology, Maryland, U.S.A.

Milli-Q deionised water (Millipore, Massachusetts, U.S.A.) or equivalent was used throughout the study.

## **2.2. INSTRUMENTATION**

The high performance liquid chromatography was carried out using an inert gradient pump (Varian Ltd., Model 9010, Warrington, U.K.). A 200  $\mu$ l injection PEEK loop was fitted to a chemically inert injection valve (Cheminert Model C1 valve, Valco Instruments Co. Inc., Texas, U.S.A.).

Initial studies were conducted on a PlasmaQuad 2 from Fisons Instruments Elemental, Cheshire, U.K. Most of the study was developed on an inductively coupled plasma-mass spectrometer PlasmaQuad 2+ (Fisons Instruments Elemental). The typical operating conditions used are shown in Table 2.1. The nickel or platinum sampler and skimmer cones (Fisons Instruments Elemental, Cheshire, U.K.) had orifices of 1.0 and 0.7 mm, respectively. Using a gas blender (Signal Instruments, Surrey, U.K.) an addition of oxygen was made to the argon nebuliser gas to avoid carbon deposition on the cones. The nebulisers employed were an Ebdon nebuliser (PS Analytical, Kent, U.K.) and a Meinhard nebuliser type A (J.A. Meinhard Associates Inc., California, U.S.A.). The spray chamber was cooled down to  $-10^{\circ}\text{C}$  with a recirculating chiller (Endocal RTE-100, Neslab Instruments, Inc., New Hampshire, U.S.A.). The ion lens settings were optimized every day to give the best performance.

### **2.2.1. Organotin speciation**

Reverse phase chromatography was evaluated with a PLRP-S column in-house packed (150x4.1 mm), 10  $\mu$ m particle size (Polymer Laboratories, Shropshire, U.K.).

The strong cation exchange analytical columns (250x4.6 mm) were packed in-house with 10  $\mu\text{m}$  Partisil SCX-10 (Thames Chromatography, Berkshire, U.K.).

A Dionex OmniPac PCX-500 column (250x4.0 mm) from Dionex, Surrey, U.K., was employed for the mixed properties chromatography.

### 2.2.2. Organolead speciation

Reverse phase ion-pairing chromatography was performed with an in-house packed Hypersil ODS column (250x4.2 mm), 5  $\mu\text{m}$  particle size, from Jones Chromatography, Mid Glamorgan, U.K.

*Table 2.1. Typical operating conditions for the ICP-MS system*

Outer gas flow (l.min <sup>-1</sup> )	15
Intermediate gas flow (l.min <sup>-1</sup> )	0.75-1.00
Nebuliser gas flow (l.min <sup>-1</sup> )	0.85
[Sheath gas flow (l.min <sup>-1</sup> )]	0.200
Oxygen bleed (%)	1.5-2.0
Forward power (W)	1500
Nebuliser	Meinhard
Spray chamber	Various



***3. HIGH PERFORMANCE  
LIQUID  
CHROMATOGRAPHIC  
SEPARATION OF THE  
ANALYTES***

### **3. HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION OF THE ANALYTES**

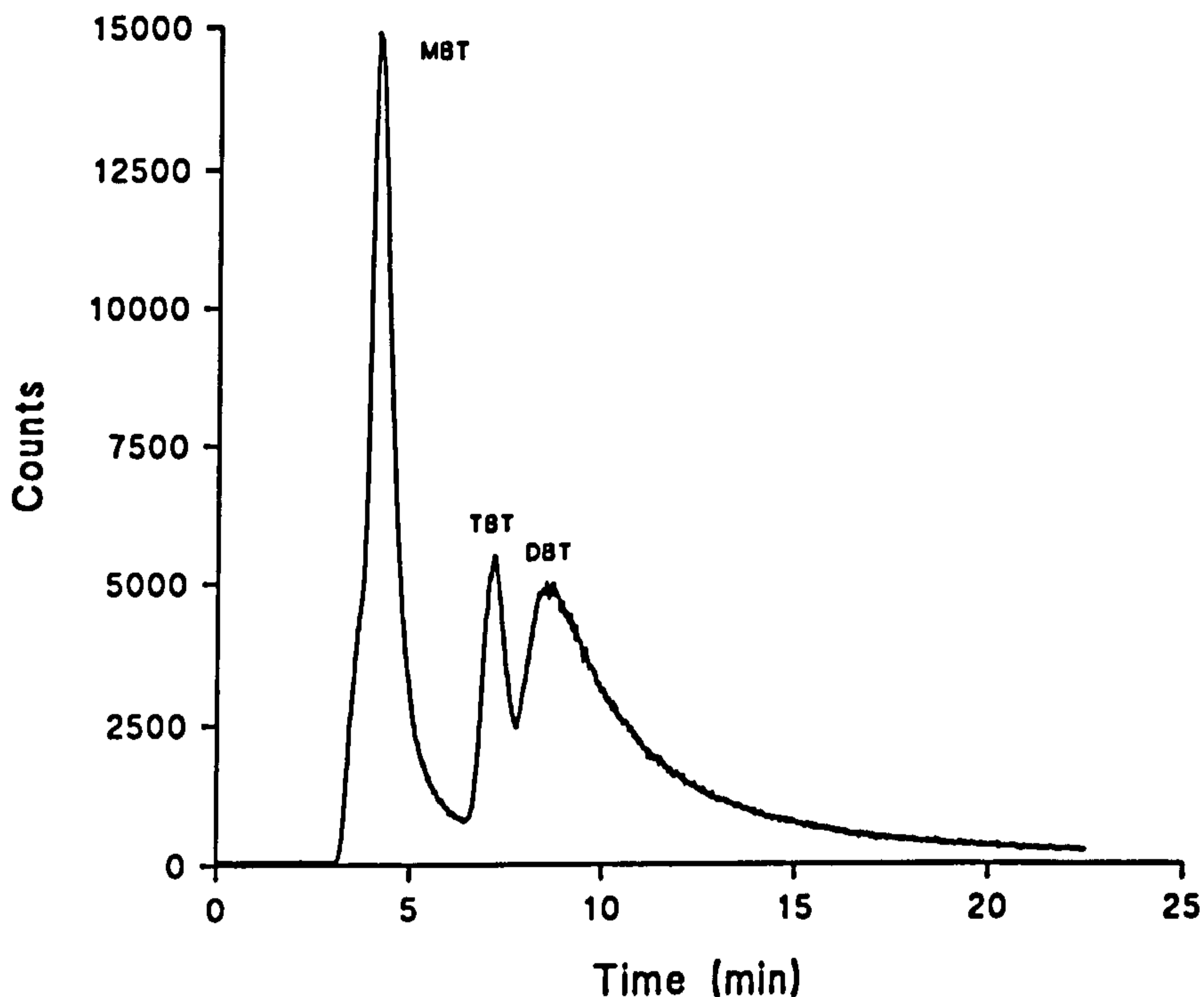
#### **3.1. ORGANOTIN COMPOUNDS**

##### **3.1.1. Reverse phase chromatography**

Separation of the different organotin species was first evaluated using a reverse phase column. There are several methods reported in the literature to separate these compounds using this form of chromatography<sup>(73-77,84,88,92,97,102,106-108)</sup>.

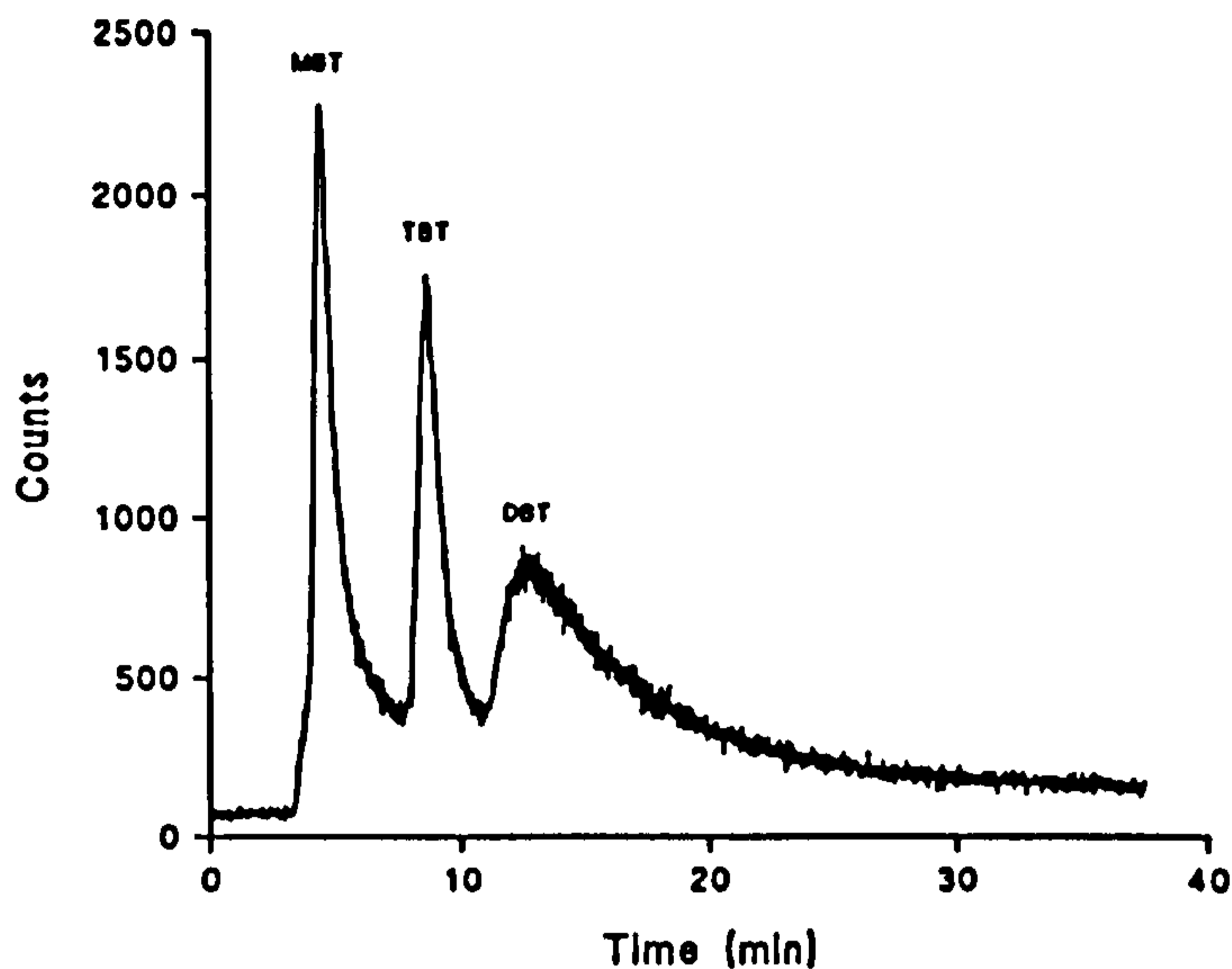
The first attempts were made using various polar mobile phases with different concentrations of methanol and buffer, acetic acid-ammonium acetate at pH 6.5. The compositions of the mobile phase ranged from 98% methanol and 2% buffer, down to 90% methanol, 2% buffer and 8% water. Using the 98% methanol, 2% buffer mobile phase system, the first species to elute was MBT, followed by TBT, and with DBT eluting last. The inorganic tin was thought to be coeluting with the MBT, a small shoulder being observed on the peak profile. A typical chromatogram obtained using this composition of mobile phase is shown in Figure 3.1.

Decreasing the methanol content of the mobile phase to 90% did not help the resolution of MBT and inorganic tin, both again coeluting as a single peak. The elution of the other species was however retarded, with a degradation in peak shape.



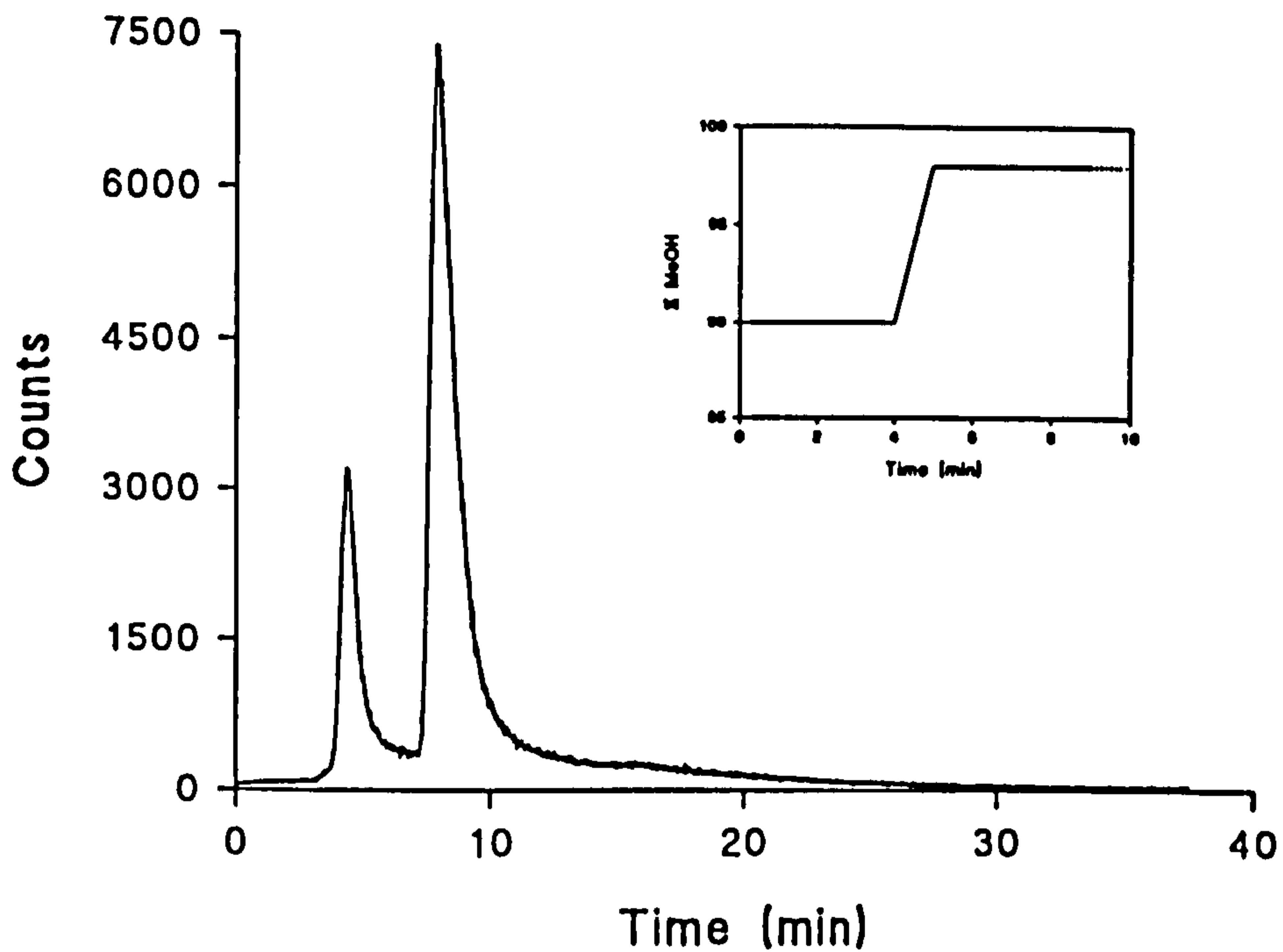
**Figure 3.1.** *Reverse phase chromatogram of inorganic tin, MBT, DBT and TBT. Mobile phase: 98% MeOH, 2% acetate buffer, pH=6.5*

A range of intermediate mobile phases with a methanol:water ratio between 90-98% were also investigated. The best performance was obtained with 93-95% methanol. However the differences between these and the chromatogram in Figure 3.1 are small, the TBT and DBT being slightly better resolved although the retention times were longer (Figure 3.2). The use of gradient elution was also evaluated in order to separate the inorganic tin and monobutyltin species. Various compositions of the mobile phase were used employing a range of flow rates and gradient steps. Examples of the chromatograms obtained are shown in Figures 3.3 and 3.4. As can be seen, little success was achieved in the separation of the different species employing the use of gradients.

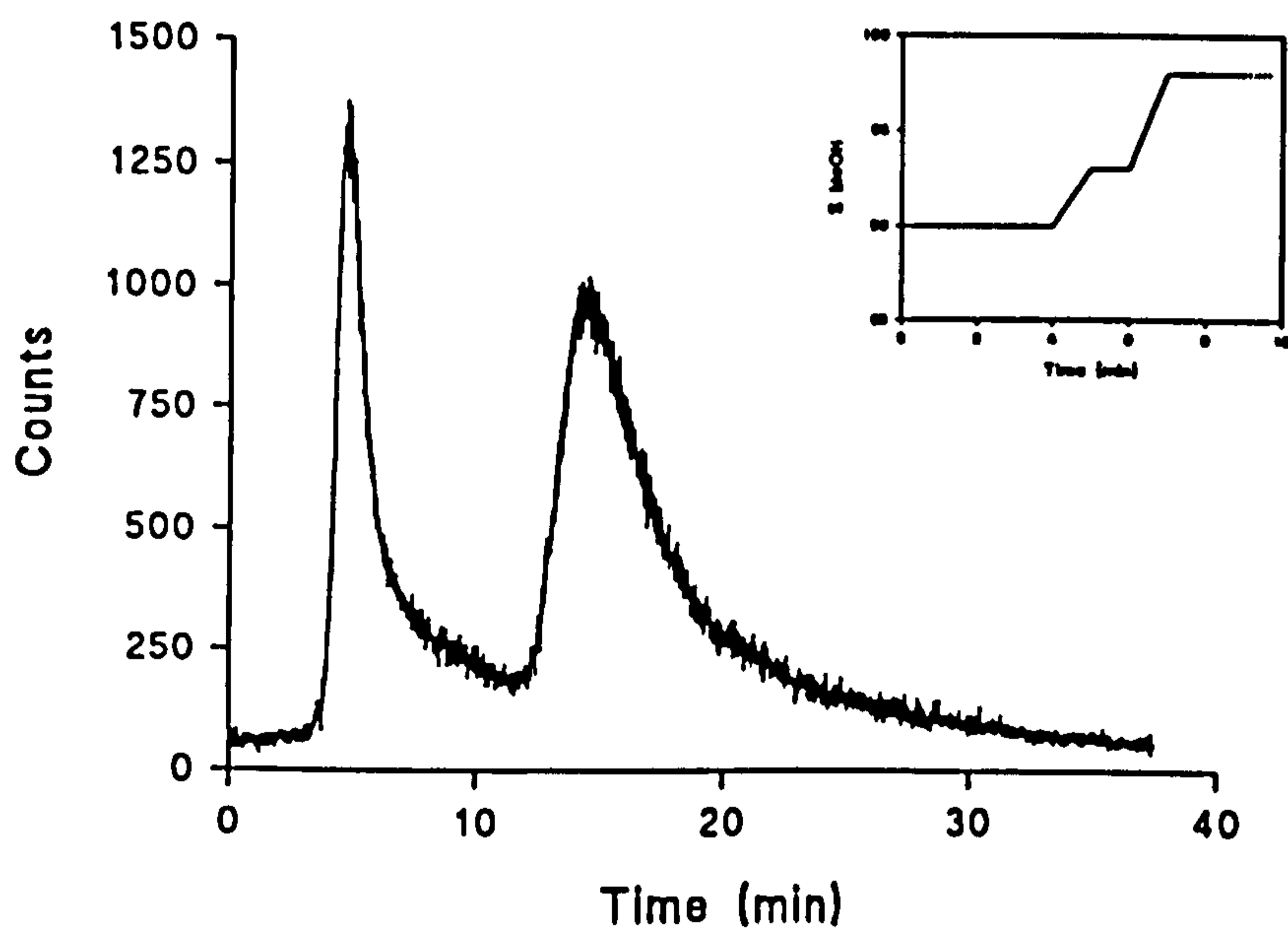


**Figure 3.2.** Reverse phase chromatogram of inorganic tin, MBT, DBT and TBT. Mobile phase: 95% MeOH, 5% acetate buffer, pH=6.5

One of the most common problems in speciation studies on organotin compounds is the low extraction efficiency of the species from the original sample. Several workers have proposed the use of a complexing agent at some stage of the extraction procedure to improve the efficiency. Tropolone is one of the best known of these complexing agents for organotin species, which has been used in several methods<sup>(50,88,101,103,106,108,109,111)</sup>. In order to study the effect of tropolone on the chromatography, a range of organotin calibrants were complexed with different amounts of tropolone. Two experiments were carried out with these calibrants; the first involved a study of the effect of adding the complexing agent to the sample and the second included the tropolone as a component of the mobile phase. Two concentrations of tropolone in two different mobile phases were tried, *i.e.* 0.0050% and 0.0075% (m/v) tropolone in mobile phases of 85 and 95% methanol/water. In both cases a single peak was obtained with all species coeluting.



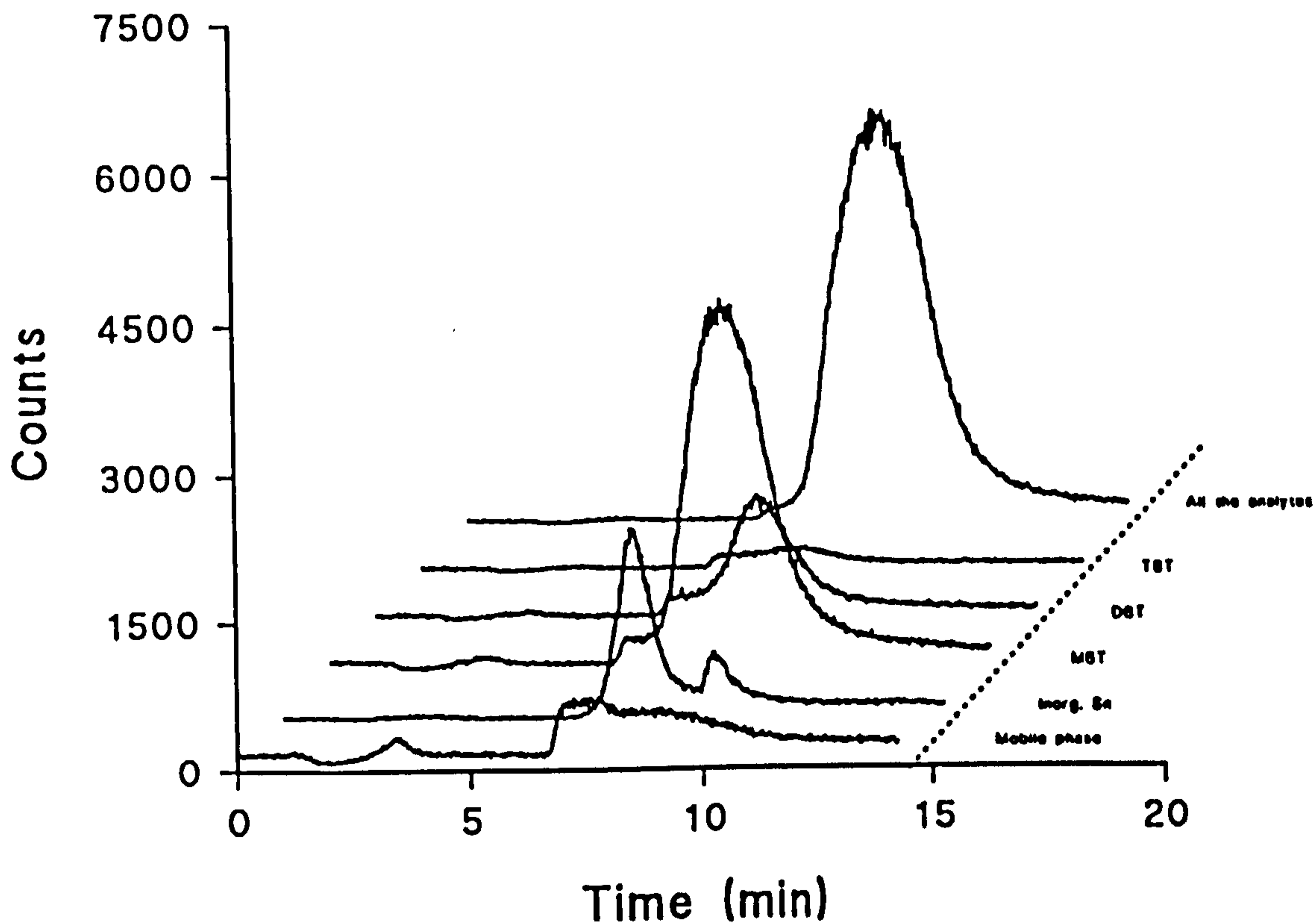
**Figure 3.3.** Reverse phase chromatogram of inorganic tin, MBT, DBT and TBT. Mobile phase: MeOH/Acetate buffer, pH=6.5. Gradient elution profile in inset



**Figure 3.4.** Reverse phase chromatogram of inorganic tin, MBT, DBT and TBT. Mobile phase: MeOH/Acetate buffer, pH=6.5. Gradient elution profile in inset



The same methanol:water mobile phases (without tropolone addition) were then used to investigate the effect of adding the tropolone (0.005 and 0.01%, m/v) to the calibrant solution only rather than also to the mobile phase. In the case of the 95% methanol with 0.005% of tropolone as complexing agent in the calibrant, again all the peaks coeluted as shown in Figure 3.5. The same was found for the same mobile phase and 0.01% of tropolone in the calibrant.



*Figure 3.5. Reverse phase chromatograms of inorganic tin, MBT, DBT and TBT complexed with 0.005% (m/v) of tropolone. Mobile phase: 95% MeOH, 5% acetate buffer, pH=6.5*

For the mobile phase of 85% methanol with 0.005 and 0.01% (m/v) of tropolone in the calibrants, the chromatograms were similar. The chromatogram obtained for the inorganic tin species showed three peaks, the first being the solvent peak. The other two may correspond to two different eluted species, one of them inorganic tin non-complexed, the second one a more polar complexed species, *i.e.* Sn(trop)<sub>n</sub> complex. This species was found to elute for both concentrations of tropolone. The chromatograms obtained are shown in Figures 3.6 and 3.7.

An alternative mobile phase system was also evaluated. The use of tetrahydrofuran, water, acetic acid and tropolone (54, 38, 8, 0.2% (m/v), respectively) has been reported for the separation of several organotin species: TPhT, DPhT, TBT, DBT, MBT, TMT, DMT, MMT<sup>(88)</sup>, using a TSK gel ODS-8TM column. The authors also suggested that the separation could be achieved with methanol, 54%, in the mobile phase instead of THF. Since this option offers advantages when the chromatography is coupled to ICP-MS detection (inductively coupled plasmas only tolerate low amounts of THF without desolvation), and additionally offers the possibility of extending the method to the separation of inorganic tin and other species of interest, the substitution of methanol for THF was evaluated. Inorganic tin, MBT, DBT and TBT were injected both as single calibrants and as a mixed calibrant. The suggested flow rate in the original paper could not be used, due to very high backpressure on the column, so it was reduced until a normal backpressure ( $\approx 14 \times 10^6$  Pa) was achieved. Unfortunately, the chromatograms obtained were again disappointing with all the peaks coeluting at or near the solvent front. The percentage of methanol in the mobile phase was both increased in steps

up to 90% and decreased to 30%. In all cases the organotin species coeluted, and no improvement was obtained.

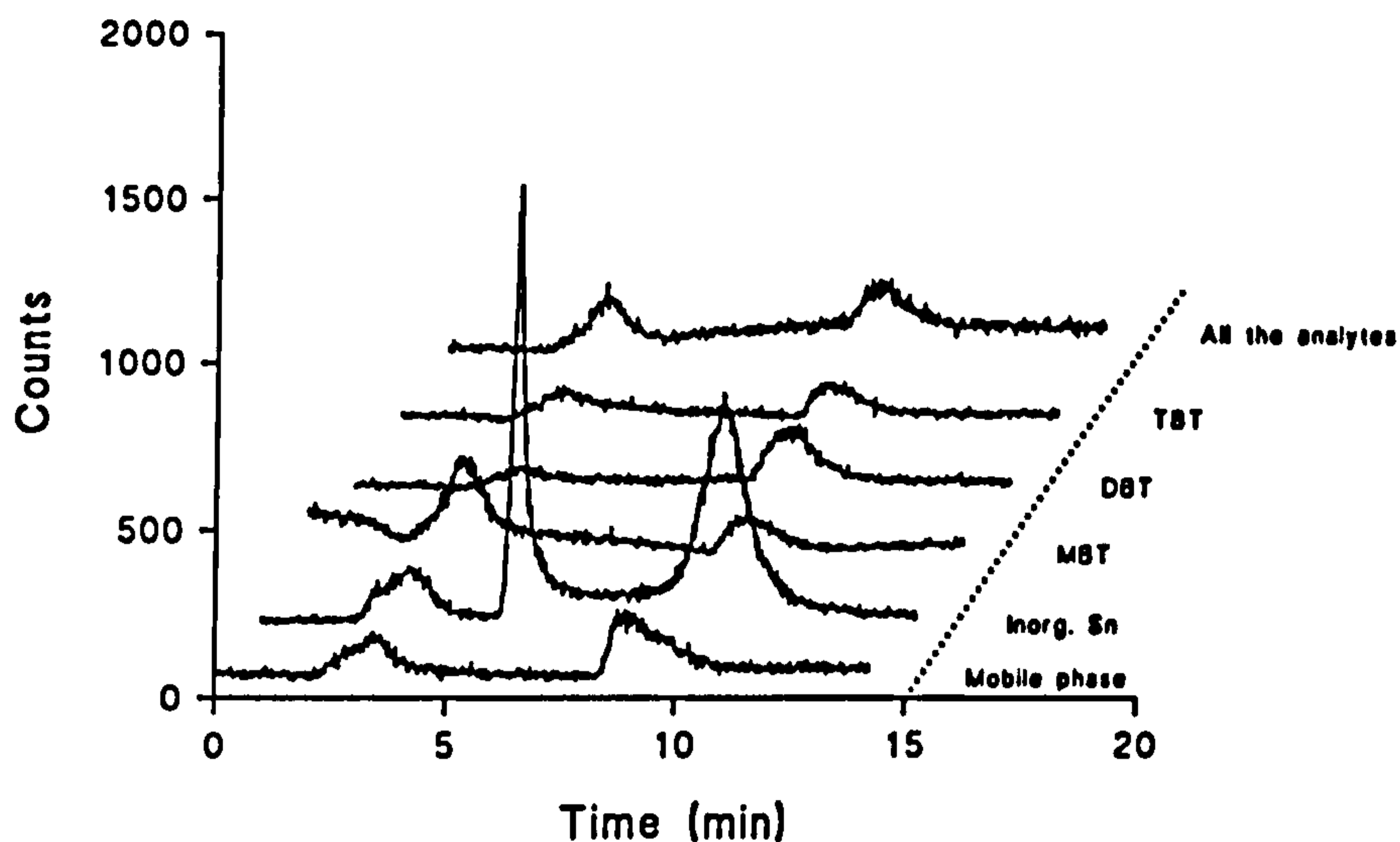


Figure 3.6. Reverse phase chromatograms of inorganic tin, MBT, DBT and TBT complexed with 0.005% (m/v) of tropolone. Mobile phase: 85% MeOH, 15% acetate buffer, pH=6.5

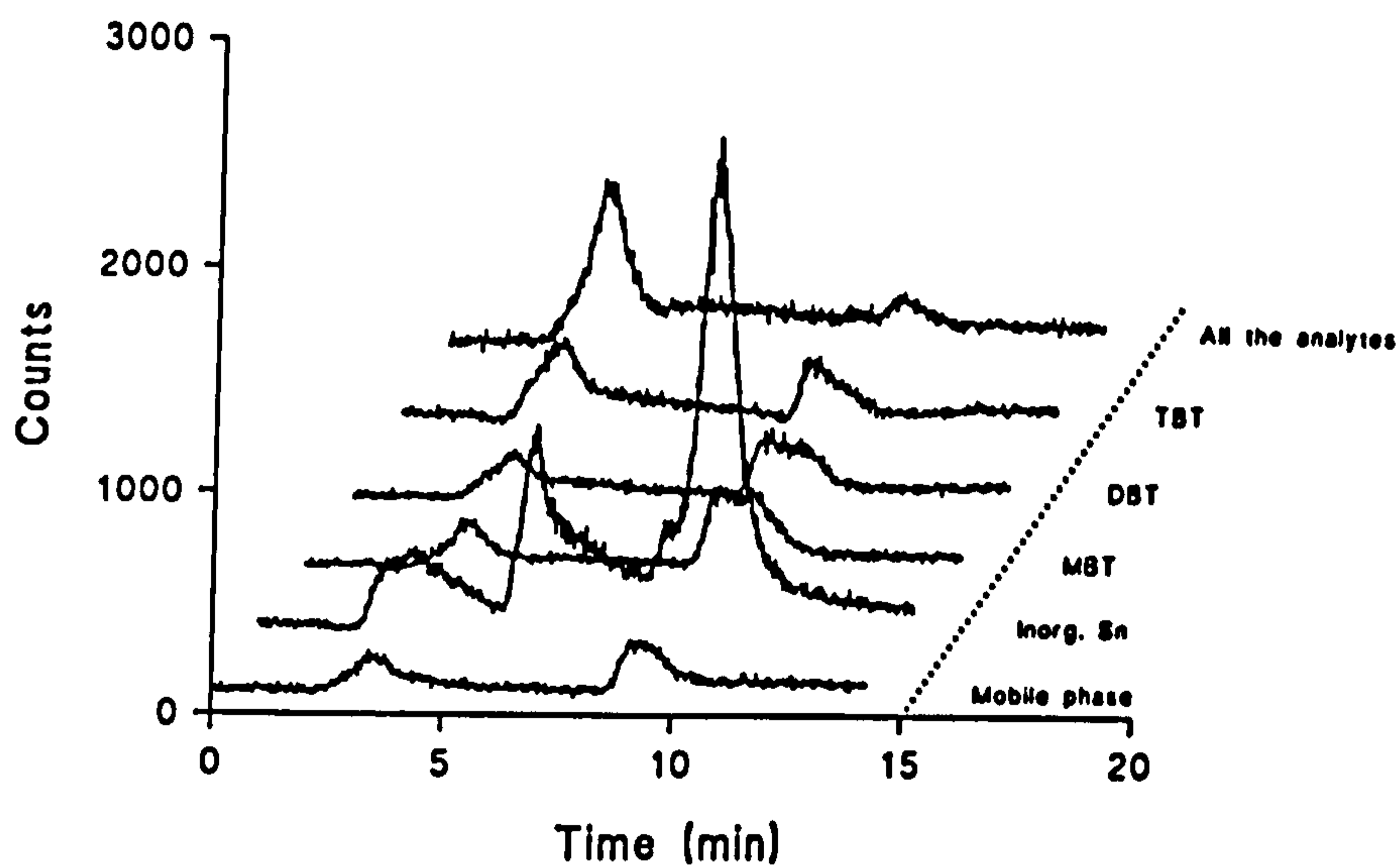
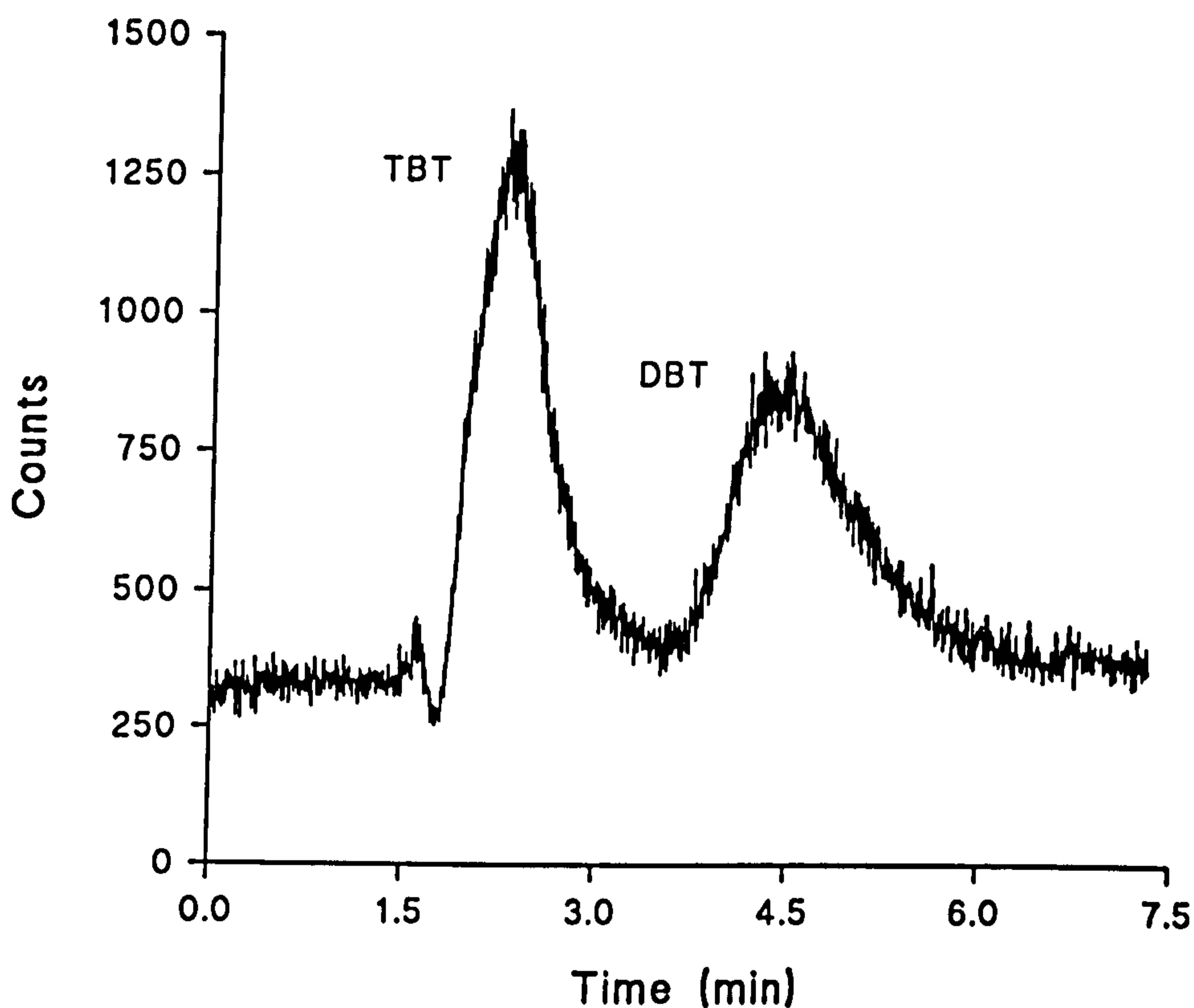


Figure 3.7. Reverse phase chromatograms of inorganic tin, MBT, DBT and TBT complexed with 0.01% (m/v) of tropolone. Mobile phase: 85% MeOH, 15% acetate buffer, pH=6.5

### 3.1.2. Ion-exchange chromatography

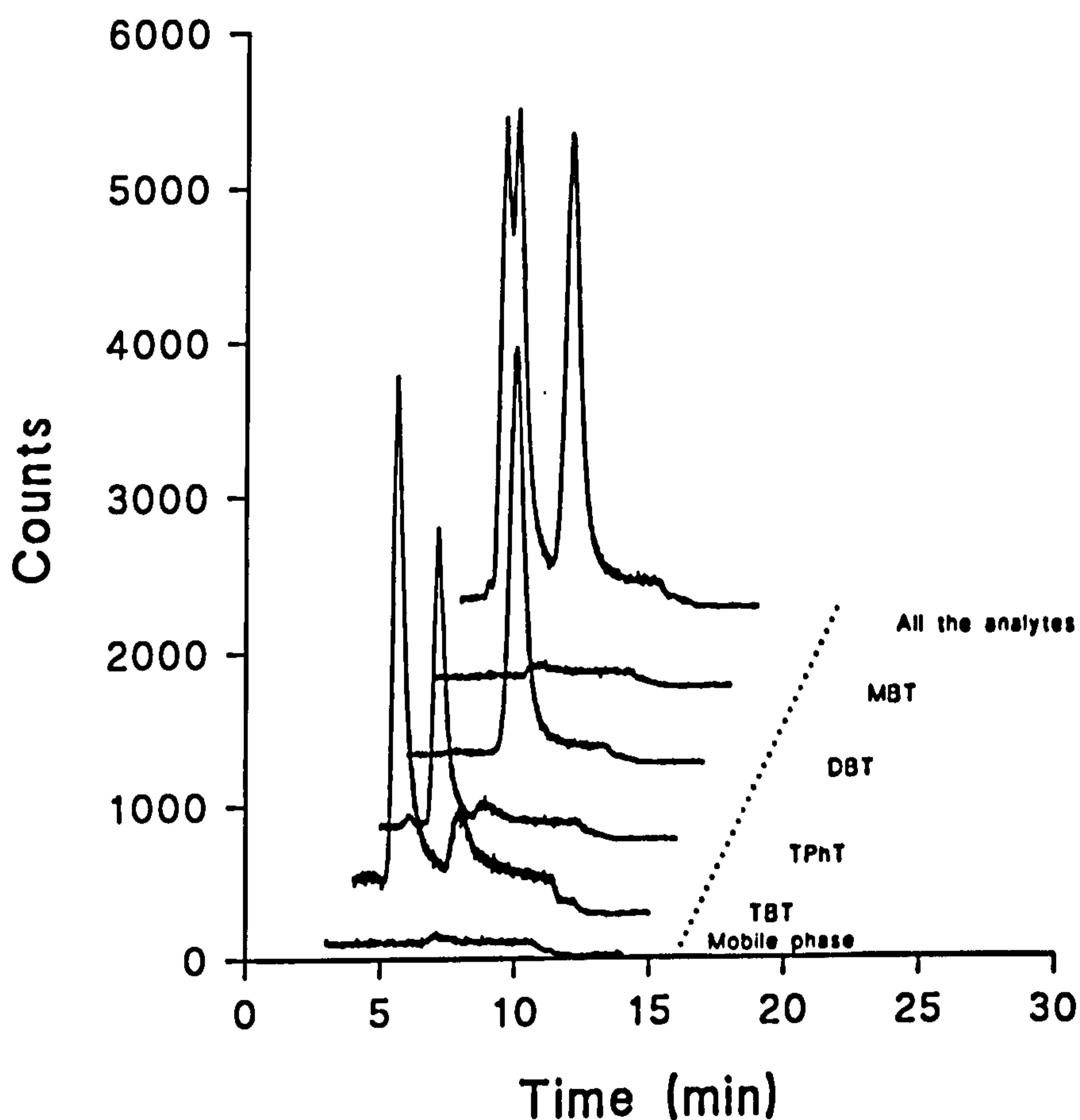
Following the disappointing results obtained using reverse phase chromatography, ion-exchange chromatography was evaluated. The separation of TBT and DBT with isocratic elution on a Partisil-10 SCX column had been previously reported<sup>(83,87,89,91,95,99,113)</sup>. An example can be seen in Figure 3.8. MBT and TPhT were included into the analytes of interest and the separation of the four species attempted.



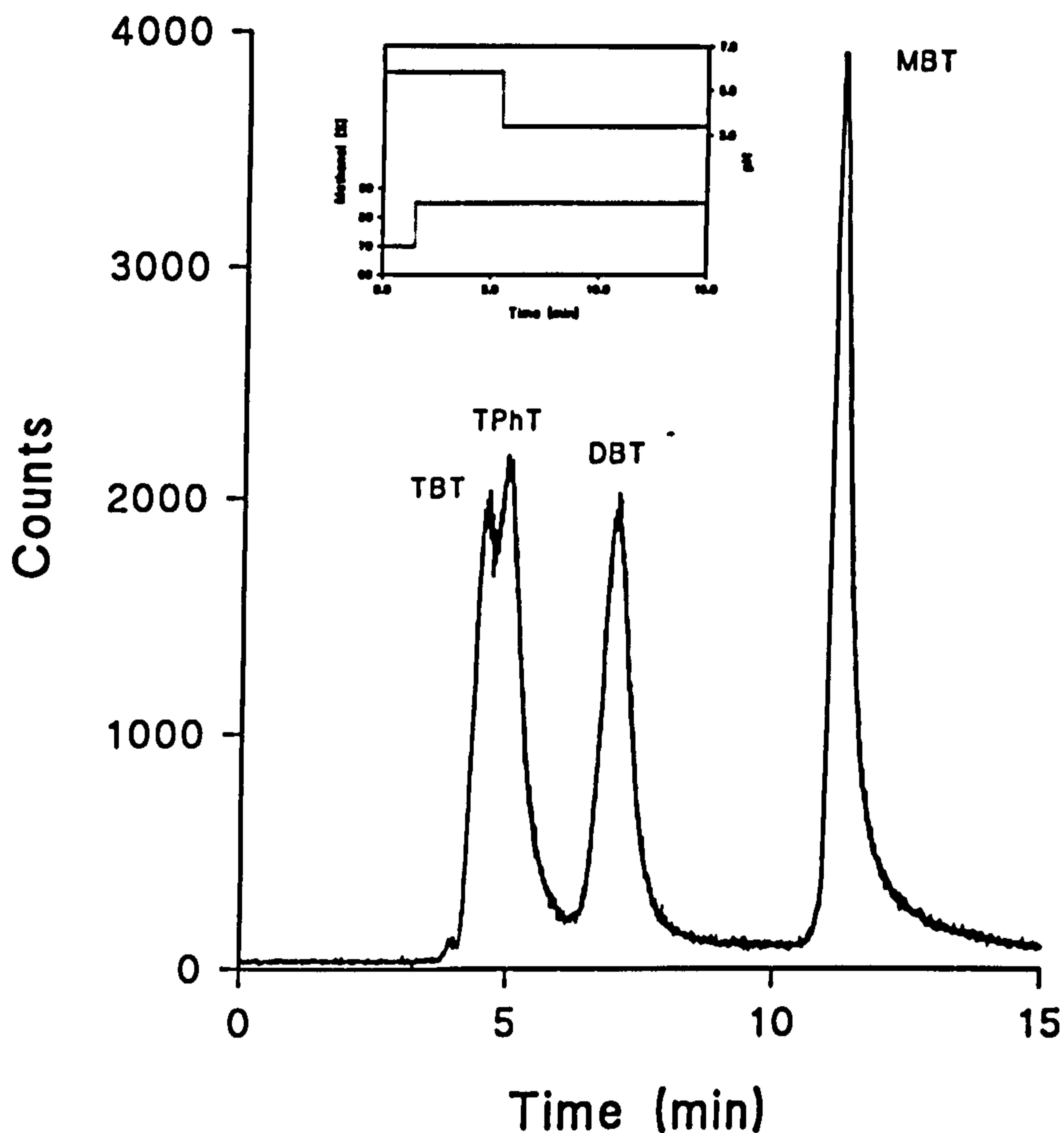
*Figure 3.8. Ion-exchange chromatogram of TBT and DBT. Isocratic elution. Mobile phase: 70% MeOH, 30% citrate buffer, pH=5.8*

One of the first refinements to be made was to reduce the retention times of the later peaks in the series and to this end a step gradient elution was applied,

increasing the amount of methanol from 70% up to 85%. This resulted in an improvement in the elution profile of DBT, but did not significantly effect the elution of MBT (Figure 3.9). Previous work<sup>(96)</sup> suggested a decrease in the pH of the eluent to overcome this problem for MBT. The results obtained can be seen in Figure 3.10. The previous study reported a large increase in the base line with the pH gradient, although as can be seen, the proposed gradient here gives only minimal background enhancement.



*Figure 3.9. Ion-exchange chromatogram of inorganic tin, TBT, TPhT, DBT and MBT. Gradient elution: 70% MeOH, 30% citrate buffer, pH=5.8 for 2.5 min; step gradient to 85% MeOH, 15% citrate buffer, pH=5.8*



**Figure 3.10.** *Ion-exchange chromatogram of inorganic tin, TBT, TPhT, DBT and MBT. Gradient elution profile in inset*

Triphenyltin was found to elute just after TBT without being truly separated although it was fully resolved from DBT. Several attempts were made to improve the separation, including modifying the percentage of methanol (from 60 up to 85%), varying the pH in the range 3.4 to 6.2 (lower or higher pH are precluded due to the type of packing material in the column), changing the concentration of the buffer in the mobile phase from 0.03 mol.l<sup>-1</sup> down to 0.015 mol.l<sup>-1</sup> and thermostating the column (0°C-60°C). Examples can be seen in Figure 3.11(a-d). However, it was



not possible to significantly enhance the separation over that shown in Figure 3.10. Increasing the buffer concentration was not evaluated due to the problems of salt loading in the mobile phase causing deposits in the injector of the torch (see section 3.1.4.3.). A system comprised of two columns (250x4.6 mm each) of the same packing material was tested. A chromatogram obtained with it is shown in Figure 3.12.

Following the method reported by Rivaro *et al.*<sup>(111)</sup> where a similar column was employed (Partisil-10 SCX) to separate TBT, TPhT, DBT and MBT with a mobile phase of methanol, ammonium acetate and tropolone, at pH=7.4, an attempt to reproduce this procedure was made. The results were disappointing. Different mobile phases were then tried, with and without tropolone in the mobile phase, but none lead to a separation of the analytes of interest.

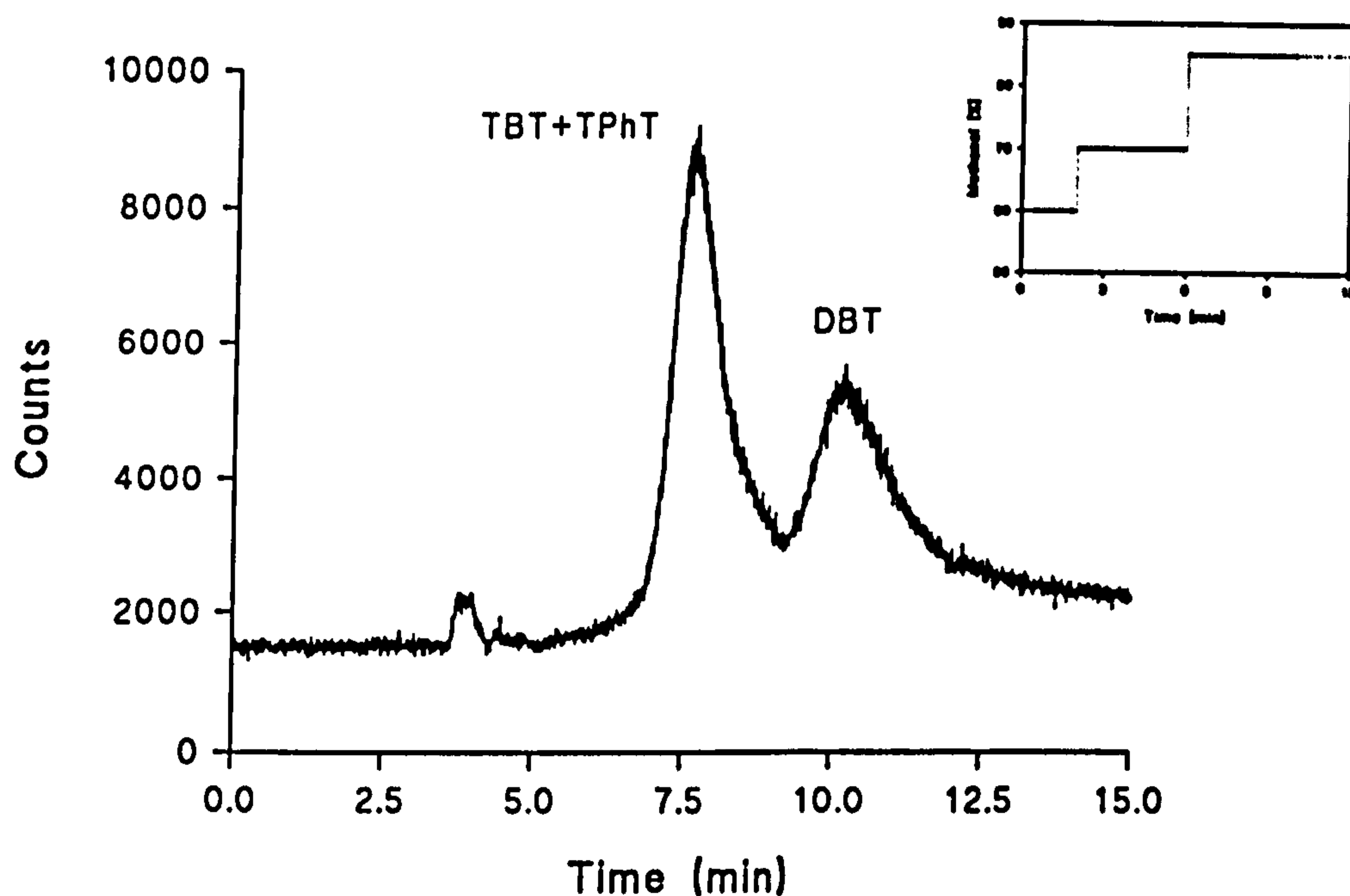
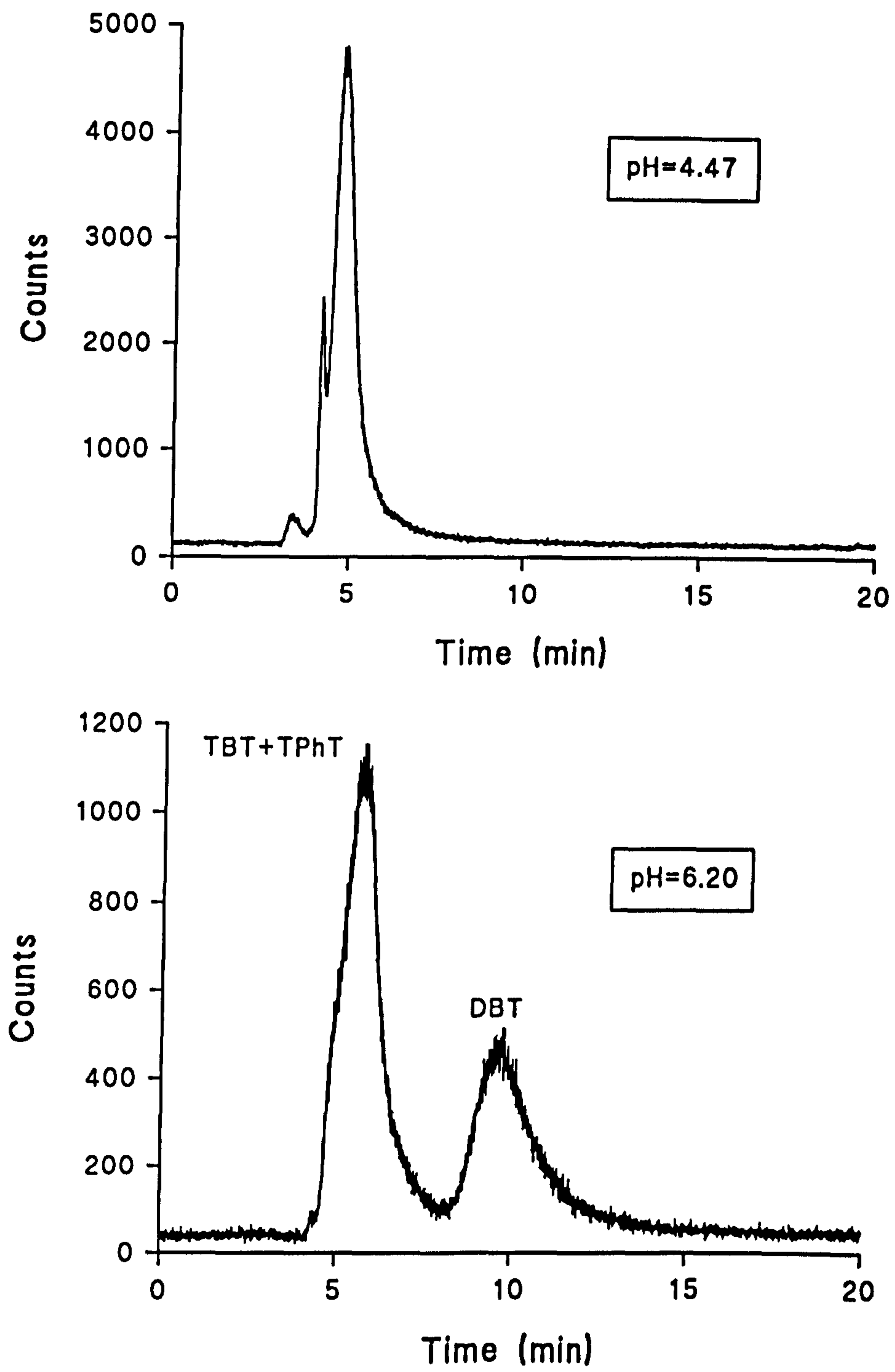
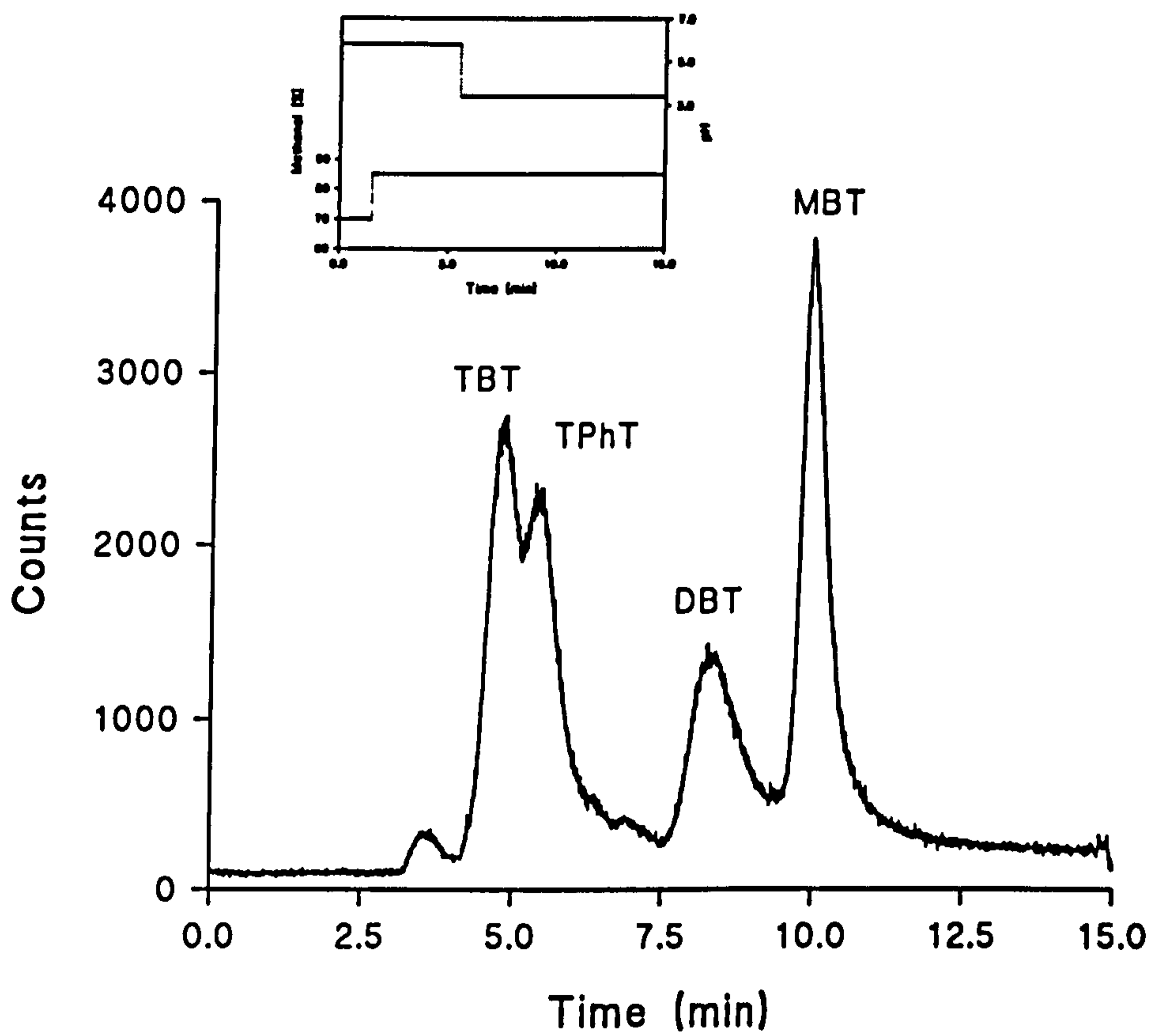


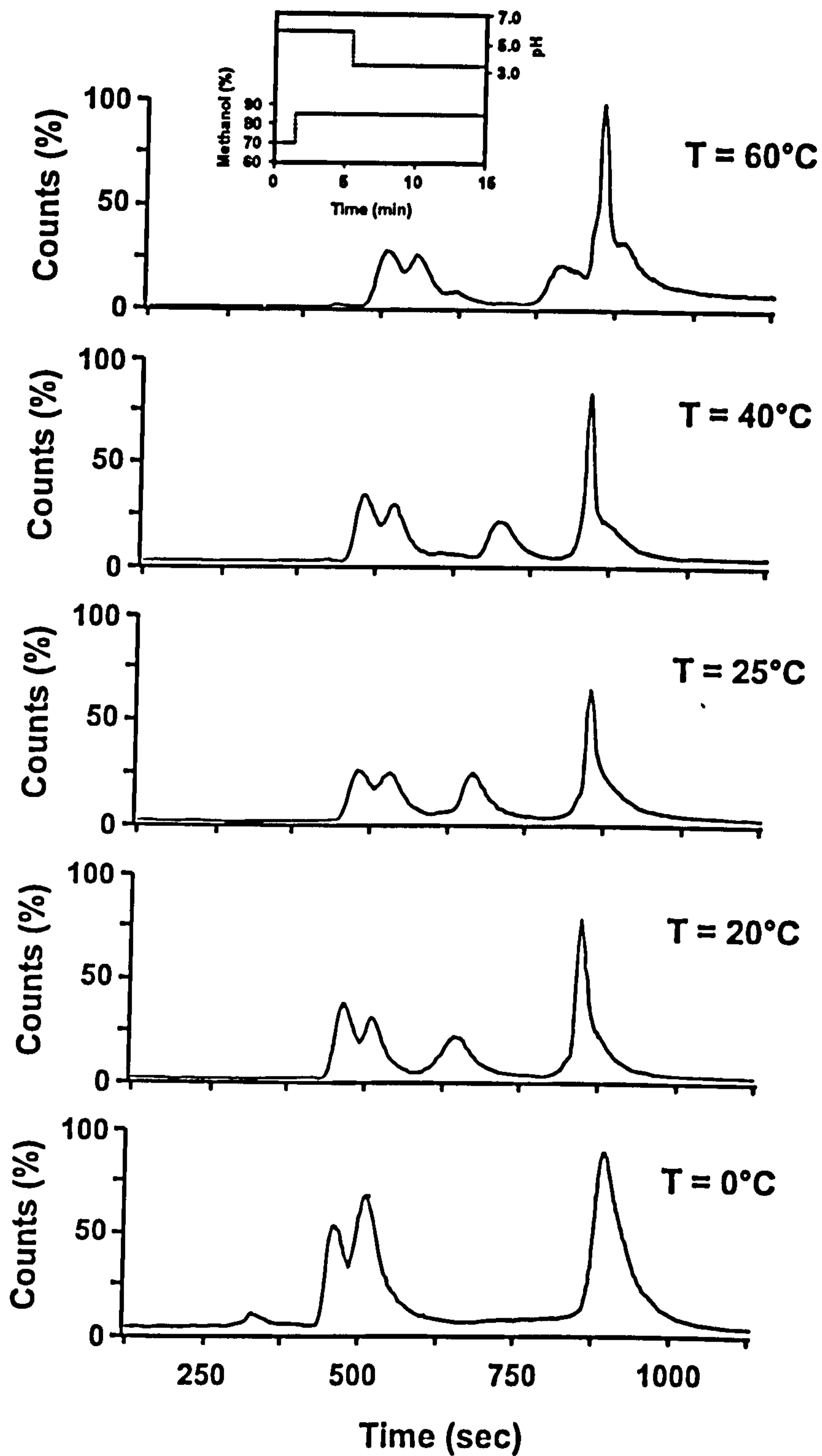
Figure 3.11(a) Ion-exchange chromatogram of TBT, TPhT, DBT and MBT.  
Gradient elution profile in inset



**Figure 3.11(b)** *Ion-exchange chromatogram of TBT, TPhT, DBT and MBT. Isocratic elution at two different pHs. Mobile phase: 70% MeOH, 30% citrate buffer*



**Figure 3.11(c)** *Ion-exchange chromatogram of TBT, TPhT, DBT and MBT. Gradient elution (profile in inset). Citrate buffer concentration: 0.015 mol.l<sup>-1</sup>*



*Figure 3.11(d) Ion-exchange chromatogram of TBT, TPhT, DBT and MBT. Gradient elution (profile in inset) at different temperatures. Mobile phase: 70% MeOH, 30% citrate buffer*

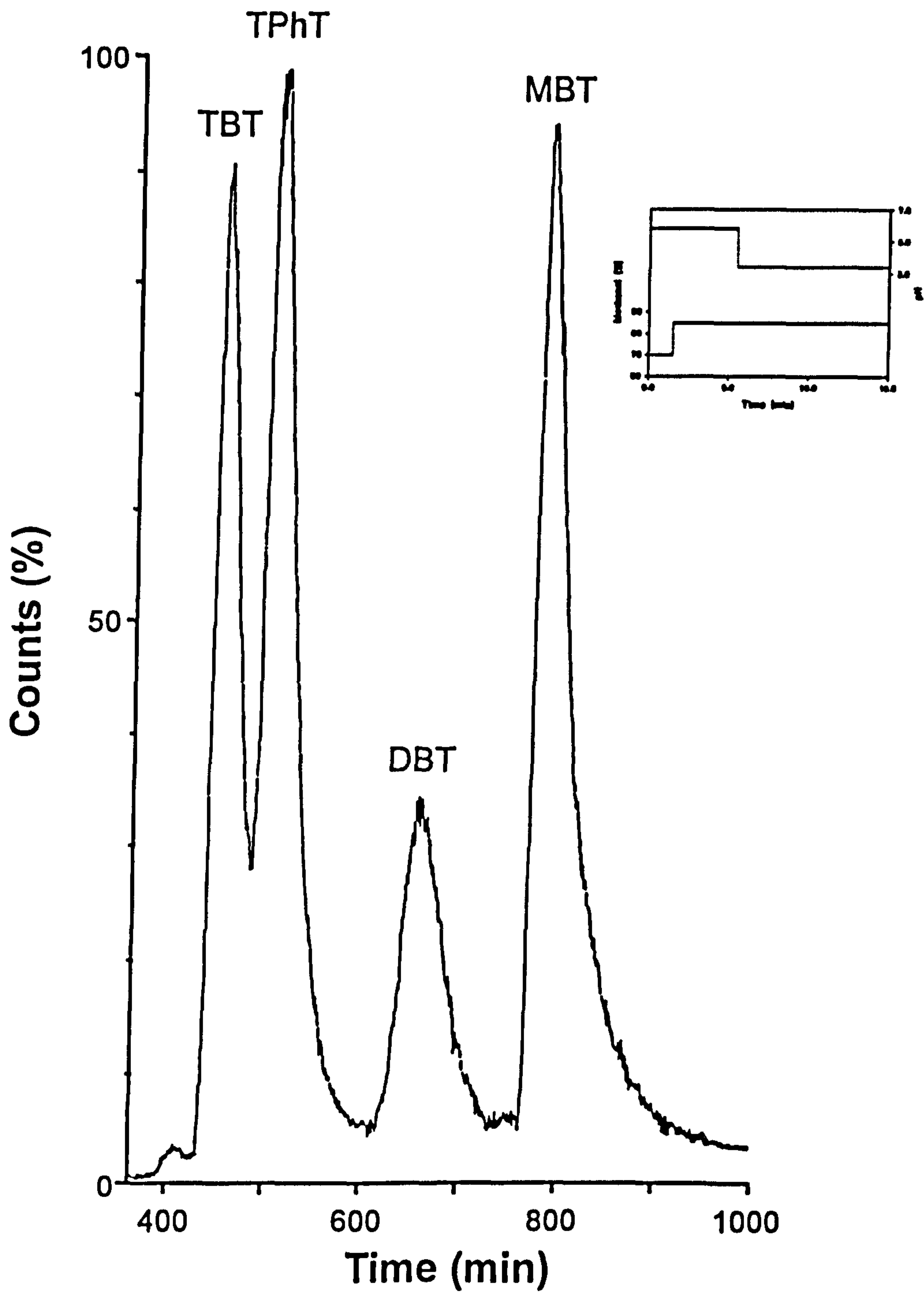
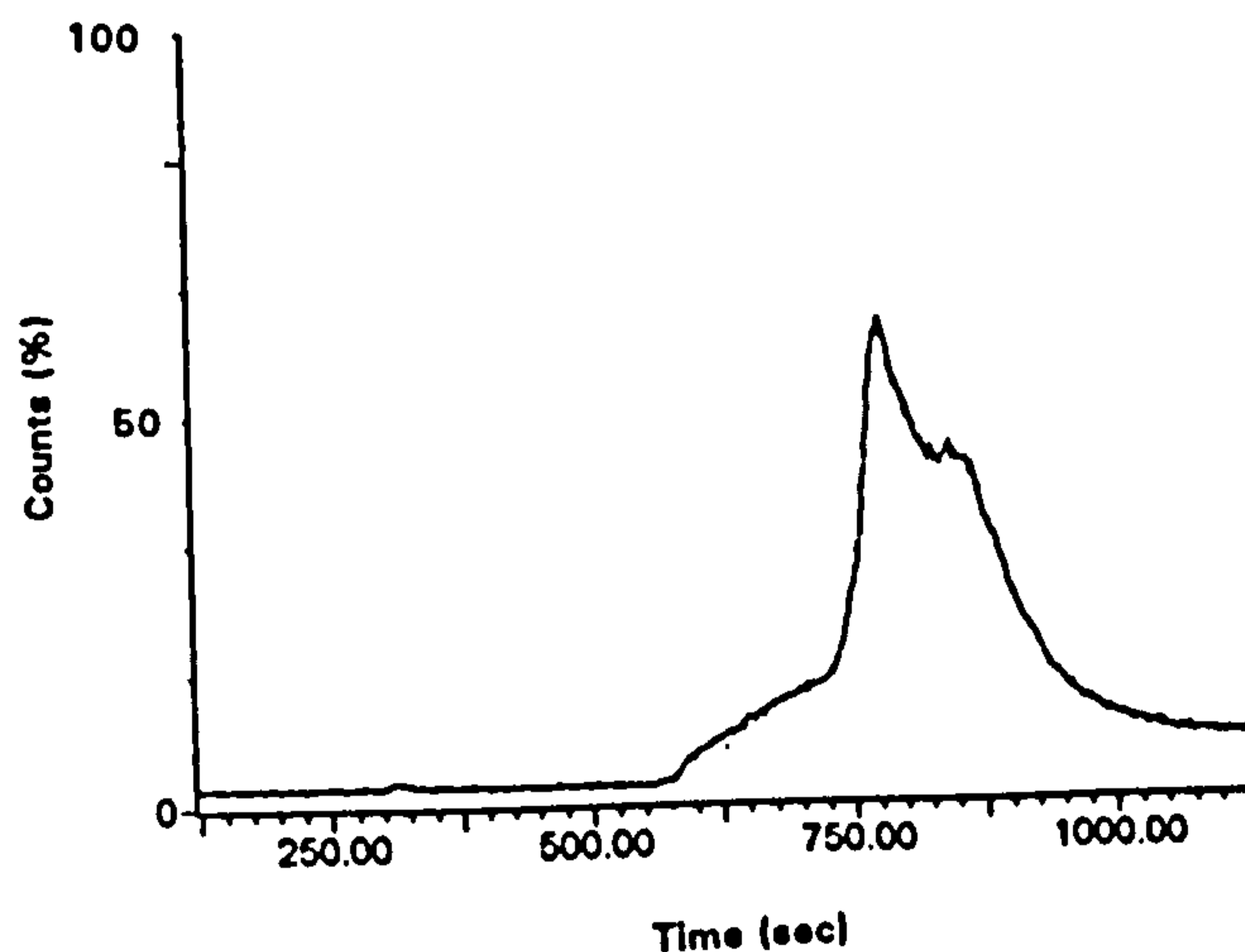


Figure 3.12. Ion-exchange chromatogram of TBT, TPhT, DBT and MBT. Gradient elution profile in inset. Two columns of 250x4.6 mm

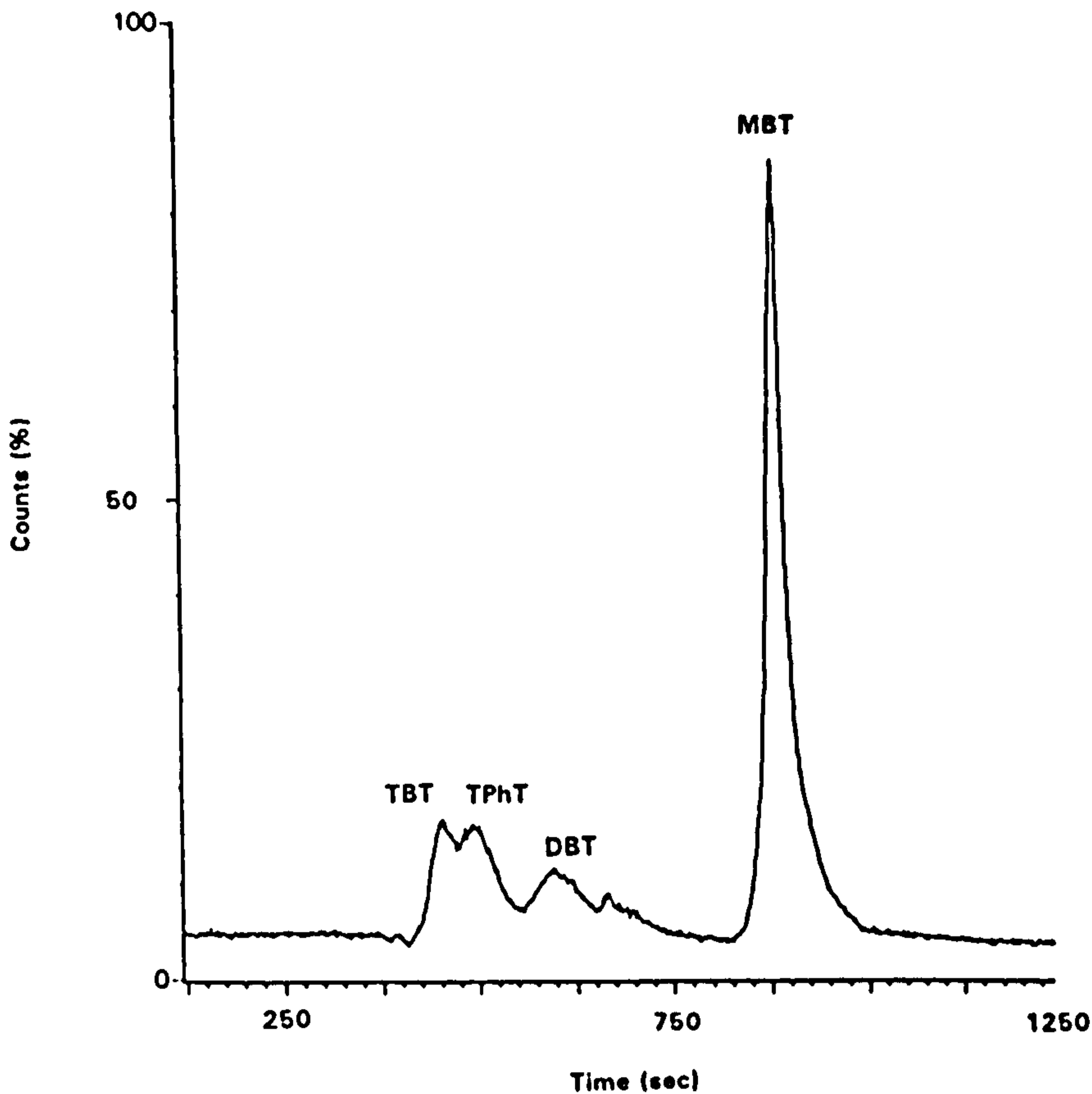
### 3.1.3. Mixed properties (reverse phase and ion-exchange) chromatography

Different systems were evaluated. The first attempts were made with an analytical column of ion-exchange properties (Partisil-10, SCX, 10  $\mu\text{m}$ ) and a guard column of reverse phase properties, silica-based (ODS) and resin-based (PRP-1, polystyrendivynylbenzene). The mobile phases employed consist of the same percentage of methanol and buffer as for the separation obtained with the ion-exchange chromatography, only trying to improve the time of analysis by using isocratic elution if possible. In neither of the cases were the results obtained better than the previous results, no advantage was obtained from using any of these dual systems. In the case of an ODS guard column and two ion-exchange columns, all the analytes coeluted under a single large peak (Figure 3.13). When a PRP-1 guard column was employed, the separation between TBT and TPhT was much degraded, as well as the separation between TPhT and DBT (Figure 3.14).



*Figure 3.13. Chromatogram of TBT, TPhT, DBT and MBT with ODS guard column and ion-exchange analytical column*

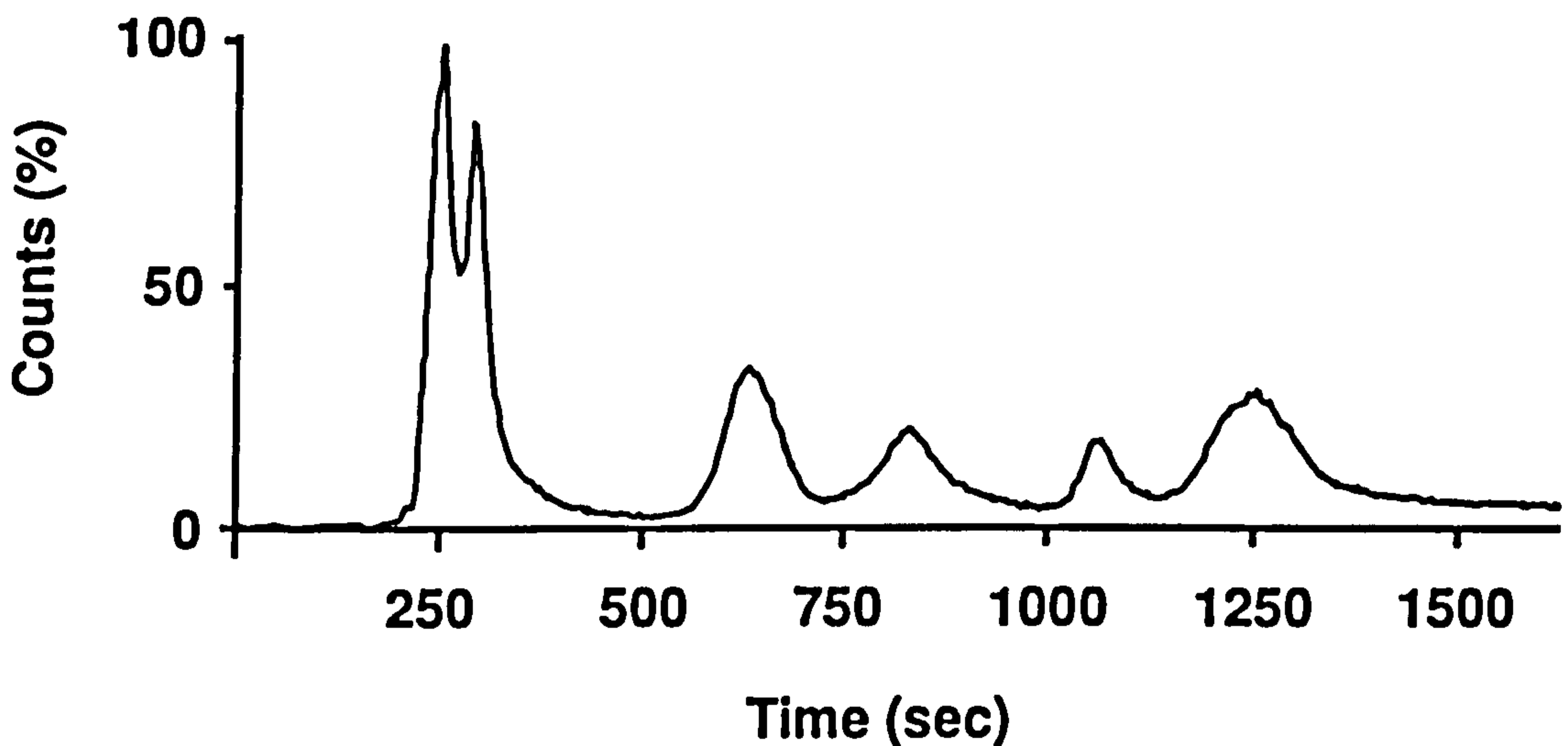




*Figure 3.14. Chromatogram of TBT, TPhT, DBT and MBT with PRP-1 guard column and ion-exchange analytical column*

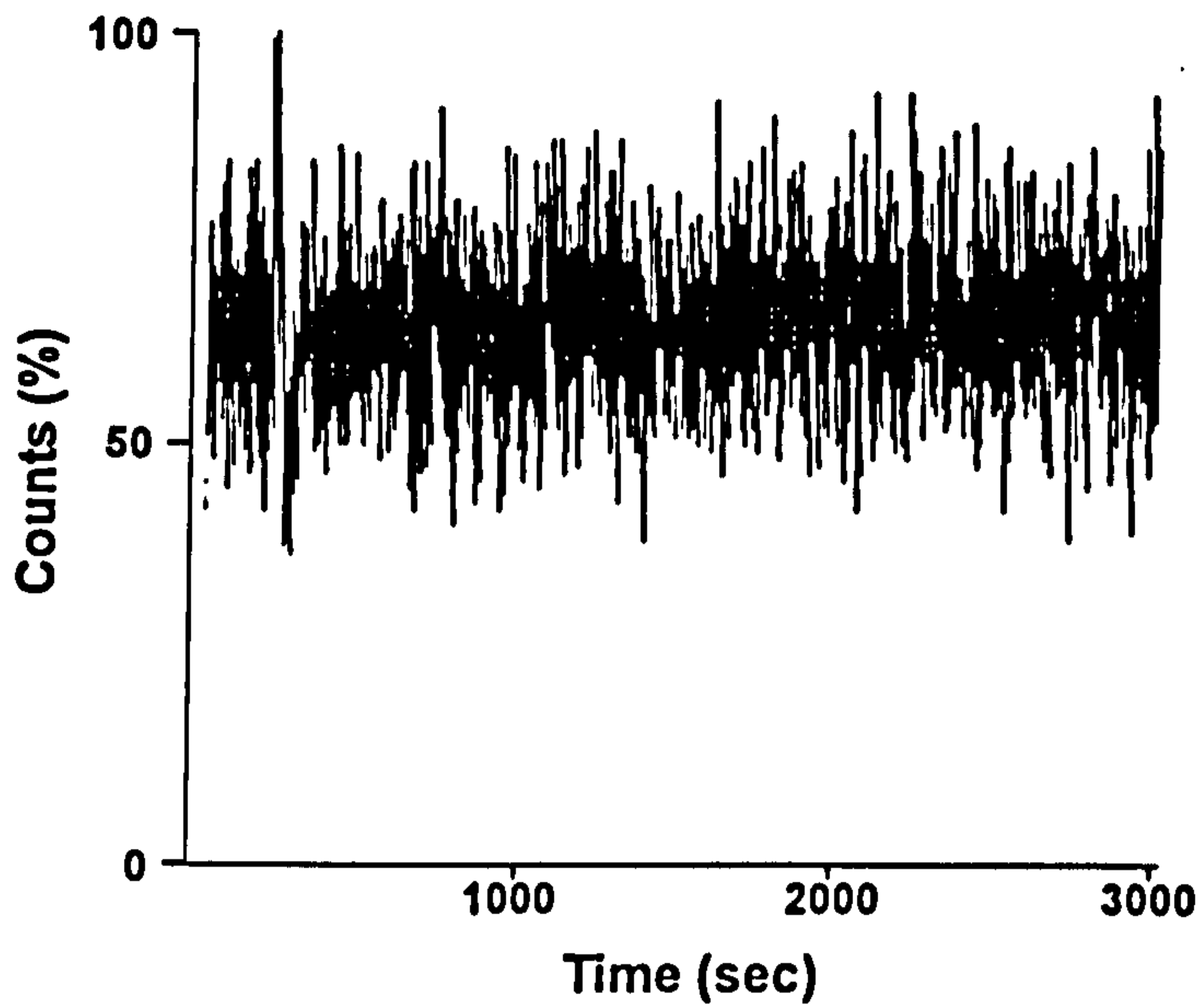
A Dionex OmniPac PCX 500 column was also evaluated. The column has cation-exchange and reverse-phase properties. Mobile phases of 65% up to 85% methanol, isocratically, with citrate buffer at pH=5.8 were first tried. Separations with different gradients and reduction in the pH of the mobile phase were also attempted. Tropolone was also included in the mobile phase (0.1%, m/v). The best results obtained were with a mobile phase of 85% MeOH+15% citrate buffer, pH=5.8, isocratic elution, with the analytes dissolved in the same mobile phase with an addition of 0.1% (m/v) tropolone. This chromatogram is shown in Figure 3.15. It can be seen that all the analytes give two peaks (the first two in the chromatogram

not being well resolved) probably due to different species of tropolone complexes, as could be the case with the reverse phase chromatography evaluated previously. Some of these complexes coelute with other species. The total run time for all the species to elute was much longer than in previous attempts. In the other elution systems the analytes either coeluted or did not elute at all.

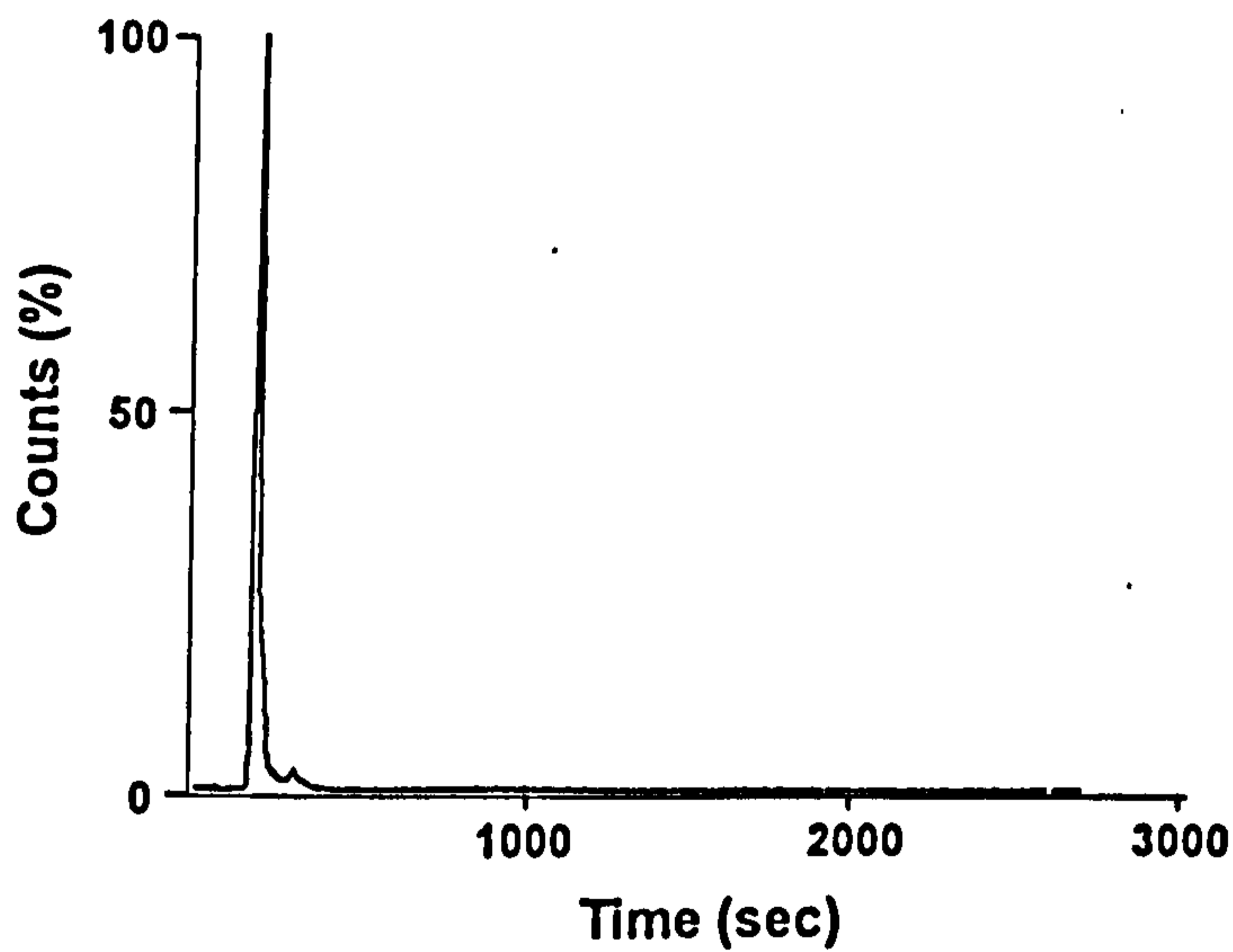


*Figure 3.15. Chromatogram of TBT, TPhT, DBT and MBT with Dionex PCX-500 mixed properties column. Isocratic elution. Mobile phase: 85% MeOH, 15% buffer citrate, 0.1% (m/v) tropolone*

Another mobile phase based on 80% methanol and ammonium acetate buffer at pH=7.4, with and without 0.1% (m/v) of tropolone, was also tried. In the case of tropolone being included in the mobile phase, the analytes did not elute, remaining firmly retained in the column (Figure 3.16). When tropolone was not included in the mobile phase, all the analytes coeluted under a single sharp peak (Figure 3.17).



**Figure 3.16.** *Chromatogram of TBT, TPhT, DBT and MBT with Dionex PCX-500 mixed properties column. Isocratic elution. Mobile phase: 80% MeOH, 20% acetate buffer, pH=7.4, 0.1% (m/v) tropolone*



**Figure 3.17.** *Chromatogram of TBT, TPhT, DBT and MBT with Dionex PCX-500 mixed properties column. Isocratic elution. Mobile phase: 80% MeOH, 20% acetate buffer, pH=7.4*

### 3.1.4. Improvements of the HPLC-ICP interface

#### 3.1.4.1. Nebuliser

Two types of nebulisers were tested: a Meinhard and a V-groove type one. The ICP-MS parameters were optimised for each system. The chromatograms obtained after the injection of a mixture of TBT, TPhT, DBT and MBT employing two Partisil-10 SCX columns, 250x4.6 mm each, with the gradient elution of MeOH and pH mentioned above, can be seen in Figure 3.18. Little difference was observed in either the resolution or sensitivity obtained with either nebuliser, although the Meinhard nebuliser is smaller and better suited to miniaturised spray chambers.

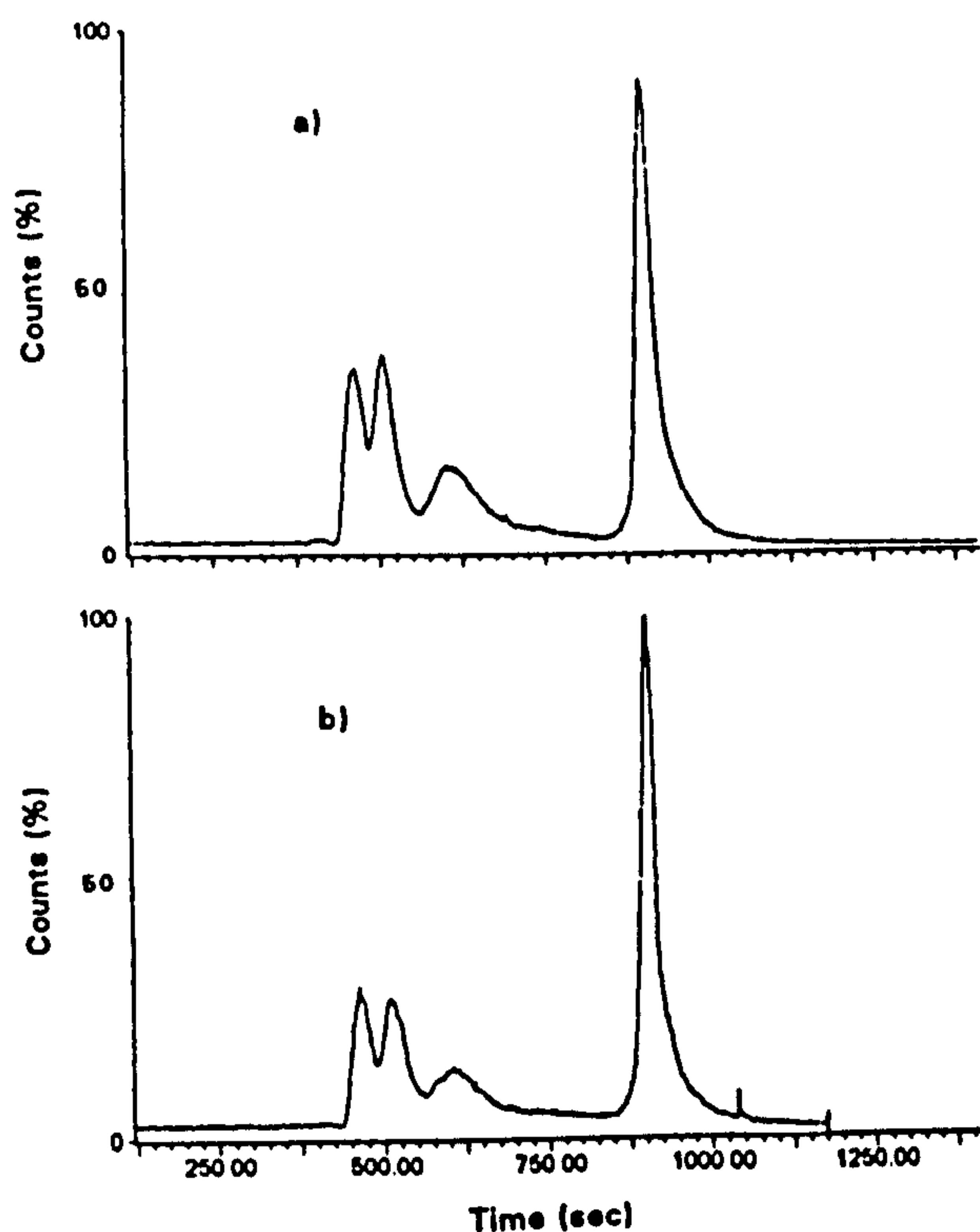


Figure 3.18. Ion-exchange chromatograms of TBT, TPhT, DBT and MBT, with two different nebulisers: (a) Meinhard, (b) V-groove

### 3.1.4.2. *Spray chamber*

The performance of a nebuliser-spray chamber system may be evaluated through its analytical performance, provided the operational parameters are fully optimised for each of the systems<sup>(183)</sup>.

Seven spray chambers (defined as A to G) were investigated in this study. They were a conventional Scott double pass (A) and a single pass (B) spray chambers, a miniaturised home-made double pass spray chamber<sup>(234)</sup> (C) and four different cyclone spray chambers. Recently, a reduced volume spray chamber of the cyclone type has been reported by Wu and Hieftje<sup>(235)</sup> for ICP-atomic emission spectrometry. The internal volume of this spray chamber was 40 ml and advantages in transport efficiency were reported. One of the cyclone type spray chambers is based on the Wu and Hieftje design but modified for ICP-MS. The first of the cyclone designs had no liquid cooling jacket (D) and the last three (E,F,G) were cooled via an integral jacket. These latter spray chambers, constructed to author's specifications, differed only in the internal volume and the shape of the indentation or "dimple". The internal volumes of the various spray chambers are given in Table 3.1, the designs and shapes of the spray chambers in Figure 3.19.

**Transport efficiency:** The silica gel trap method<sup>(188,193)</sup> was employed in all the cases to obtain the transport efficiency. An argon flow rate of 1 l.min<sup>-1</sup> was used with a sample uptake rate intake of 1 ml.min<sup>-1</sup> (achived using a peristaltic pump). The spray chamber was cooled (except types C and D) to -4°C. The experiment was conducted in triplicate. The results obtained are shown in Table 3.2.

**Table 3.1. Internal volumes of the spray chambers**

<b>Spray chamber</b>	<b>Internal volume (ml)</b>
<b>(A) Scott type double pass</b>	<b>88</b>
<b>(B) Scott type single pass</b>	<b>40</b>
<b>(C) Miniaturized double pass</b>	<b>13</b>
<b>(D) Cyclone (no jacket)</b>	<b>20</b>
<b>(E) Cyclone (jacketed)</b>	<b>40</b>
<b>(F) Cyclone (jacketed)</b>	<b>27</b>
<b>(G) Cyclone (jacketed)</b>	<b>22</b>

**Table 3.2. Transport efficiency and signal to noise ratio values for each spray chamber**

<b>Spray chamber</b>	<b>Transport Efficiency (% ± SD)</b>	<b>Signal (TBT) to noise ratio</b>
<b>(A) Scott type double pass</b>	<b>2.45 ± 0.07</b>	<b>39</b>
<b>(B) Scott type single pass</b>	<b>2.92 ± 0.20</b>	<b>59</b>
<b>(C) Miniaturized double pass</b>	<b>1.74 ± 0.05</b>	<b>41</b>
<b>(D) Cyclone (no jacket)</b>	<b>8.05 ± 0.12</b>	<b>50</b>
<b>(E) Cyclone (jacketed)</b>	<b>3.95 ± 0.07</b>	<b>56</b>
<b>(F) Cyclone (jacketed)</b>	<b>5.85 ± 0.10</b>	<b>56</b>
<b>(G) Cyclone (jacketed)</b>	<b>7.53 ± 0.04</b>	<b>108</b>



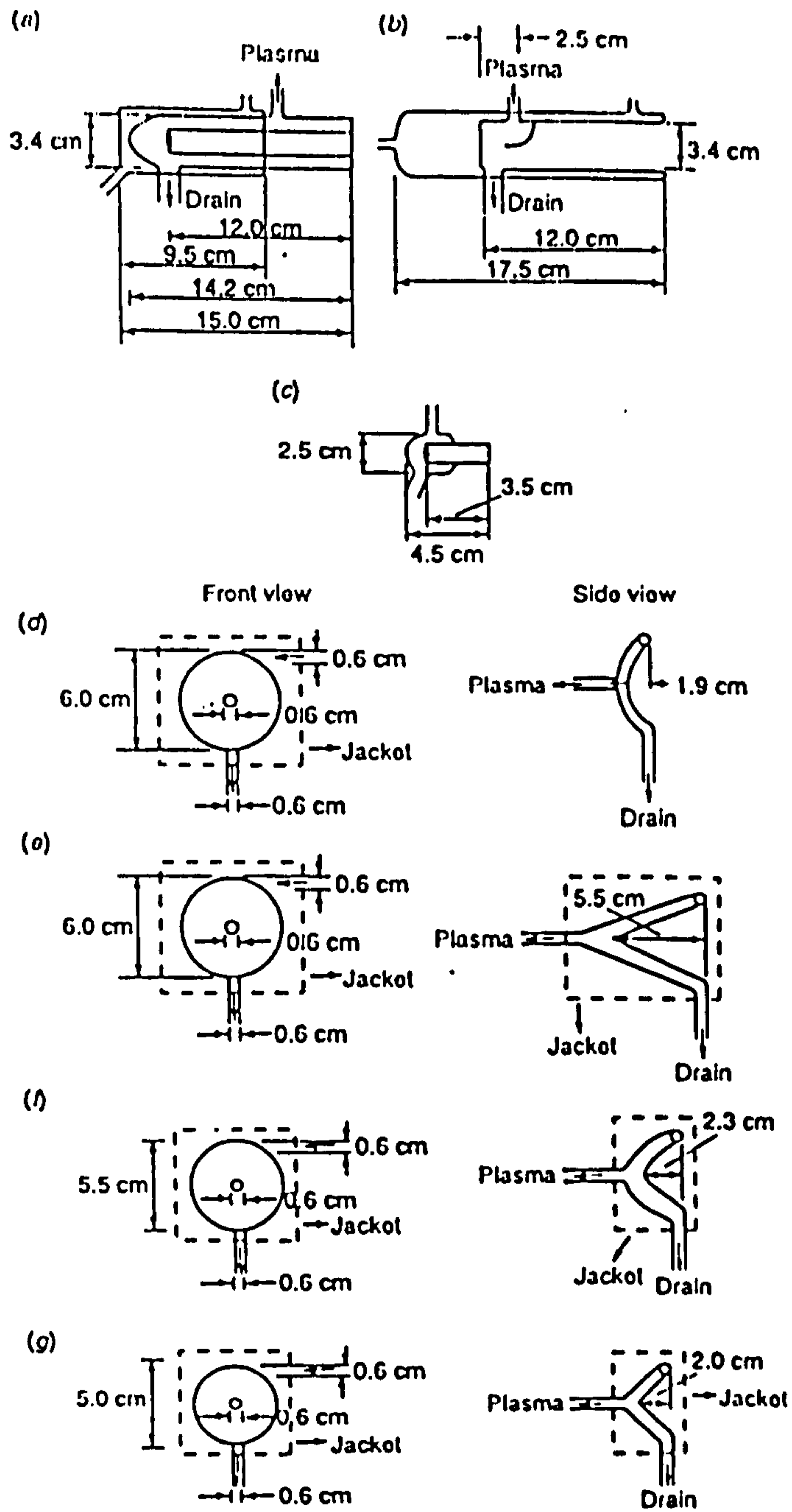


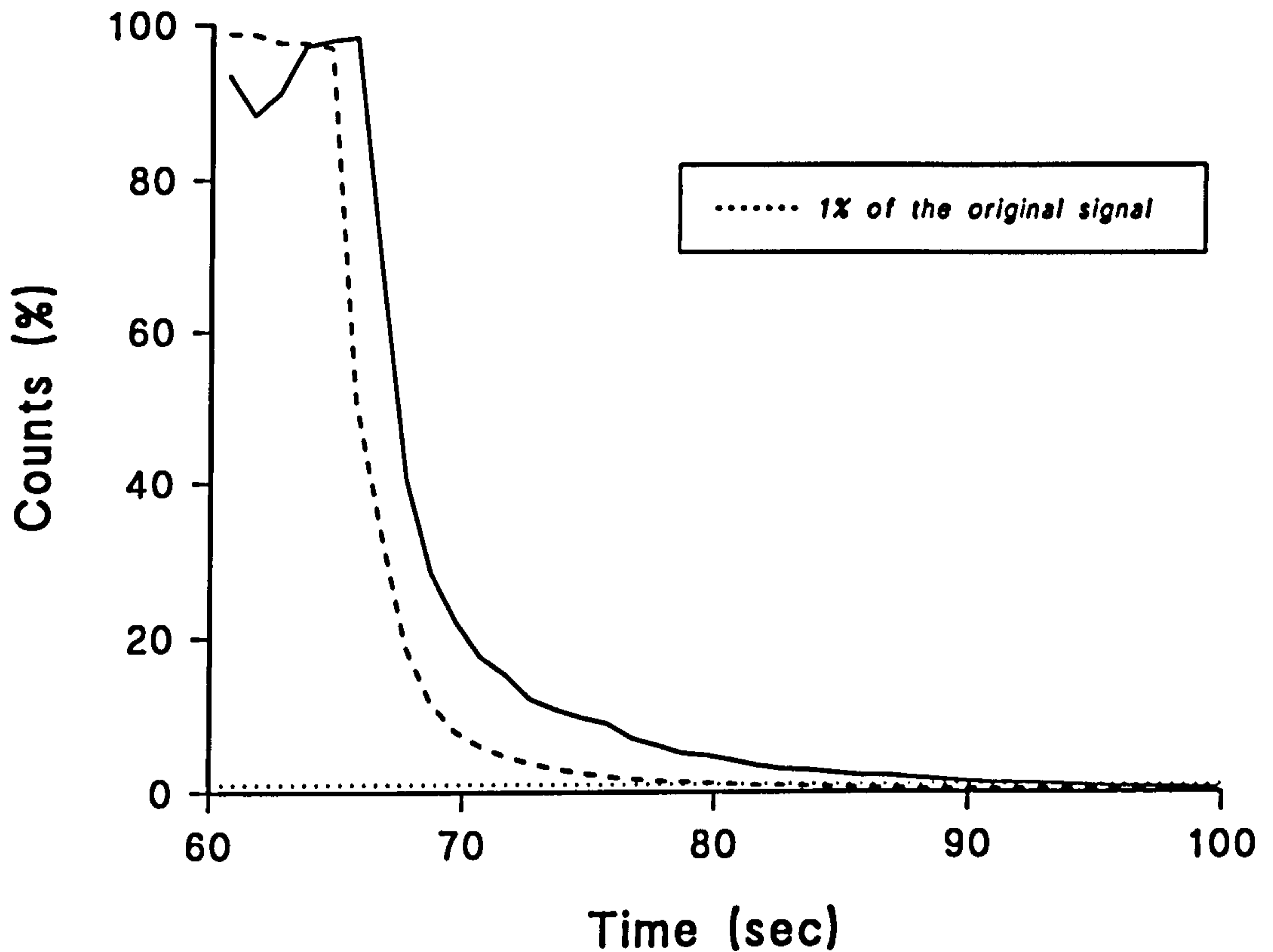
Figure 3.19. Types of spray chamber used: (a) Scott-type double pass, (b) Scott-type single pass, (c) miniaturised double pass, (d) cyclone without cooling jacket, (e), (f) and (g) cyclones with cooling jackets

As expected, the cyclone spray chambers give better transport efficiency, possibly as a result of allowing bigger drops to pass through the system. The lowest transport efficiency was found for the miniaturised double pass spray chamber, in this case probably because of the large ratio of impaction surface to internal volume and the more contorted gas flow necessary to exit this small spray chamber.

It is also interesting to note that although the uncooled cyclone spray chamber (D) had the highest transport efficiency, the signal to noise ratio obtained from the chromatogram was poor. Cooling of the spray chamber (e.g. type G), although reducing the transport efficiency, much improved the signal to noise ratio. The extent of this improvement was influenced by the overall design (Table 3.2).

**Effect of internal volume on washout time and resolution:** Clearly, the internal volume of the spray chamber can increase the dead volume of the system. The washout time was calculated by monitoring the decrease in the signal of a solution of 100 ng.ml<sup>-1</sup> of <sup>115</sup>In in 70% MeOH after being replaced with a 70% MeOH solution without In.

The washout curves obtained for both the Scott type double pass (A) and the new cyclone spray chamber (G) can be seen in Figure 3.20. Surprisingly the washout time in the cyclone spray chamber is slightly longer (24 sec) than in the much larger internal volume double pass spray chamber (15 sec). This would indicate that there are pockets of stagnation within the cyclone spray chamber which are not being efficiently removed. Optimising the size of the dimple is intended to minimise this problem.



*Figure 3.20. Wash-out curves for 100 ng.ml<sup>-1</sup> of <sup>115</sup>In in 70% MeOH for cyclone spray chamber G (solid line) and Scott-type double pass spray chamber A (dashed line)*

Using the chromatographic system employed in this study (70% MeOH+30% citrate buffer, pH=5.8, for 2.5 min.; step gradient to 85% MeOH+15% citrate buffer, pH=5.8, for 2 min.; step gradient to 85% MeOH+15% citrate buffer, pH=3.4, for 6.5 min.) TBT and TPhT are not base-line resolved. Any loss of resolution in the system will adversely affect the separation of species. The resolution ( $R_s$ ) between these two species was calculated for each of the spray chambers.

The ICP-MS experimental parameters (*i.e.* gas flow rates, torch position and lenses settings) were sequentially optimized for each system in order to obtain the best signal (cps) using a solution of  $^{115}\text{In}$ ,  $100 \text{ ng.ml}^{-1}$ , made up in the mobile phase (70% methanol, 30% water). The results obtained are shown in Table 3.3. As can be seen, all the spray chambers gave similar resolution, although the spray chambers with the smallest internal volumes tended to give slightly better results.

**Table 3.3.** Resolution between TBT and TPhT for each spray chamber

Spray chamber	Resolution
(A) Scott type double pass	0.81
(B) Scott type single pass	0.83
(C) Miniaturized double pass	0.84
(D) Cyclone (no jacket)	0.74
(E) Cyclone (jacketed)	0.76
(F) Cyclone (jacketed)	0.75
(G) Cyclone (jacketed)	0.83

The system giving the best performance using the above parameters was selected for further study. The cyclone spray chamber with cooling jacket (G) was one of the three spray chambers giving the best resolution between TBT and TPhT and the transport efficiency was also second highest of the tested. Using the cyclone spray chamber (D), although better transport efficiency was achieved, the resolution was not so good as with the spray chamber G. The miniaturized double pass spray

chamber (C) gave good resolution but the sensitivity obtained was inferior to that for the spray chamber (G). This was attributed to the lower transport efficiency. An additional disadvantage of spray chamber C was the noisy signal obtained as a result of the small internal volume.

Following these considerations, the cyclone spray chamber G was chosen as the best for use in this application. Examples of the chromatograms obtained with both the Scott type double pass spray chamber (A) and spray chamber G can be seen in Figure 3.21.

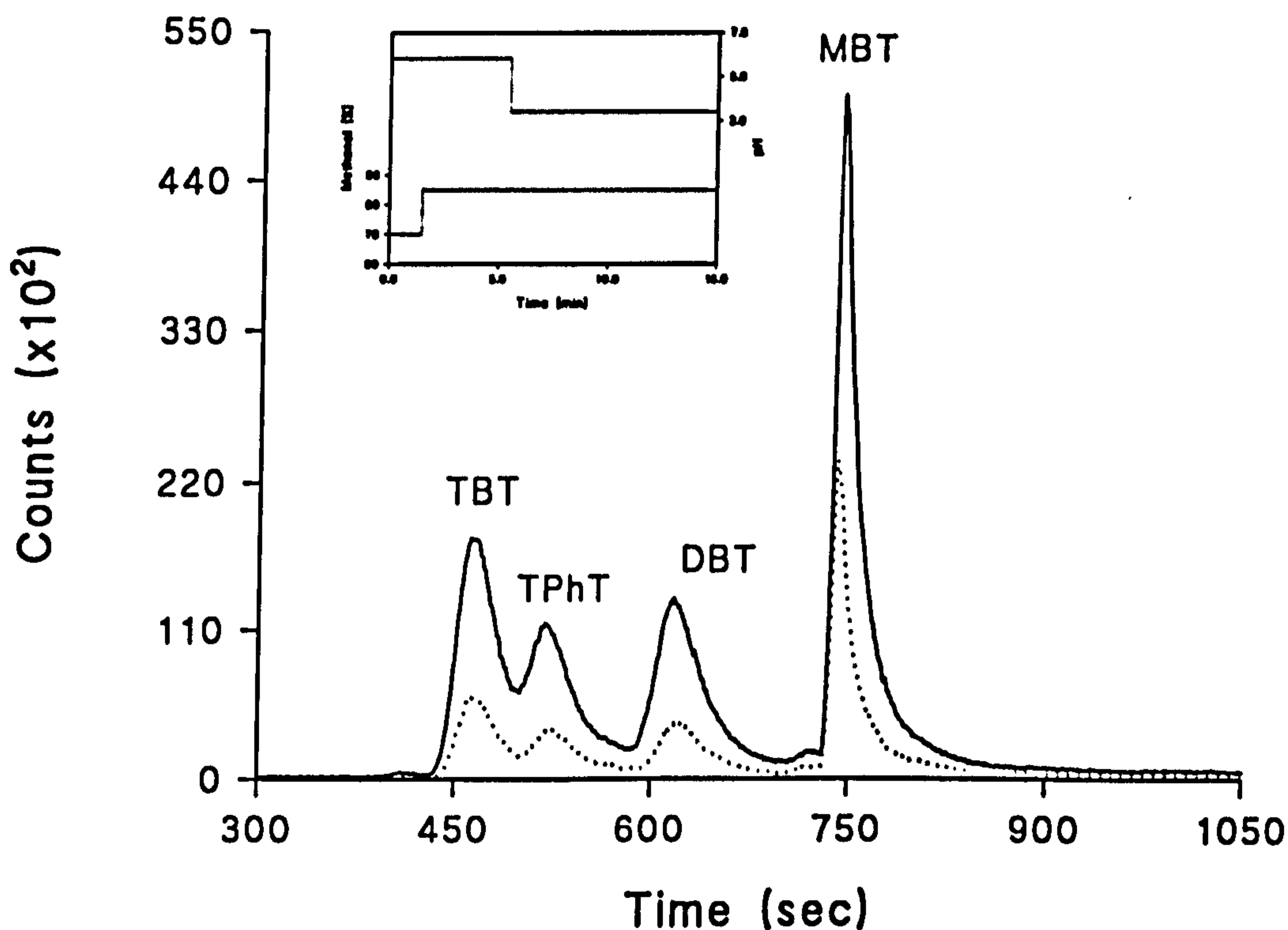


Figure 3.21. Chromatograms obtained with new cyclone spray chamber (solid line) and Scott-type double pass (dotted line). Gradient elution profile in inset

### 3.1.4.3. Torch injector and sheath gas

Under the elution conditions required for the gradient elution with the two cation-exchange columns, a major problem was encountered. The injector of the torch blocked with salt deposits after 3-4 hours using the system. Different types of injectors were tested with a demountable torch and their effect on the reflected power (RP), the amount of oxygen required, the nebuliser flow rate and the effect on the signal of  $^{115}\text{In}$  in a 70% MeOH solution evaluated.

Different injector sizes were tried (1.0, 1.2 and 1.5 mm i.d.). Also stepped and straight injectors were tested. The plasma extinguished with the straight injector of 1.0 mm and with the stepped of 1.5 mm. The results obtained can be seen in Figure 3.22(a-d). It can be concluded that:

- 1/ The nebuliser flow rate required for the same injector size is lower for a straight injector and increases with the internal diameter, probably due to the better punching of the plasma with smaller injector size (Figure 3.22-a).

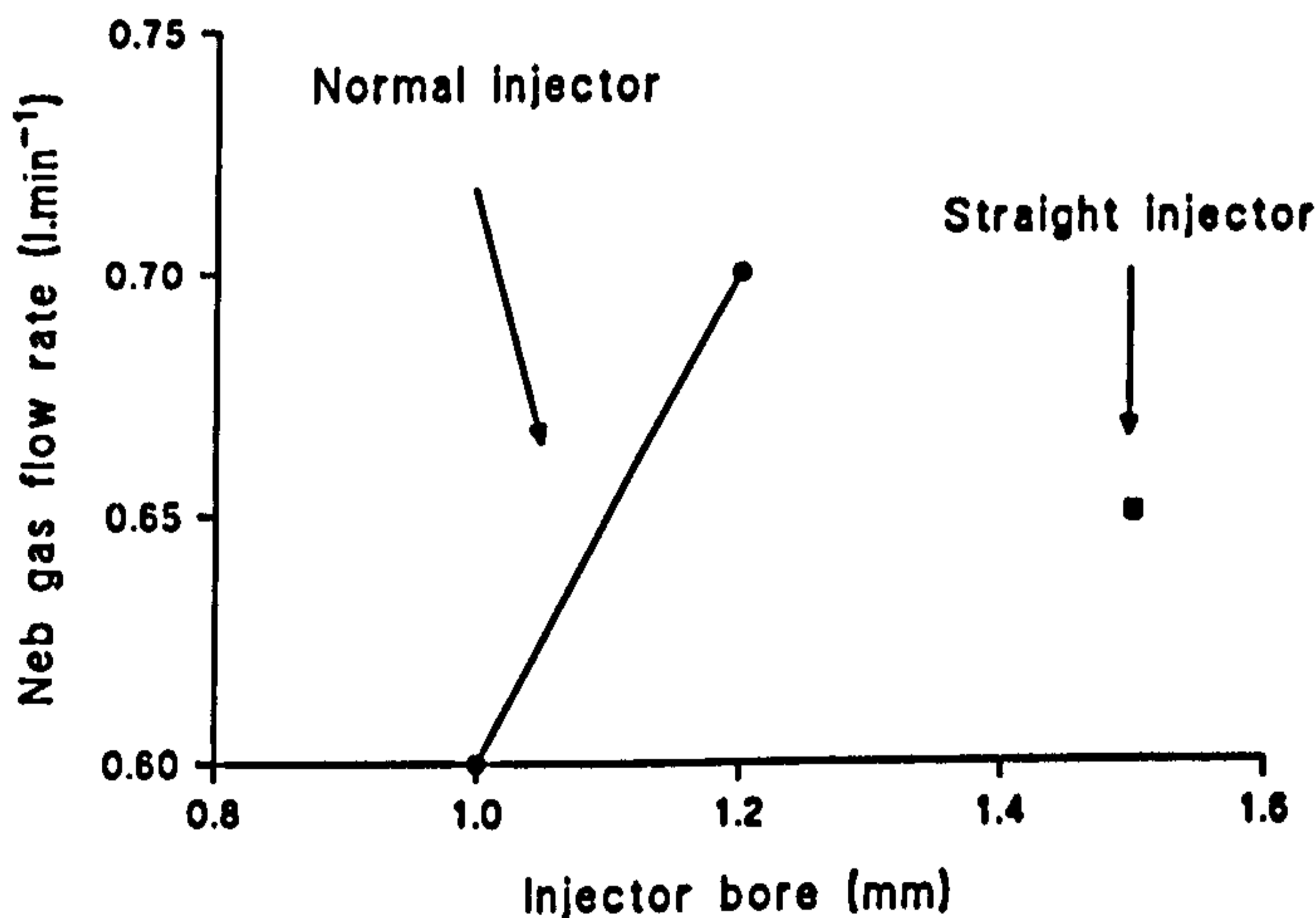


Figure 3.22(a) Effect of injector type and bore on the nebuliser gas flow rate for a solution of 100 ng.ml<sup>-1</sup> of  $^{115}\text{In}$  in 70% MeOH



- 2/ The reflected power observed is higher the larger the injector bore (Figure 3.22-b) and smaller with a straight injector than a normal stepped one, presumably this is due to methanol loading in the plasma.

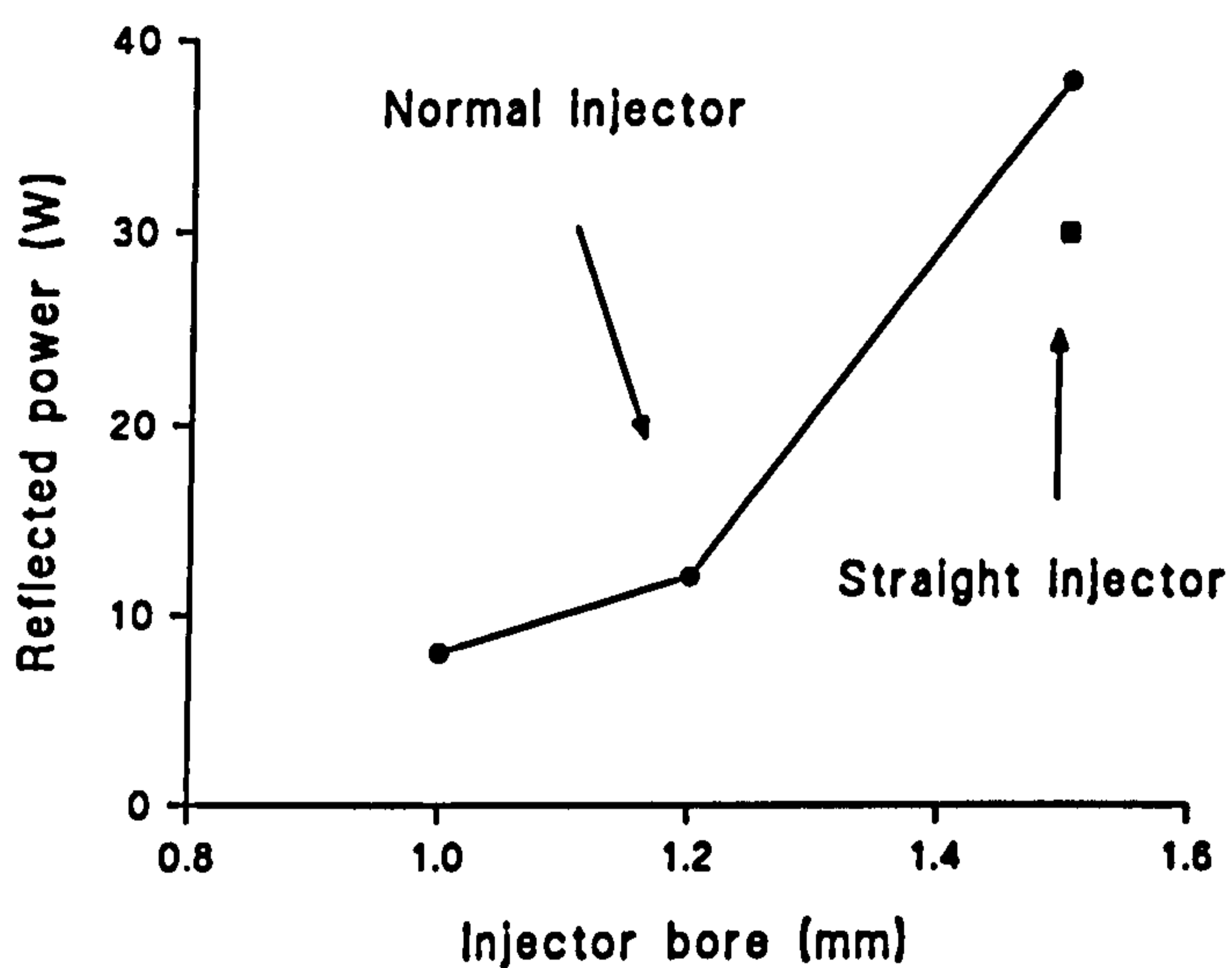


Figure 3.22(b) Effect of injector type and bore on reflected power for a solution of  $100 \text{ ng.ml}^{-1}$  of  $^{115}\text{In}$  in 70% MeOH

- 3/ More oxygen is needed with straight injectors (Figure 3.22-c). This may have the same cause as for the observed effect on the reflected power.
- 4/ The signal obtained for a solution of  $100 \text{ ng.ml}^{-1}$  of  $^{115}\text{In}$  in 70% MeOH is better the larger the internal diameter but comparable between straight and stepped injectors (Figure 3.22-d).

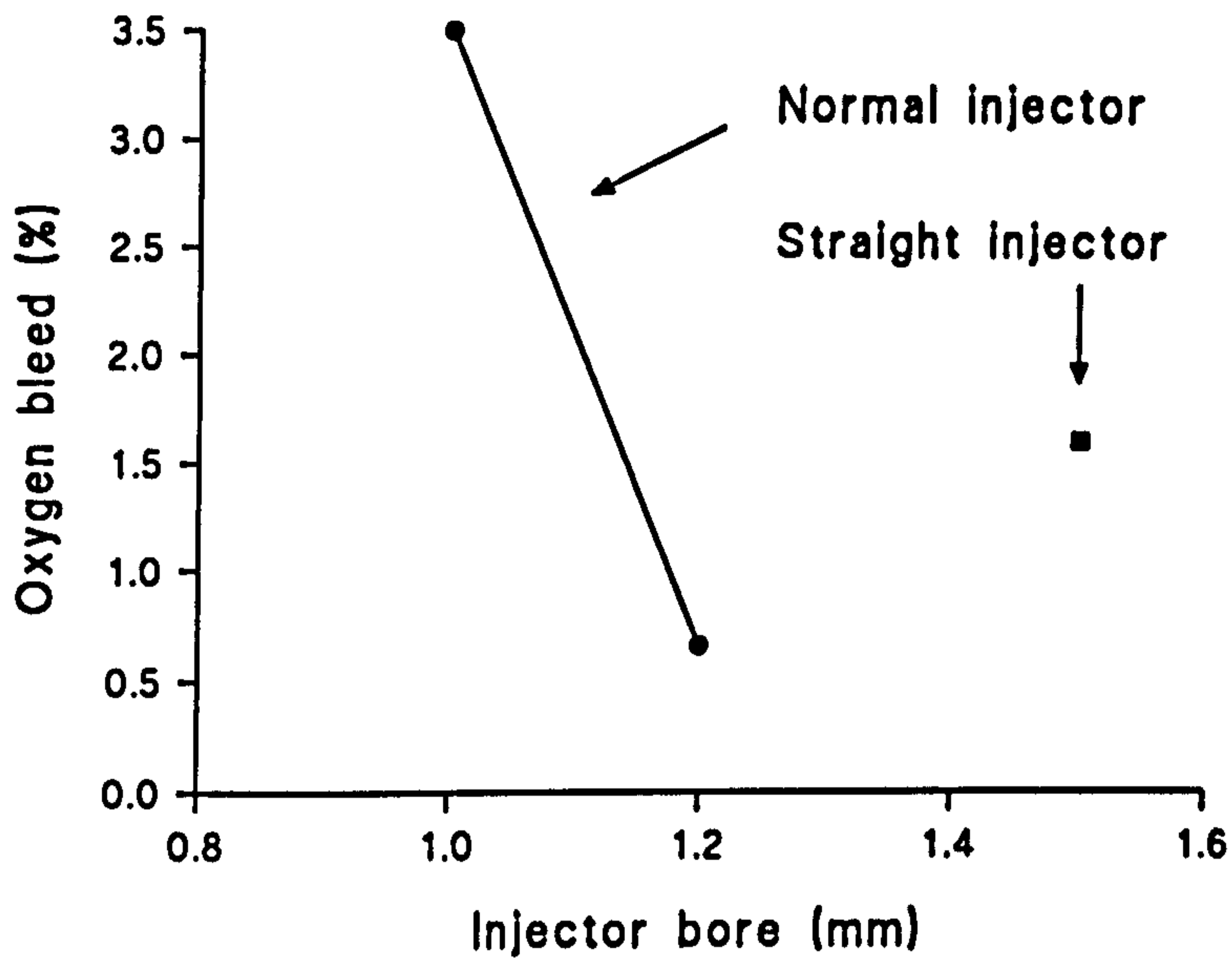


Figure 3.22(c) Effect of injector type and bore on oxygen bleed when analysing a solution of  $100 \text{ ng.ml}^{-1}$  of  $^{115}\text{In}$  in 70% MeOH

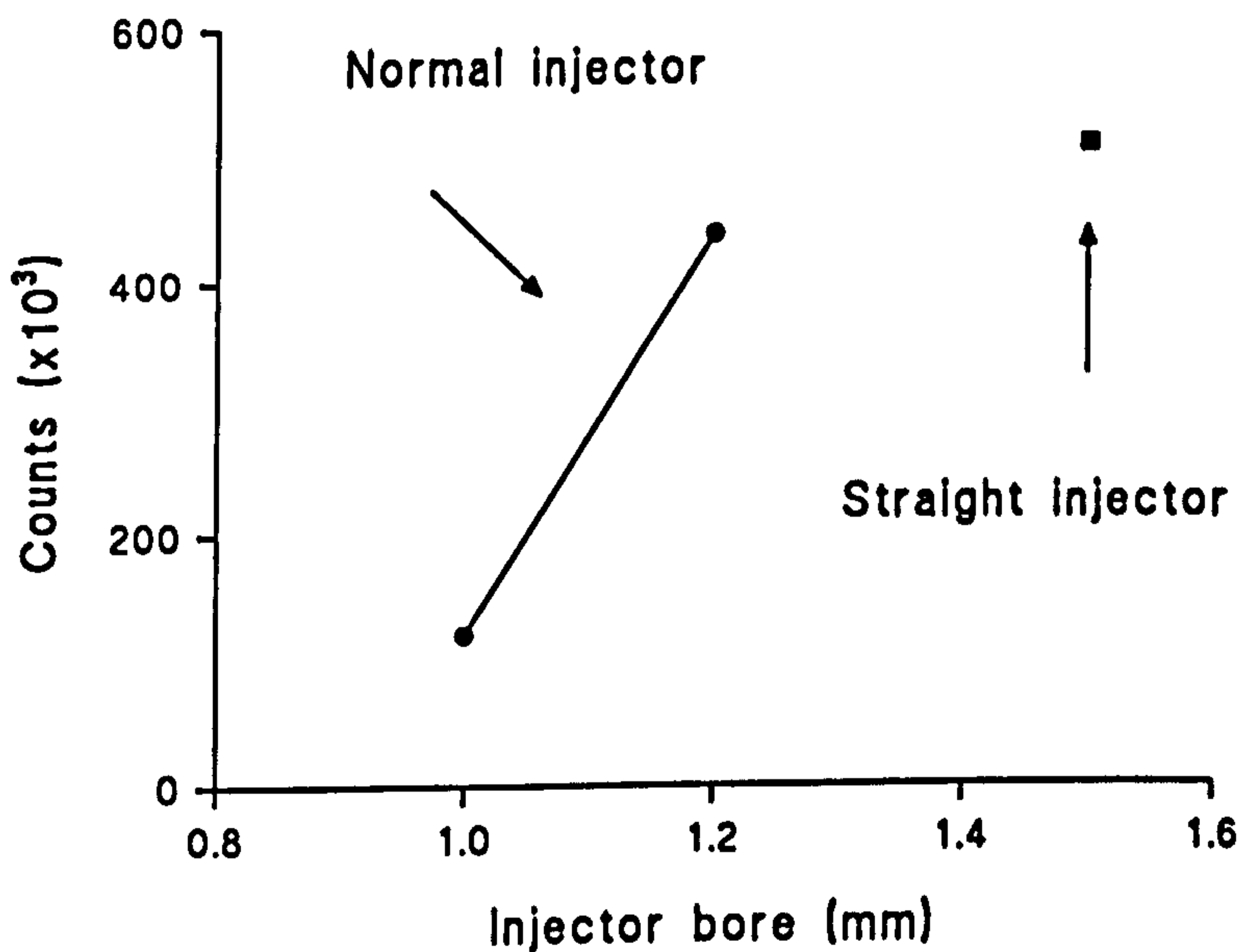


Figure 3.22(d) Effect of injector type and bore on sensitivity

After this study, a straight injector of 1.5 mm i.d. was selected for further studies. No solid deposits were observed with this type of injector even after 24 h of continuous use.

The reflected power constituted a problem since it was at the limit tolerable level by the RF generator (30 W). The use of an auxiliary Ar sheath gas was then evaluated in order to see its effect on the salt deposition in the injector and the analyte signal. A tapered tip injector torch was used and of smaller i.d. (1.2 mm), to help reduce the reflected power. A minimum sheath gas flow of 0.2 l.min<sup>-1</sup> was necessary to avoid deposits of solids in the injector. Increasing flow rates of sheath gas did not greatly affect the reflected power observed, although this was much smaller with the sheath gas present in the system.

Thus, the final set-up system chosen was a Meinhard nebuliser, a cyclone type spray chamber (G) cooled to -10°C, a sheath gas flow of 0.2 l.min<sup>-1</sup>, a tapered injector torch and a bleed of oxygen of 1.5-2.0% in the nebuliser.

### **3.1.5. Conclusions**

The best separation of TBT, TPhT, DBT and MBT was achieved using two Partisil-10 SCX columns, 10 µm particle size, 250x4.6 mm each, with a guard column of the same packing material (25x4.6 mm). The elution system of the analytes was a gradient elution with two step gradients. The first mobile phase was 70% MeOH+30% citrate buffer, 0.03 mol.l<sup>-1</sup>, pH=5.8, for 2.0 min; the second one was 85% MeOH+15% citrate buffer, 0.03 mol.l<sup>-1</sup>, pH=5.8, for 2.5 min; the third and final mobile phase was 85% MeOH+15% citrate buffer, 0.03 mol.l<sup>-1</sup>, pH=3.4,

for the remaining time until equilibration of the column (6-7 min).

The interface between the HPLC and the ICP was a Meinhard nebuliser, a cyclone type spray chamber (G) cooled at  $-10^{\circ}\text{C}$ , with  $0.2\text{ l}\cdot\text{min}^{-1}$  sheath gas and 1.6% oxygen bleed. The torch was a demountable one with a tapered injector of 1.2 mm i.d.

To evaluate possible interferences on the separation of these organotin species, other organotin compounds commercially available (TET, DMT, TMT, DPhT and MPhT) were tested. The conditions used are the same mentioned above, but only one analytical column was used in this study. Di- and monosubstituted species coelute with MBT at the pH gradient used, TET coelutes with TPhT, while TMT is a separated peak after DBT. The chromatograms can be seen in Figure 3.23.

The detection limits were based on peak height and were calculated as three times the standard deviation of the noise of the base line. The linear calibration range is higher than  $5\text{ }\mu\text{g}\cdot\text{g}^{-1}$  as Sn for all the analytes. The detection limits and the repeatability ( $n=6$ ) at three different concentration levels for each analyte can be seen in Tables 3.4 and 3.5, respectively.

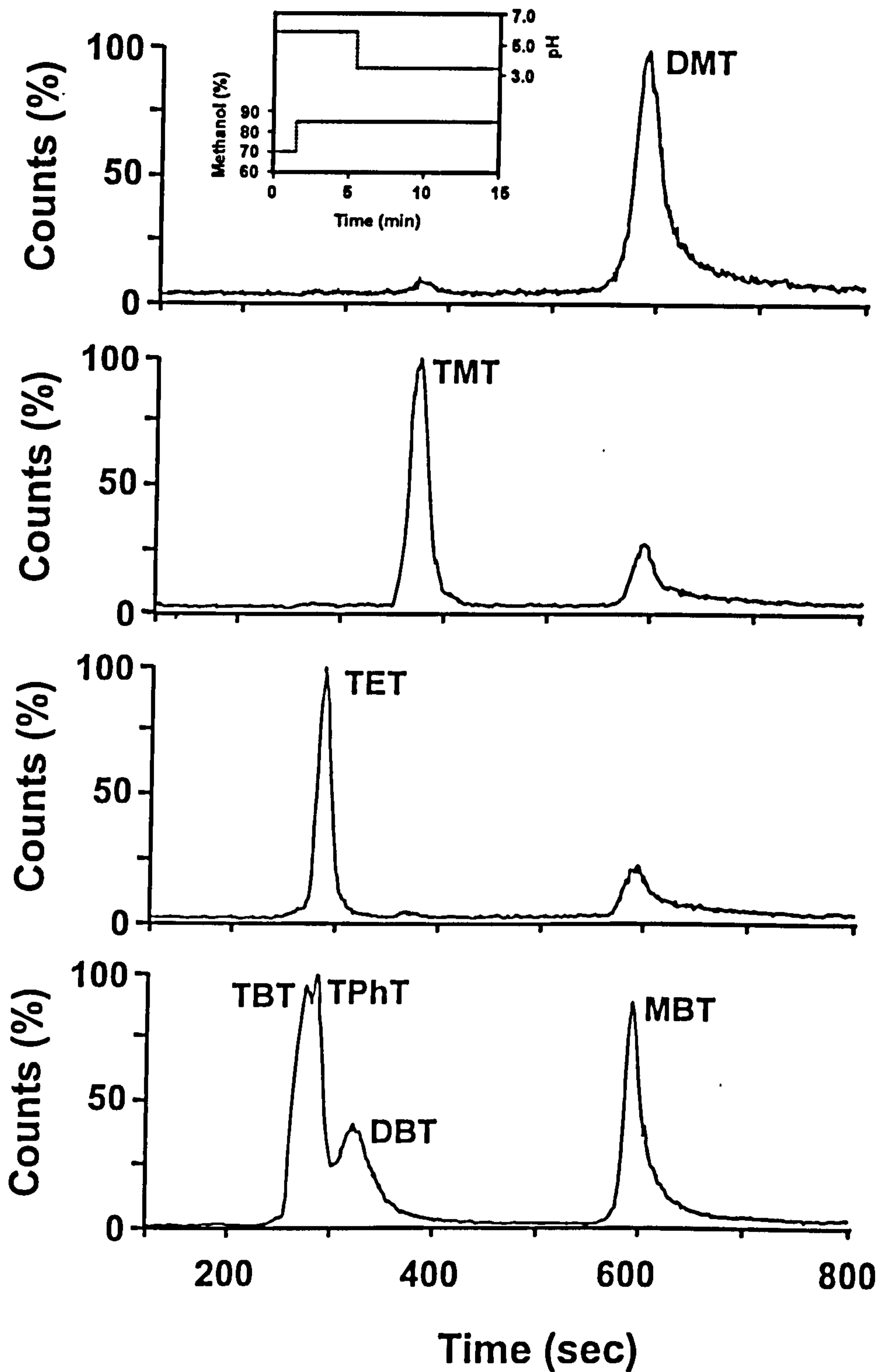


Figure 3.23. Chromatograms of DMT, TMT, TET and a mixture of TBT, TPhT, DBT and MBT. Gradient elution profile in inset

**Table 3.4.** *Limits of detection for TBT, TPhT, DBT and MBT with Scott-type double pass spray chamber (A) and the new cyclone spray chamber (G)*

Spray chamber	Limits of detection (ng.g <sup>-1</sup> )			
	TBT	TPhT	DBT	MBT
(A) Scott type double pass	1	1	2	1
(G) Cyclone	0.44	0.26	1.40	0.23

**Table 3.5.** *Repeatability for cyclone spray chamber (G) using 3 chromatographic runs at each of the concentrations (RSD)*

Analyte	Relative Standard Deviation (%)		
	Nominal concentration investigated (ng.g <sup>-1</sup> )		
	10	100	500
TBT	8.3	4.2	2.7
TPhT	8.0	4.5	4.0
DBT	6.5	3.6	2.0
MBT	7.3	10.5	7.6



## **3.2. ORGANOLEAD COMPOUNDS**

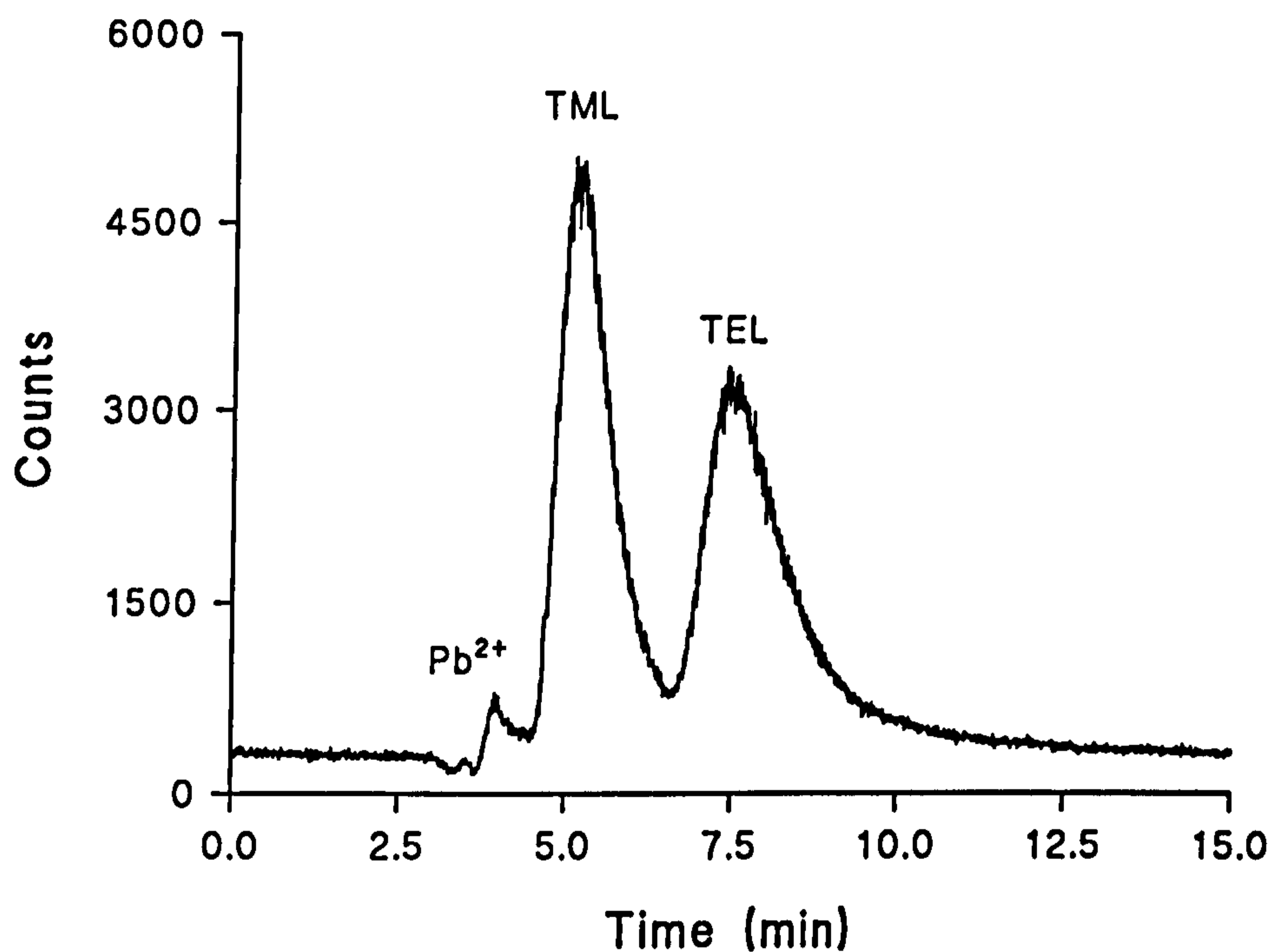
### **3.2.1. Ion-pairing chromatography**

Previous studies have detailed the use of ion-pairing chromatography for the separation of lead species. One of them<sup>(236)</sup> made use of gradient elution, finding problems due to the different characteristics of the plasmas when different amounts of organic solvents were run. Thus, an isocratic elution of  $Pb^{2+}$ , TML and TEL under similar elution conditions to those used by Brown *et al.*<sup>(236)</sup> was attempted. These authors reported great problems of coelution of  $Pb^{2+}$  with TML. In this study, although  $Pb^{2+}$  was not base line resolved from TML, the overlapping of both peaks is minimal when in equal concentrations (Figure 3.24). Quantification of the TML peak is still possible at a ratio  $Pb^{2+}/TML$  of 20. Different pHs (4.1, 4.6 and 5.1) and different percentages of MeOH (50, 60 and 70%), as well as some gradient elution at different times were tried in order to improve the separation. The best performance was obtained with a mobile phase of 60% MeOH+40% buffer, this being prepared by mixing a solution of acetic acid, 0.1 mol.l<sup>-1</sup>, and sodium 1-pentanesulphonic acid (SPSA), 4 mmol.l<sup>-1</sup>, with a solution of NaAc, 0.1 mol.l<sup>-1</sup>, and SPSA, 4 mmol.l<sup>-1</sup>, the pH of the mixture was 4.6.

### **3.2.2. Conclusions**

The separation of inorganic lead, TML and TEL under these conditions was achieved within 10 minutes and no time for equilibration of the column was required since no gradient elution was employed. The detection limits obtained (calculated as for the organotin species) were 3 ng.g<sup>-1</sup> as Pb for TML and 14 ng.g<sup>-1</sup> as Pb for TEL. The linear calibration range was up to more than 1000 µg.g<sup>-1</sup> as Pb for all the

species. The repeatability ( $n=6$ ) for each species can be seen in Table 3.6.



**Figure 3.24.** *Isocratic ion-pairing chromatogram of inorganic lead, TML and TEL. Mobile phase: 60% MeOH, 40% acetate buffer, pH=4.6, 0.4 mmol.l<sup>-1</sup> SPSA*

**Table 3.6.** *Repeatability for TML and TEL ( $n=6$ ) at different concentrations of the linear range*

Analyte	Relative Standard Deviation (%)				
	Nominal concentration investigated (ng.g <sup>-1</sup> )				
	50	100	250	500	1000
TML	4.3	4.4	n.m.	n.m.	4.0
TEL	n.m.	n.m.	14	12	9

(n.m.: not measured)

***4. EXTRACTION  
PROCEDURES FROM  
ENVIRONMENTAL  
SAMPLES***

## **4. EXTRACTION PROCEDURES FROM ENVIRONMENTAL SAMPLES**

### **4.1. DETERMINATION OF ORGANOTIN SPECIES**

#### **4.1.1. Introduction**

Although the use of TBT in antifouling paints was banned in the U.K. in 1982 and also in some other countries in the European Union, there is still great concern about the effect these species may have in the environment. The half life of TBT in waters is very small, but it is much longer in, for example, sediments. Marine organisms are severely affected<sup>(33,91,176)</sup> and can bioaccumulate these toxic species. Thus, a way of monitoring the effect of organotin compounds in the environment can be through the analysis of sediments and shellfish.

#### **4.1.2. Analysis of sediments**

To verify the suitability of the HPLC separation developed for the organotin compounds, the analysis of real samples (sediments) was performed. An extraction method had previously been developed and employed in this laboratory for the analysis of sediments<sup>(237,238)</sup>. The procedure is as follows: approximately 1 g of sediment is accurately weighed, 25 ml of glacial acetic acid added and the mixture is shaken for 4 hours. After this time, the sample is centrifuged at 2000 rpm for 15 min and the supernatant liquid separated from the solid. This is washed with acetic

acid twice, 5 ml each. The aqueous phase is diluted to 50 ml with purified water and the organotins extracted into 30 ml of toluene. This organic phase is rotary evaporated until dryness and the analytes dissolved in the mobile phase prior to the analysis.

In order to improve the recovery of the extraction of the species the method was slightly modified: the acetic acid extraction was performed in duplicate. The extraction was helped by heating the sample at 30°C when shaking. And finally, the extraction into toluene was performed by triplicate, 10 ml of organic solvent each time.

A sediment sample was collected in the vicinity of Plymouth. The sediment was dried at 105°C for 24 hours, cleaned, ground and sieved through 710 µm mesh. There was no trace of organotin species in this sample following the above extraction procedure and HPLC-ICP-MS detection. A recovery study was carried out with this "clean" sediment. Approximately 2 g of sediment was weighed in a centrifuge tube and spiked with approximately 250 ng of each analyte (as Sn in methanol). The same volume of methanol was spiked in the non-spiked samples and in the blanks. The sediment was left overnight to equilibrate. The results obtained are shown in Table 4.1.

The Canadian sediment PACS-1, certified for tributyltin, dibutyltin and monobutyltin was also analysed. 0.5 g of sediment were employed in each extraction. The sediment was spiked with TBT, DBT, MBT and TPhT in order to correct the values obtained for the efficiency of the extraction. The results obtained

are shown in Table 4.2.

**Table 4.1.** Recovery studies for the "clean" sediment (n=4)

Analyte	TBT	TPhT	DBT	MBT
Extraction efficiency (%)	103	61	33	12
Standard deviation (%)	23	9	3	4

**Table 4.2.** Analysis of sediment certified reference material PACS-1 (n=6)

Analyte	TBT	TPhT	DBT	MBT
Certified value ( $\mu\text{g}\cdot\text{g}^{-1}$ as Sn)	$1.27 \pm 0.22$	not certified	$1.16 \pm 0.18$	$0.28 \pm 0.17$
Recovery ( $\pm$ SD, %)	$24 \pm 8$	-	$27 \pm 5$	$34 \pm 6$
Analysis value ( $\mu\text{g}\cdot\text{g}^{-1}$ as Sn)	$1.31 \pm 0.14$	$0.06 \pm 0.01$	$0.64 \pm 0.13$	$0.69 \pm 0.18$

#### 4.1.3. Analysis of mussels

There have been three main types of extraction procedures of organotin compounds from biological samples: acidic<sup>(176)</sup>, basic<sup>(239)</sup> and enzymatic<sup>(239)</sup>. The best extraction would be the one which, at the same time as extracting the analytes, would destroy the high content of lipids and proteins in the biological samples and thus made the analysis easier and more suitable for HPLC analysis.

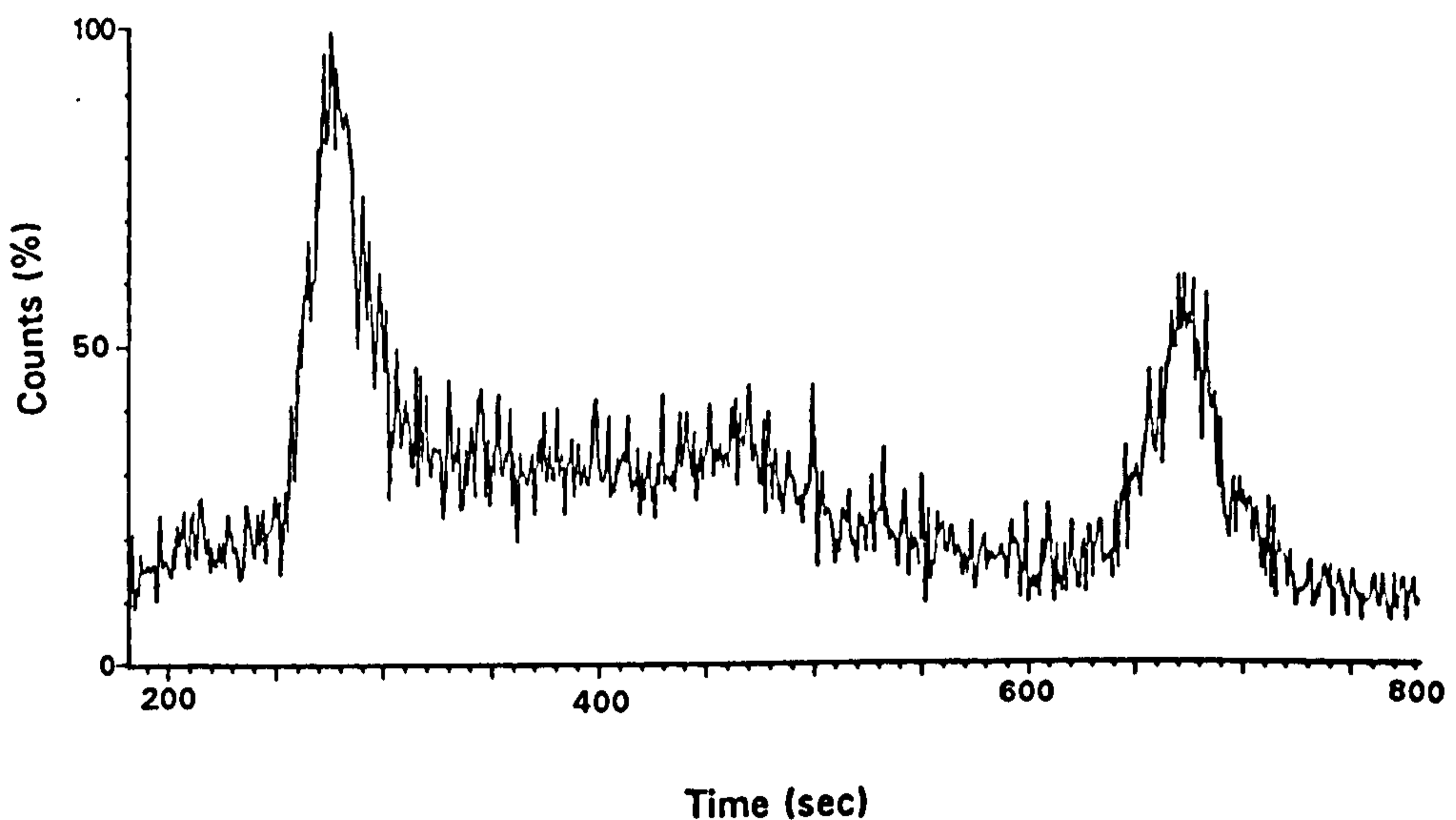
In order to develop an extraction method, some mussels were purchased in



the local market. They were frozen and shucked. The tissue was then freeze dried, ground and sieved through 1 mm mesh size.

#### 4.1.3.1. *Acid extractions*

The first extraction procedure was the same as that followed for the analysis of sediments and described in section 4.1.2. Approximately 1 g of mussel tissue was weighed. Some of the samples were spiked with TBT, TPhT, DBT and MBT ( $\approx 250$  ng of each analyte as tin) and some were analysed as such as control samples. The latter showed no significant amount of organotin species after this extraction procedure and HPLC-ICP-MS analysis. The values for the recoveries for TBT were not reproducible, being between 45 and 81%. An example of a chromatogram can be seen in Figure 4.1.

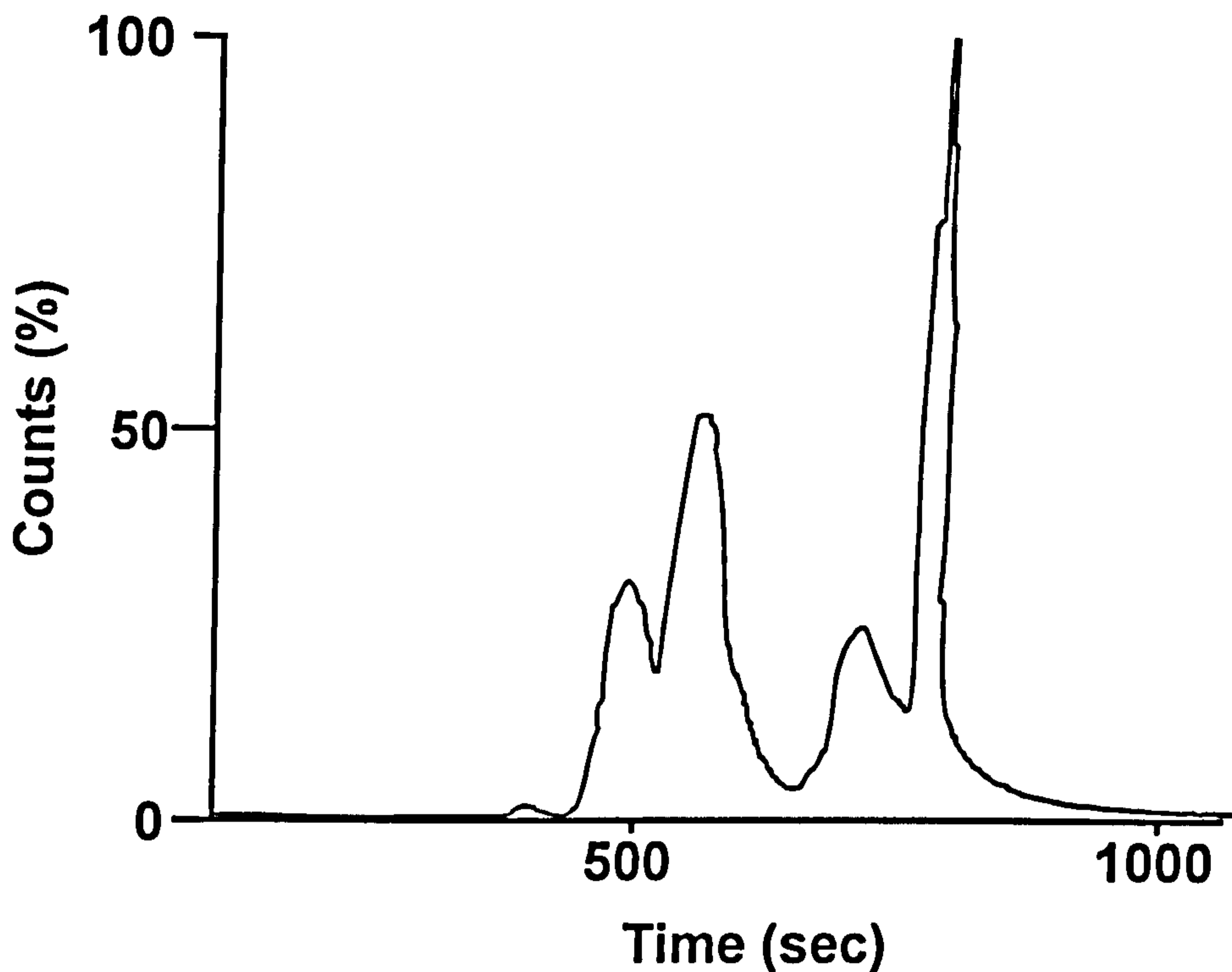


*Figure 4.1. Typical chromatogram of mussel sample extracted with acetic acid and toluene as organic solvent*

In order to see if the extraction efficiency was improved, toluene (instead of mobile phase) was employed to dissolve the dry extracts. No improved results were obtained since the resolution between the different analytes was lost. This was probably due to the amount of lipids and/or proteins present in the sample that passed through the extraction procedure.

A second acid extraction procedure tested was the method previously used by Ebdon *et al.*<sup>(176)</sup> for the analysis of organotin compounds in oysters. Approximately 1 g of mussel was weighed and some of the samples were spiked with a methanolic solution of  $\approx 250$  ng in each analyte as Sn. Methanol alone was spiked in the samples used as controls. The mussels were left at least overnight to equilibrate. The extraction procedure was as follows: 30 ml of hydrochloric acid (35%, 1.18 sp.gr.) were added to the mussels and these were shaken for 30 min. 20 ml of hexane were added to the mixture after this time and left shaking for a further 60 min. This mixture was centrifuged at 2000 rpm for 15 min. The organic layer was separated and rotary evaporated until dryness. Prior to the determination by HPLC-ICP-MS the samples were dissolved in  $\approx 2$  ml of toluene.

The method did not prove successful for mussels since the recovery values for all the analytes were very low (TBT-7%, TPhT-0%, DBT-12%, MBT-30%). An example of a chromatogram can be seen in Figure 4.2. No further studies with this method were performed.



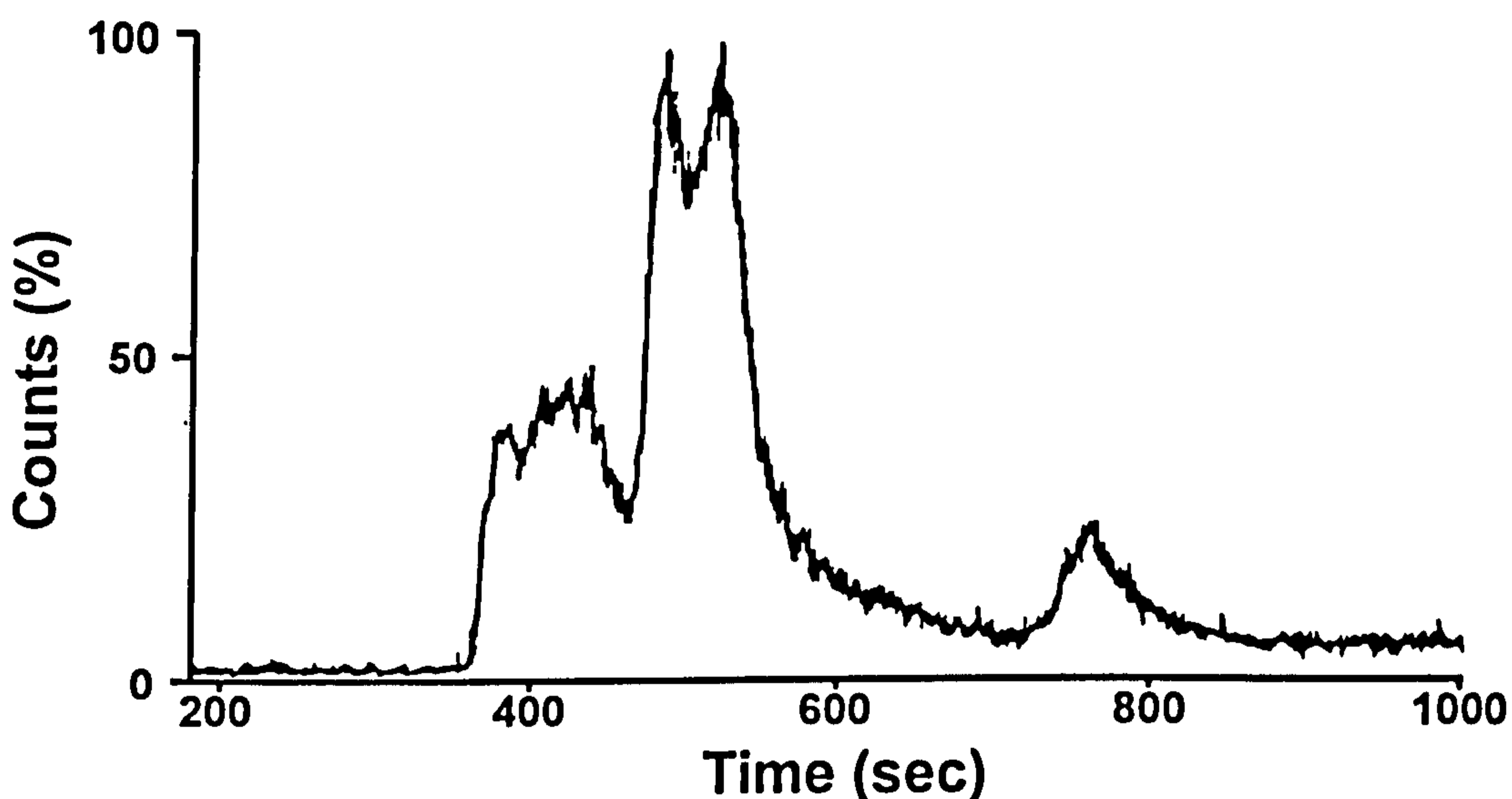
*Figure 4.2. Typical chromatogram of mussels sample extracted with hydrochloric acid and hexane as organic solvent*

#### *4.1.3.2. Basic extraction*

Tetramethylammonium hydroxide (TMAH) has been previously employed for the analysis of mussels<sup>(239)</sup> with gas chromatographic separation of the analytes. Again, the procedure was modified to make it suitable for analysis by HPLC. 0.1 g of mussel tissue was mixed with 5 ml of TMAH and left shaking for 4 h at 60°C. The aqueous phase was then extracted into toluene (3x10 ml each time) and rotary evaporated until dryness. Prior to the analysis, the extracts were dissolved in 1 ml of methanol. Recovery experiments were conducted after spiking some of the samples with approximately 500 ng of each analyte as Sn.

An example of the chromatogram obtained for one of the spiked samples can be seen in Figure 4.3. The shape of the peaks is much worse than for any previous extraction procedure and the quantification of the peaks is clearly difficult. The peak for DBT has disappeared completely under the second peak and in general a tendency to split peaks can be observed.

An estimate of the recovery for TBT can be made, this being of approximately 10%. This extraction procedure, thus, was considered not to be successful.



*Figure 4.3. Typical chromatogram of mussels sample extracted into tetramethylammonium hydroxide and toluene*

#### *4.1.3.3. Enzymatic extractions*

Enzymes have been previously used for the extraction of different analytes from biological materials, for organotin compounds<sup>(239)</sup> and also for arsenic species<sup>(240)</sup>.

The first step to be followed in any enzymatic extraction is the study of the suitability of these procedures for speciation purposes. No change in the species during the extraction should occur. In order to test this, the procedure had to be followed with individual organotin compounds. In none of the cases studied a change in the speciation was observed.

Different enzymes were employed: trypsin, pancreatin and a mixture of lipase and protease (based on a procedure by Ceulemans *et al.*<sup>(239)</sup>). The general procedure followed is basically the same for the three systems. An accurately weighed amount of sample was placed in a tube together with buffer solution at the desired pH and left shaking. The aqueous phase was then extracted into an organic solvent and rotary evaporated until dryness prior to the analysis.

**Trypsin extraction:** the optimal pH for this enzyme is about 7.6. A 0.1 mol.l<sup>-1</sup> buffer solution of ammonium bicarbonate was dissolved in water and diluted to 1000 ml. The pH of this solution is 8, which is valid for the enzyme. 0.2 g of trypsin were used in the extraction together with 30 ml of buffer solution. The samples were left in a shaker overnight at 37°C. The aqueous phase was extracted afterwards into toluene (3x10 ml) and rotary evaporated until dryness. The dry extract was dissolved in the methanol prior to the analysis by HPLC-ICP-MS.

**Pancreatin extraction:** the same procedure as for trypsin was followed with the only difference being the use of 0.5 g of enzyme, since pancreatin is a mixture of several enzymes (amylase, trypsin, lipase, ribonuclease and protease).

**Lipase and protease extraction:** the buffer employed was a mixture of 21

g of citric acid, 14 g of ammonium dihydrophosphate and 64 ml of ethanol diluted to 1000 ml of water. The pH of this mixture was adjusted to 7.5 with ammonia. 0.1 g of each enzyme were used. The procedure was the same as for the previous enzymatic systems. The analytes were placed with the enzymes and 40 ml of buffer solution in a test tube. This was left overnight shaking at 37°C. The aqueous phase was extracted into an organic solvent (3x10 ml) and rotary evaporated until dryness before analysis.

This system proved to be the more effective one in this preliminary study and thus further investigations were conducted to optimise this procedure. The values for the extraction efficiency of the four calibrants with the three systems employing toluene as organic solvent, can be seen in Table 4.3.

**Table 4.3.** *Recovery values for different enzymatic extraction procedures*

<b>Enzymatic system</b>	<b>Trypsin</b>	<b>Pancreatin</b>	<b>Lipase+Protease</b>
<b>TBT</b>	47±3%	n.e.	61±3%
<b>TPhT</b>	58.1±0.4%	n.e.	68±9%
<b>DBT</b>	10.53±0.07%	n.e.	11.93±0.07%
<b>MBT</b>	n.e.	n.e.	n.e.

(n.e.: not extracted)

In following studies with lipase and protease several organic solvents were employed: toluene, hexane, chloroform and dichloromethane. Solvents of different polarity were selected in order to improve the extraction efficiency of DBT and



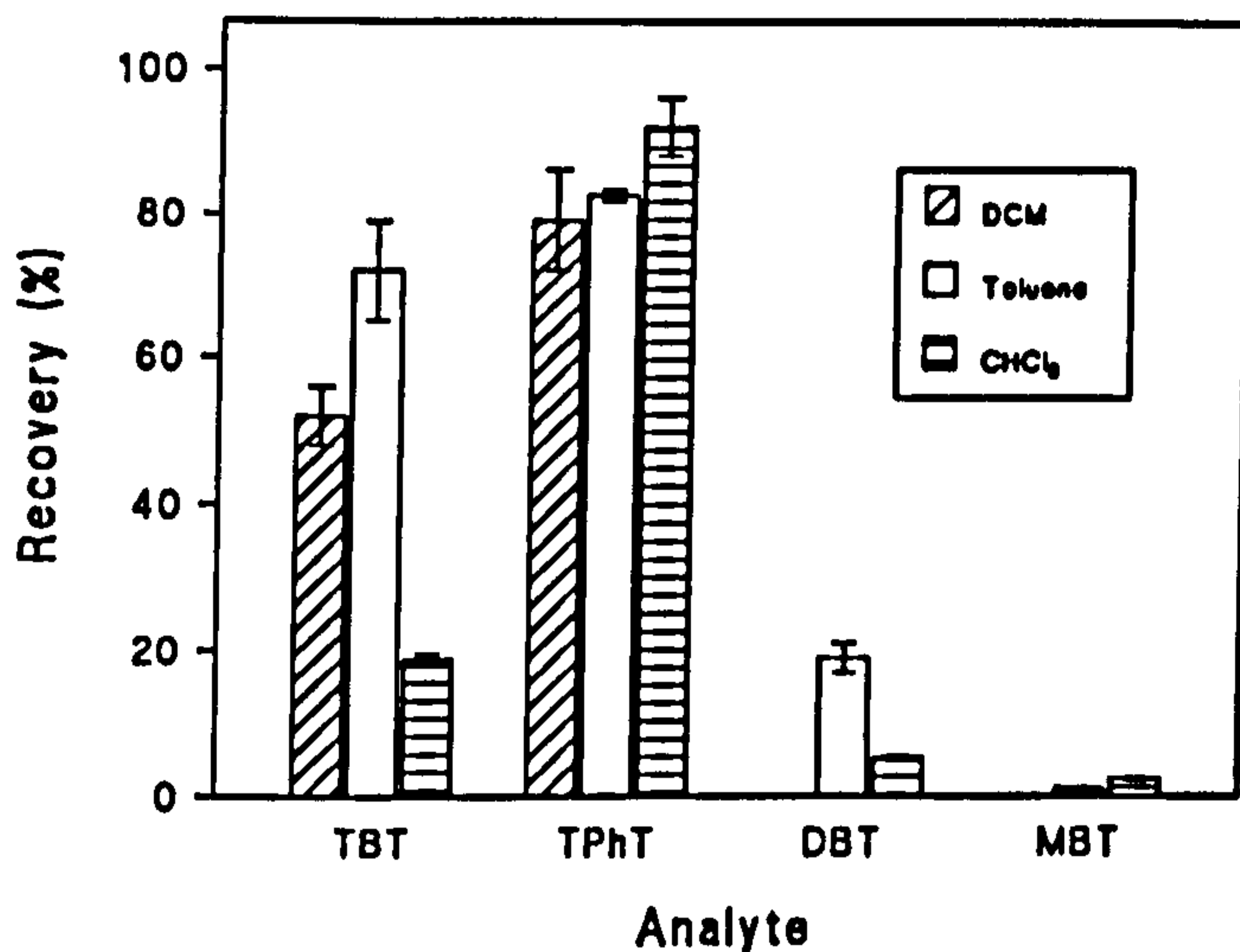
MBT especially. Hexane offered lower efficiencies for all the analytes (TBT:  $58 \pm 2\%$ , TPhT:  $51 \pm 3\%$ , DBT:  $4.3 \pm 0.5\%$ , MBT:  $0\%$ ) than toluene. Salting out the aqueous phase with sodium chloride also improved the extraction efficiency (Table 4.4). The values for the efficiencies for the four analytes with lipase and protease with different organic solvents, with salting out the aqueous phase, are shown in Figure 4.4. Toluene and dichloromethane gave the best results, especially for TBT ( $72\%$  and  $52\%$ , respectively) and TPhT ( $82.5\%$  and  $79\%$ , respectively). In none of the cases the recoveries for MBT were significant (always  $< 2\%$ ). Chloroform gave a very good extraction efficiency for TPhT ( $92 \pm 4\%$ ) but it was much worse for the other analytes than the other organic solvents.

*Table 4.4. Recovery values for the extraction with lipase+protease enzymatic system, extracting into toluene, with and without salting out*

Analyte	TBT	TPhT	DBT	MBT
Without salting out	$61 \pm 3\%$	$68 \pm 9\%$	$11.93 \pm 0.03\%$	$0\%$
With salting out	$72 \pm 7\%$	$82.5 \pm 0.8\%$	$19 \pm 2\%$	$1.07 \pm 0.03\%$

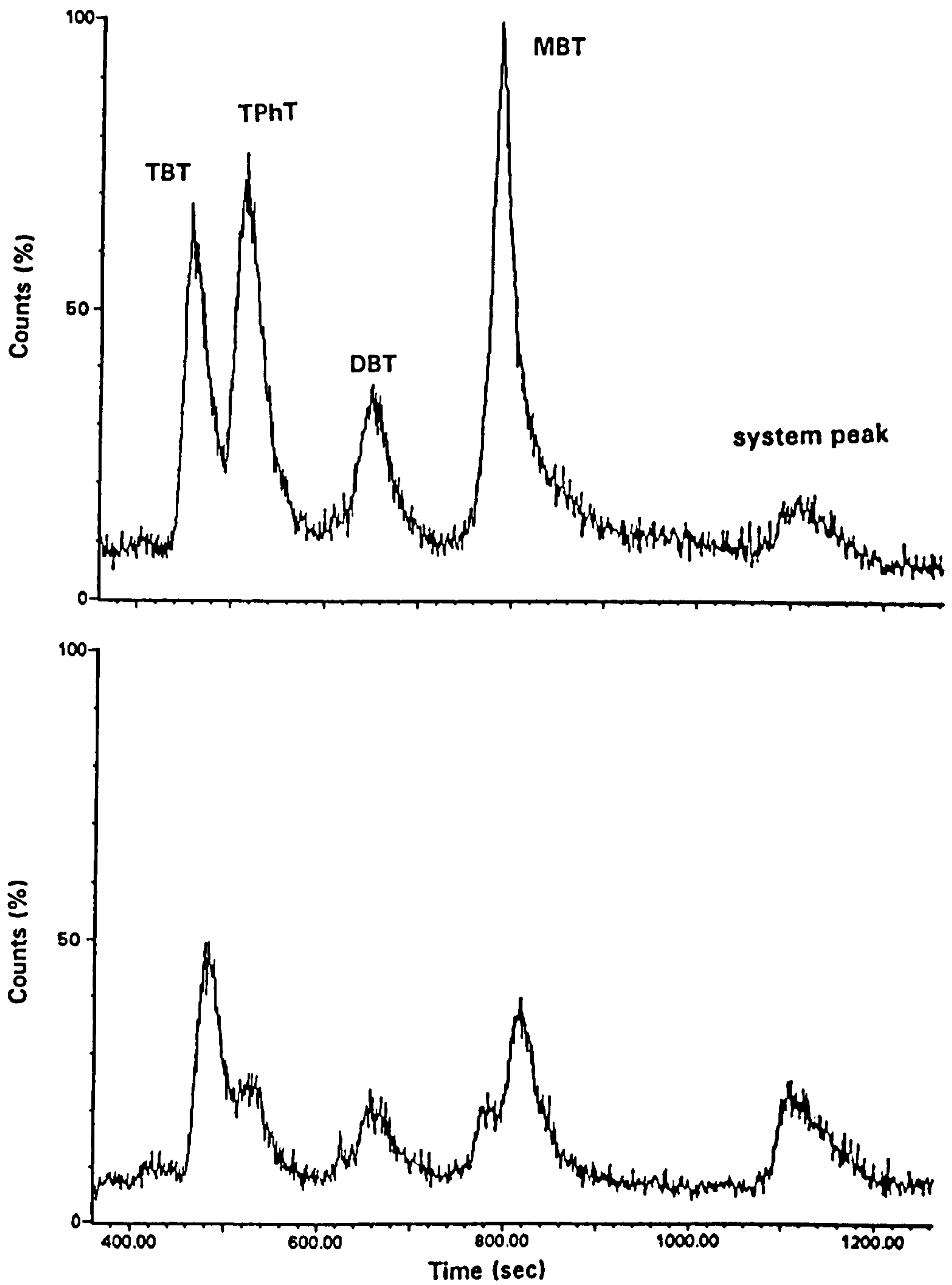
Extracting into dichloromethane and toluene, salting out the aqueous phase, was then tested in real samples. Approximately 1 g of wet mussel was weighed. Some samples were spiked with approximately 200 ng of each analyte as Sn. 0.1 g of each enzyme and 40 ml of citric/phosphate buffer were added. This mixture was left shaking overnight at  $37^\circ\text{C}$ . The aqueous phase was salted out and then extracted into dichloromethane or toluene, rotary evaporated until dryness and dissolved in 1.5 ml of the first mobile phase of the gradient prior to the analysis. The results were

very disappointing since the recoveries for all the analytes were extremely low. Examples of the chromatograms of the spiked samples, compared with the chromatogram of a calibrant solution of approximately 5 ng.g<sup>-1</sup> as Sn in each analyte, can be seen in Figure 4.5(a) and 4.5(b).

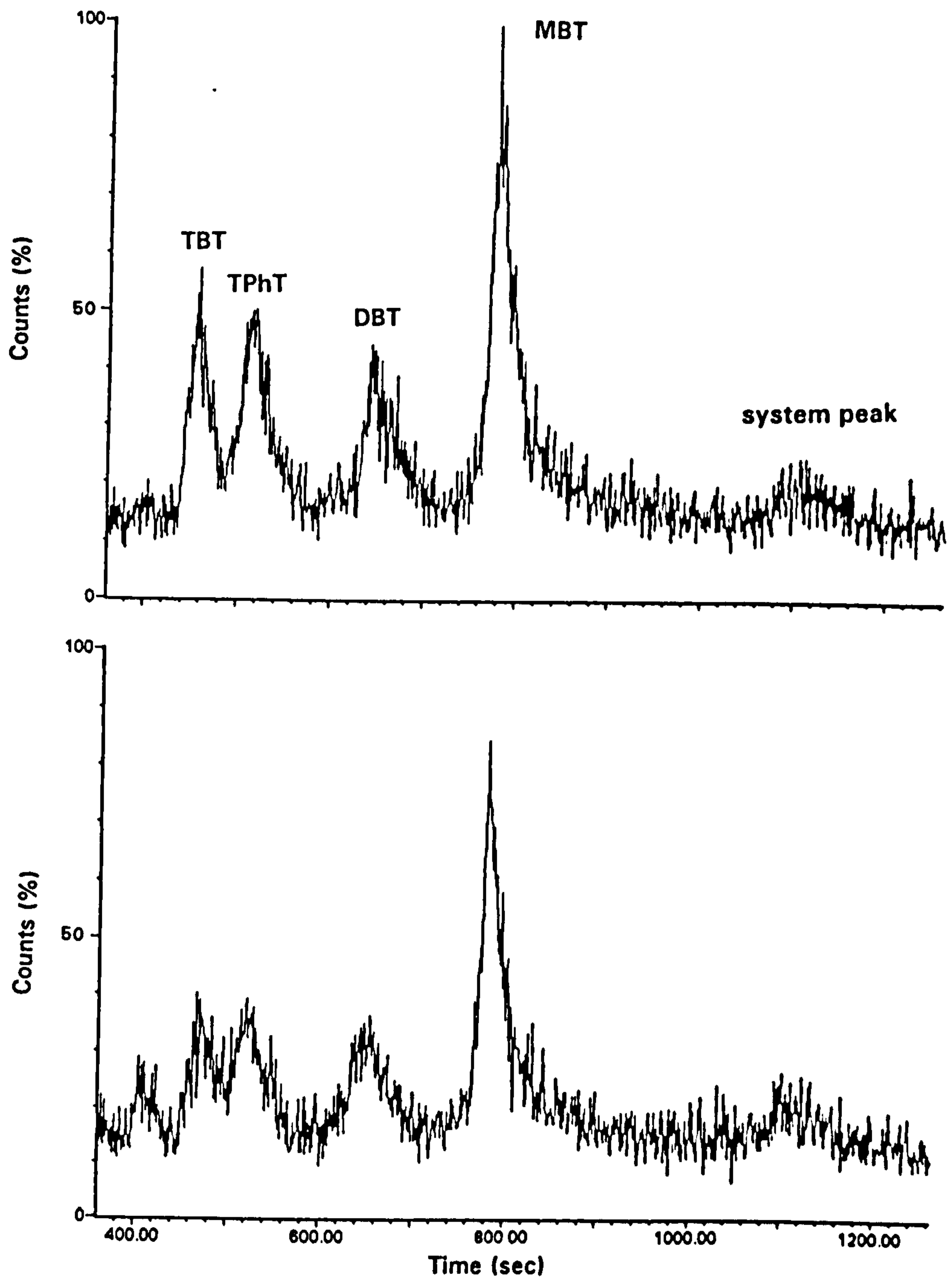


**Figure 4.4.** Recovery values for each analyte with lipase and protease enzymatic extraction with three different organic solvents to extract into from the aqueous phase

The dry extracts from the extraction with toluene seemed to fully dissolve in the mobile phase. This was not the case, though, for the extracts using dichloromethane. A mixture of 1 ml of methanol and 0.5 ml of mobile phase was then used and this seemed to dissolve all the dry extract. A study on the efficiency dissolving in this mixture was performed. The values obtained can be seen in Table 4.5. The use of a Potter homogeniser was also tested in an attempt to make the mixture more homogeneous and thus improve the extraction. As can be seen from the values in Table 4.5, the Potter homogeniser improved the recoveries especially for TBT and TPhT. Thus, its use was adopted from now on.



*Figure 4.5(a) Typical chromatogram of mussels sample extracted with lipase and protease and toluene as organic solvent (bottom chromatogram) compared with chromatogram of calibrant solution of approx. 5 ng.g<sup>-1</sup> of each analyte (top chromatogram)*

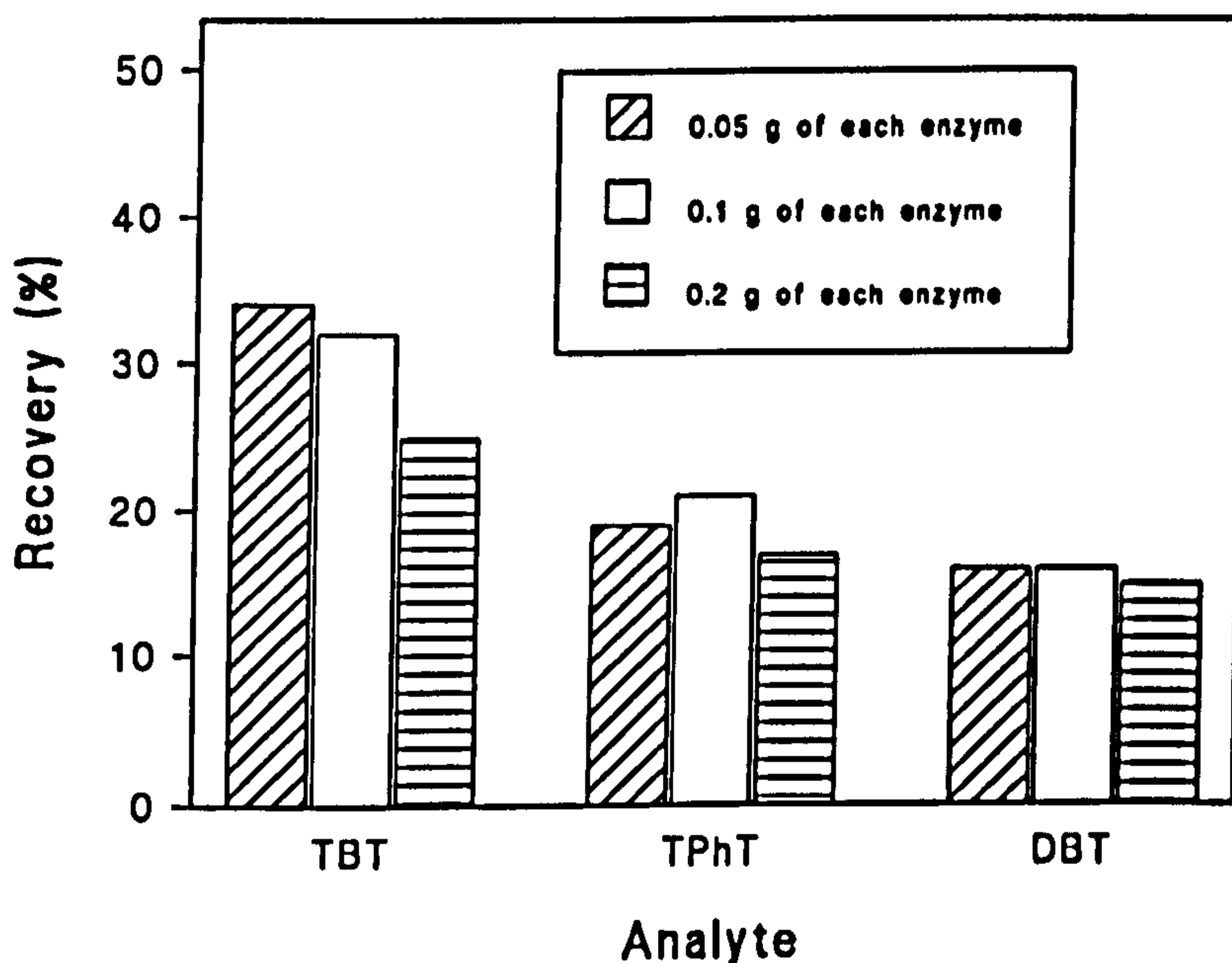


**Figure 4.5(b)** *Typical chromatogram of mussels sample extracted with lipase and protease and dichloromethane as organic solvent (bottom chromatogram) compared with chromatogram of calibrant solution of approx. 5 ng.g<sup>-1</sup> of each analyte (top chromatogram)*

**Table 4.5.** Recovery values for the extraction with lipase+protease enzymatic system, extracting into dichloromethane, without and with a Potter homogeniser

Analyte	TBT	TPhT	DBT	MBT
Without homogeniser	34%	19%	16%	0%
With homogeniser	40%	34%	15%	0%

The amount of enzyme required was also studied. Three amounts were tried: 0.05, 0.1 and 0.2 g of each enzyme. The results can be seen in Figure 4.6. There was no significant difference between them and thus 0.05 g of each enzyme were chosen.



**Figure 4.6.** Recovery values for the enzymatic extraction with lipase and protease with three different amounts of enzymes

The use of the enzymes sequentially, adding lipase first and protease after at least 4 h, was also studied. The aim of this experiment was to check that the protease was really acting on the mussels tissue and not on the lipase only. The results for TBT were no different, being 45 % for the sequential extraction and 46 % for the simultaneous. This latter approach was chosen since it simplifies the method.

The summary of the final extraction procedure is as follows: the necessary amount of sample (about 1 g) was accurately weighed. 0.05 g of lipase and 0.05 g of protease and 40 ml of citric/phosphate buffer solution (pH=7.5) were added. This mixture was homogenised with a potter homogeniser until an homogeneous paste is formed. This mixture is transferred to test tubes and left shaking overnight at 37°C. The aqueous phase was then extracted into 10 ml of dichloromethane, three times. This organic phase was rotary evaporated until dryness and dissolved in 1 ml of methanol and 0.5 ml of mobile phase (70 % MeOH-30 % buffer) prior to the analysis by HPLC-ICP-MS.

A repeatability study on this extraction procedure was carried out. The samples were spiked with approximately 200 ng of each analyte (TBT, TPhT, DBT and MBT) as tin in methanol. This mixture was left for at least 12 h to equilibrate before adding the enzymes and the buffer. The values obtained can be seen in Table 4.6. A typical chromatogram is shown in Figure 4.7.

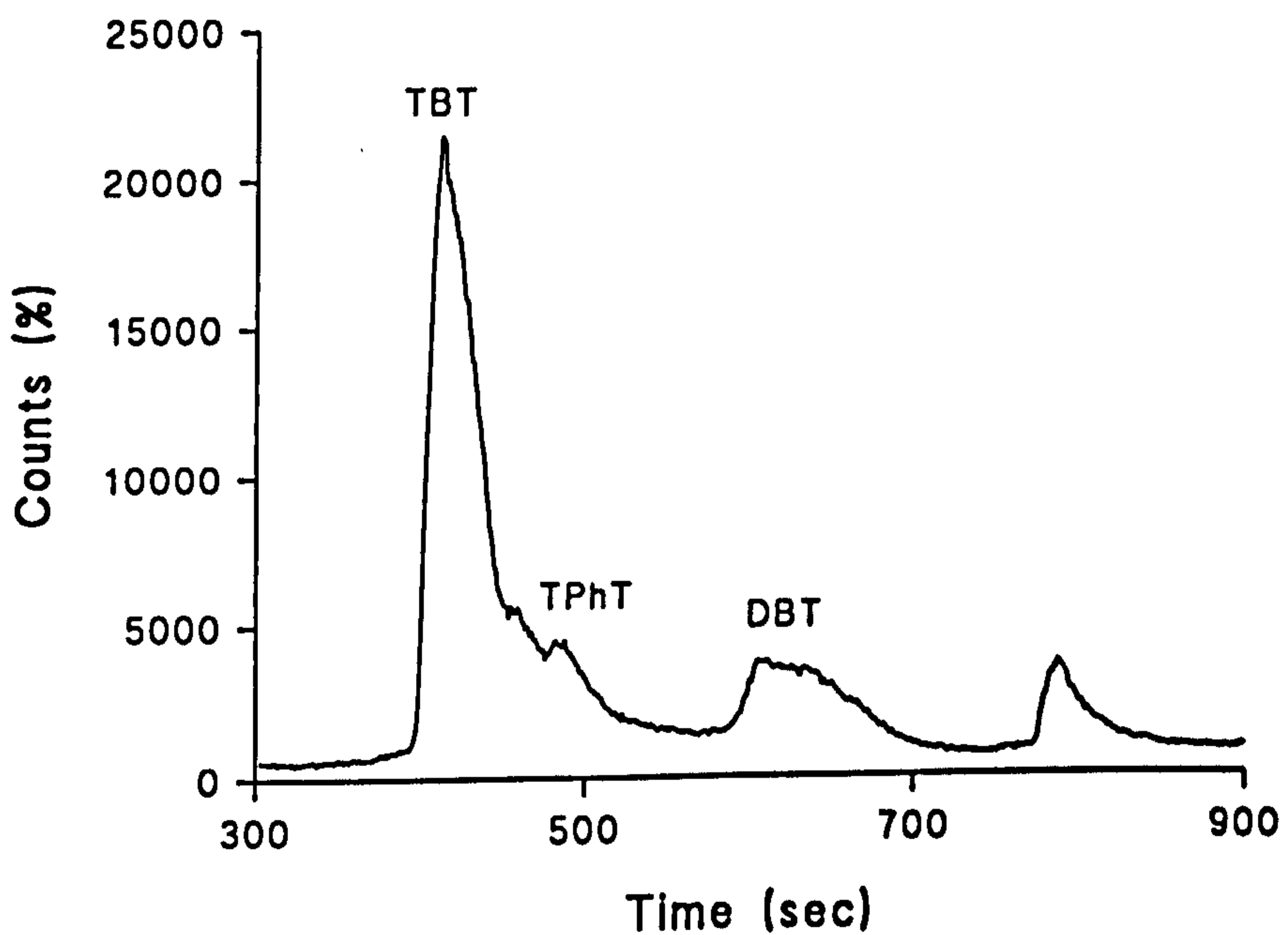
The analysis of fish tissue NIES-11, certified for TBT ( $1.3 \mu\text{g.g}^{-1}$  as TBTCI) and with an indicative value for TPhT ( $6.3 \mu\text{g.g}^{-1}$  as TPhTCI) was analysed to validate the method. This material was the only biological material available for



speciation purposes of organotin compounds. The enzymatic method proved to be adequate for the analysis of this material. The result obtained for TBT (n=6) following this procedure ( $1.29 \pm 0.05 \mu\text{g.g}^{-1}$  as tributyltin chloride) is in good agreement with the certified value.

*Table 4.6. Repeatability values for the enzymatic extraction (see text for details) of mussels (n=10)*

Analyte	Recovery (%)	SD (%)
TBT	36	12
TPhT	40	16
DBT	26	12



*Figure 4.7. Typical chromatogram of mussel sample extracted with lipase and protease enzymatic system (see section 4.1.3)*

A candidate reference material from the BCR (CRM 477) was also analysed following this procedure. This material is under its certification process and although the final certified values are not known, the results for all the analytes were incorporated in the certification campaign of the candidate CRM 477. The results obtained can be seen in Table 4.7.

*Table 4.7. Analysis of mussel candidate reference material CRM 477 (n=6)*

Analyte	TBT ( $\mu\text{g TBT}\cdot\text{g}^{-1}$ dry mussel)	TPhT ( $\mu\text{g TPhT}\cdot\text{g}^{-1}$ dry mussel)	DBT ( $\mu\text{g DBT}\cdot\text{g}^{-1}$ dry mussel)
Mean	2.24	0.84	1.22
S.D.	0.57	0.34	0.33

The concentration values obtained for TBT and TPhT have been calculated using peak height, since these two peaks were not base line resolved. In the case of DBT the peak did not coelute with any other and, thus, peak area was used.

#### 4.1.4. Conclusions

There are several conclusions to be drawn from the previous extraction studies.

In the analysis of sediments, the sample matrix is a very important factor. The organic substances present in the sediment drastically affect the extraction efficiency. The extraction values for the four analytes from the "clean" sediment

from Plymouth (Table 4.1) are very good for TBT and TPhT. The efficiencies are not so good for DBT and MBT; the difficulties on extracting this species are well known and this is one of the reasons for employing complexing agents in the extraction procedure by many authors<sup>(50,88,101,103,106,108,109,111)</sup>. The recoveries for the same analytes from the sediment from the National Research Council of Canada (PACS-1) are much lower for all the analytes (*i.e.* TBT-24% vs 103%). However, once the results are corrected for recovery, the value obtained for TBT is within the certified range. TPhT is not certified in this material and, thus, no conclusion can be drawn from its value. The results for DBT and MBT are lower for DBT and higher for MBT than the certified values. This could be due to the degradation with time of DBT into MBT. Besides, other organotin species coelute with this last peak, which would also account for the higher value found.

The enzymatic extraction with lipase and protease has proved to be the best one for the analysis of mussel tissue. The difference in the recovery values between the analysis of calibrants and real samples show, once more, the difficulties of the analysis of solid samples for speciation purposes. The use of another chromatographic system which would allow the use of complexing agents to help the extraction of the different analytes might improve the overall results. Meanwhile, the extraction procedure shown here, as long as the results are corrected for the recovery, is a valid method for the determination of organotin species in biological samples. This has been proved by the incorporation of the results for TBT, TPhT and DBT in the certification campaign of the candidate mussels tissue CRM 477.

## **4.2. DETERMINATION OF ORGANOLEAD SPECIES**

### **4.2.1. Introduction**

The use of organolead compounds as antiknock agents in petrol is decreasing rapidly. Despite this, and due mainly to the cumulative effect of lead compounds in humans, the monitoring of its concentration remains important. Rain water could be used as one of the sample types that will give an idea of the concentration of organolead species in the environment.

The European Union, through the Standards, Measurements & Testing Programme, promoted several intercomparison exercises between different European laboratories for the analysis of TML in artificial rain water in preparation for a certification exercise.

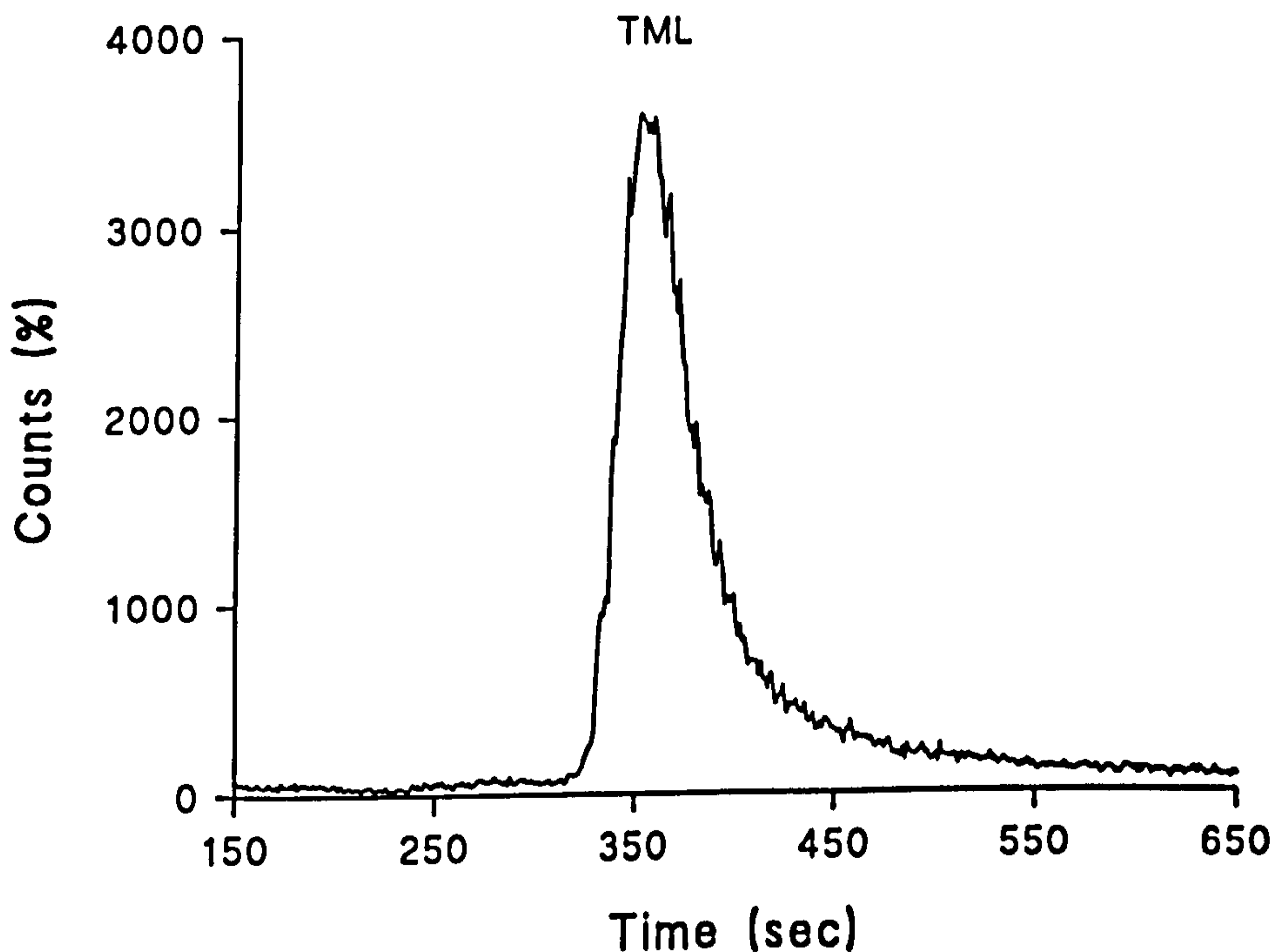
### **4.2.2. Analysis of artificial rain water**

Two solutions were available, the first one being of about 50 ng.ml<sup>-1</sup> of TML as lead, the second of about 5 ng.ml<sup>-1</sup> of TML as lead. Only the first one is above the limit of determination for the HPLC method (30 ng.g<sup>-1</sup> of TML as Pb) without preconcentration. Since aqueous solutions could be injected in the liquid chromatographic system, no extraction procedure was required.

The artificial rain water was analysed using external calibration, employing TML calibrants diluted in 2% nitric acid. 200 µl of the sample were injected. The concentration found following this analysis was of 173 ± 10 ng.ml<sup>-1</sup> of TML as lead. A chromatogram of this sample can be seen in Figure 4.8.

### 4.2.3. Conclusions

There was a big difference between the found and the expected value in the artificial rain water analysis. No errors could be found in the calibration procedure and thus no explanation due to this could be found for this result at this stage. No spectral interference on lead was known. A matrix dependant interference could be speculated. This could be corroborated by the isotope dilution analysis-HPLC-ICP-MS of the same sample (see section 5.2).



*Figure 4.8. Typical chromatogram of artificial rain water*

***5. ISOTOPE DILUTION ANALYSIS-  
HIGH PERFORMANCE LIQUID  
CHROMATOGRAPHY-  
INDUCTIVELY COUPLED  
PLASMA-MASS SPECTROMETRY  
(IDA-HPLC-ICP-MS)***



## **5. ISOTOPE DILUTION ANALYSIS-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETRY (IDA-HPLC-ICP-MS)**

### **5.1. INTRODUCTION**

Isotope dilution analysis is acknowledged to be the best calibration technique. Provided the sample is homogenised with the calibrant (spike) this calibration procedure compensates for different sources of variability, such as loss of analyte during the sample preparation or drifts in the sensitivity of the instrument. When used in conjunction with a powerful separation technique, such as high performance liquid chromatography, and a sensitive detection system (ICP-MS), the final coupling offers a way to speciate different analytes in samples of environmental interest at the low concentration level (frequently below ppb) found in these samples. The overall accuracy and precision of any analysis will be improved by the use of this technique.

Isotope dilution analysis and its coupling with ICP-MS or HPLC-ICP-MS have been described in more detailed in sections 1.6 and 1.7.

## **5.2. PREPARATION OF ISOTOPICALLY ENRICHED COMPOUNDS**

### **5.2.1. Tributyltin iodide**

Tributyltin iodide was selected to be prepared instead of tributyltin chloride due to the greater volatility and toxicity of the required precursor for the reaction,  $\text{SnCl}_4$ . This compound also hydrolyses easily. All these properties and the small amount of isotopically enriched metal Sn available lead to the decision to synthesise tributyltin iodide (TBTI).

Standard tin metal enriched in  $^{116}\text{Sn}$  was obtained from AEA Technology, Oxfordshire, U.K. The isotopic composition of this material was reported to be:  $^{112}\text{Sn}$ -<0.02%;  $^{114}\text{Sn}$ -<0.01%;  $^{115}\text{Sn}$ -0.04%;  $^{116}\text{Sn}$ -98.00%;  $^{117}\text{Sn}$ -0.68%;  $^{118}\text{Sn}$ -0.70%;  $^{119}\text{Sn}$ -0.09%;  $^{120}\text{Sn}$ -0.31%;  $^{122}\text{Sn}$ -0.04%;  $^{124}\text{Sn}$ -0.14%.

The synthesis was based on the methods reviewed by Moedritzer<sup>(24)</sup>. The first step in the synthesis was to prepare  $\text{SnI}_4$  from the isotopically enriched tin, mixing it with iodine in glacial acetic acid.  $\text{SnI}_4$  is an orange solid. This product was purified by recrystallisation from chloroform. The butyl compounds were obtained after Grignard reaction of  $\text{SnI}_4$  with butylmagnesium bromide ( $\text{BuMgBr}$ ) prepared from butylbromide ( $\text{BuBr}$ ) and magnesium in ether. A mixture of tetrabutyltin, tributyltin iodide, dibutyltin iodide and monobutyltin iodide was obtained. This mixture was purified by silica gel separation with dichloromethane. Tetrabutyltin was thus separated from the other species. A disproportionation reaction was then performed by reacting the TetraBT with the remaining  $\text{SnI}_4$  isotopically enriched at  $220^\circ\text{C}$  under nitrogen to avoid decomposition of the products. Again, a mixture of

TBTI (major product), TetraBT (second major product) and DBTI<sub>2</sub> and MBTI<sub>3</sub>, was obtained. This mixture was run through a silica column and eluted with heptane. This procedure separated TetraBT and TBTI from DBTI<sub>2</sub> and MBTI<sub>3</sub>, but the first two species could not be separated from each other by this column chromatographic system.

The purity of the mixture was checked by nuclear magnetic resonance spectrometry (NMR) of <sup>1</sup>H and <sup>13</sup>C and compared with commercially available calibrants. The <sup>13</sup>C spectrum of this mixture can be seen in Figure 5.1. A relevant characteristic of this spectrum is the absence of satellite peaks. The isotopically enriched tin in <sup>116</sup>Sn has a very low abundance of the spin active isotopes (<sup>115</sup>Sn, <sup>117</sup>Sn and <sup>119</sup>Sn) and their effect is not observed. The mixture consists of tributyltin iodide and tetrabutyltin, being 72% of TBTI and 27% of TetraBT with less than 1% of other impurities. The overall yield of the reaction for TBTI was 18%.

**Modifications in the chromatographic procedure:** The chromatographic system described in section 3.1 had to be modified in order to separate TetraBT from TBTI. With a mobile phase of 70% MeOH and 30% citrate buffer, pH=5.8, TetraBT elutes at the solvent front. This peak is broad enough to effectively coelute with TBTI (Figure 5.2). A decrease in the methanol content of the first step of the gradient was studied with isocratic elution. This amount of methanol should not be so low as to retard the elution of TBT so much as to make this peak coelute with the TPhT species which may exist in the samples analysed. At 60% MeOH content in the mobile phase, TetraBT was separated from TBT (Figure 5.3). From this chromatogram and previous studies (Figure 3.11-a) it can be concluded that an

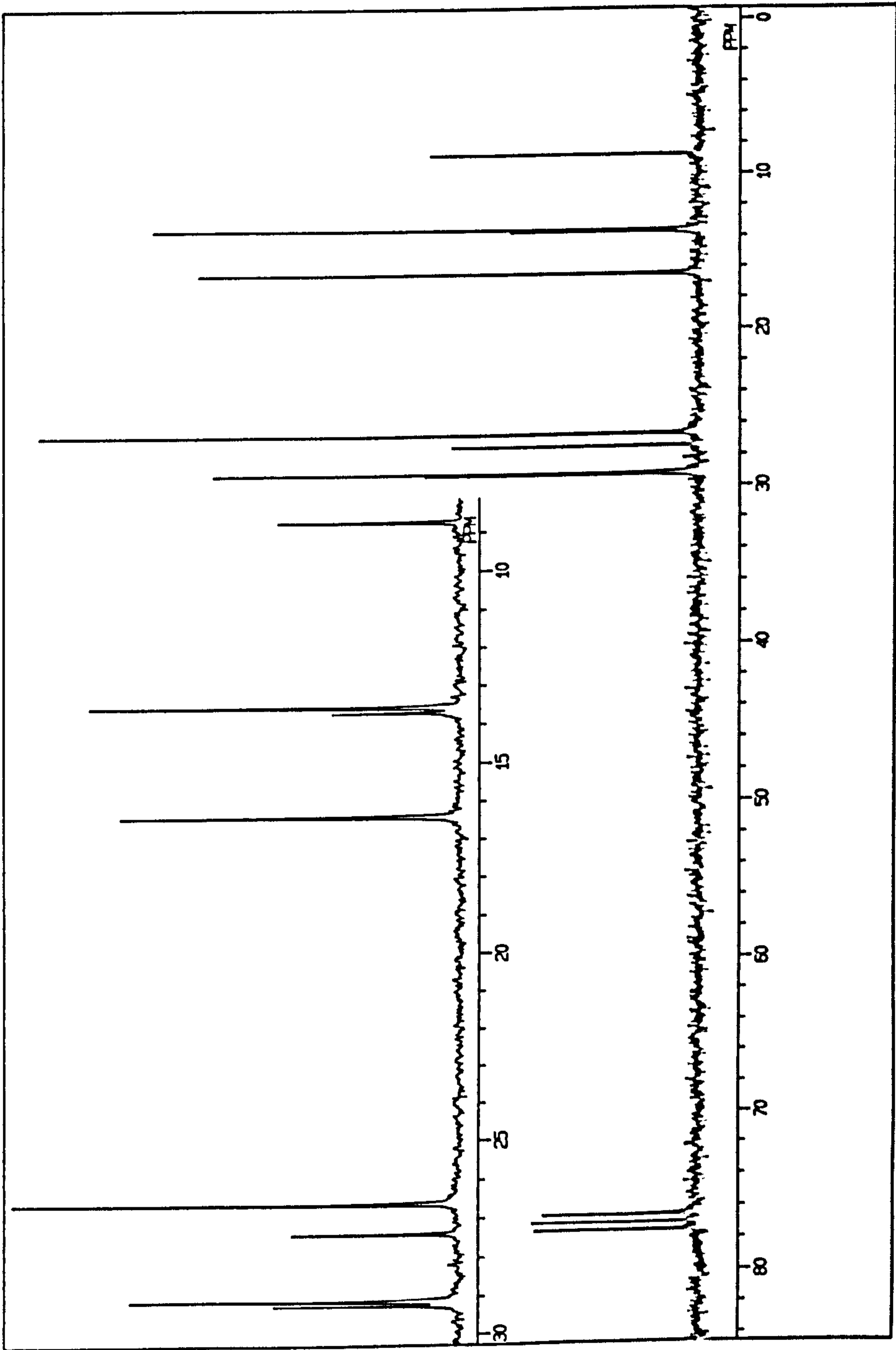
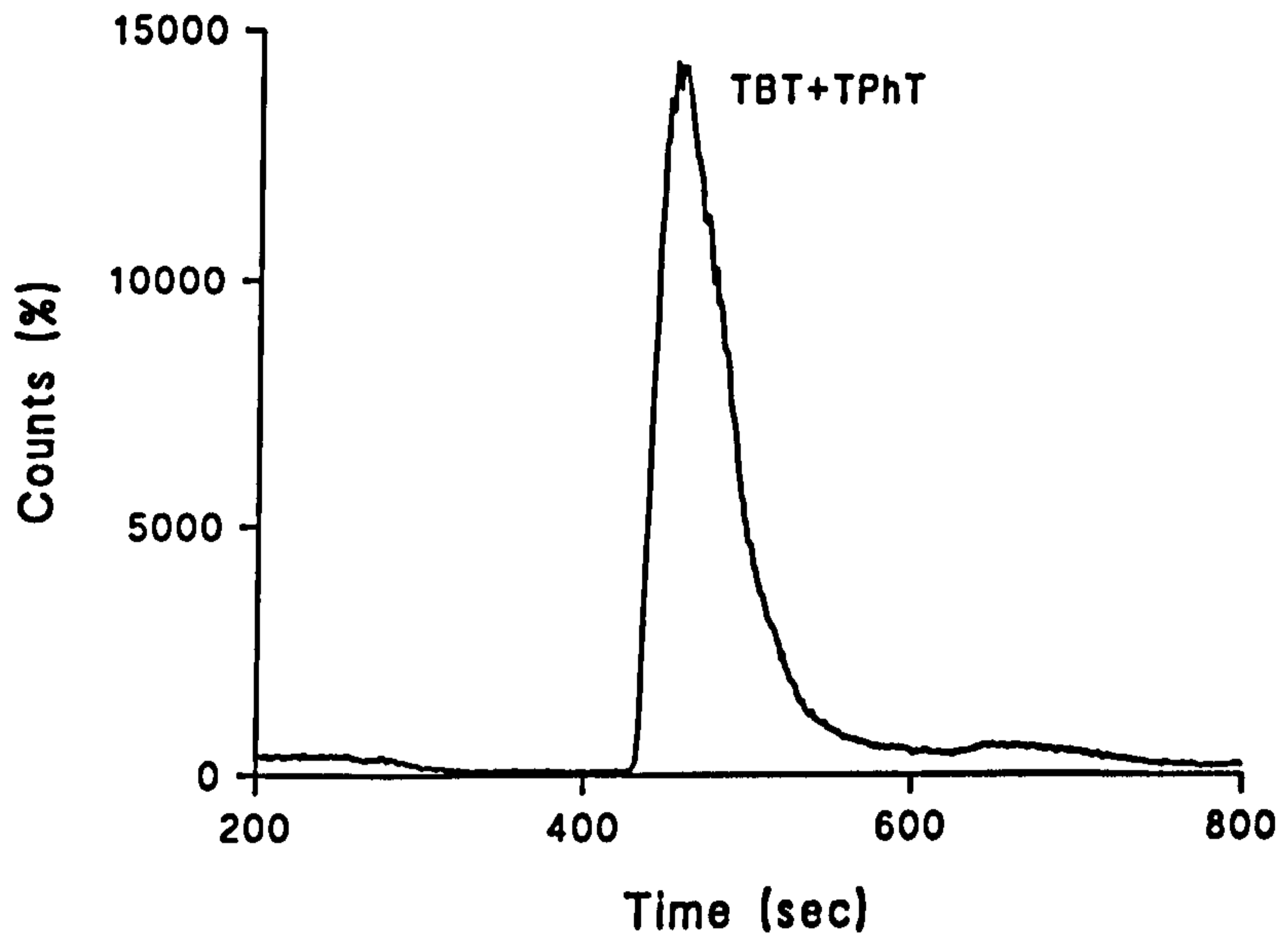
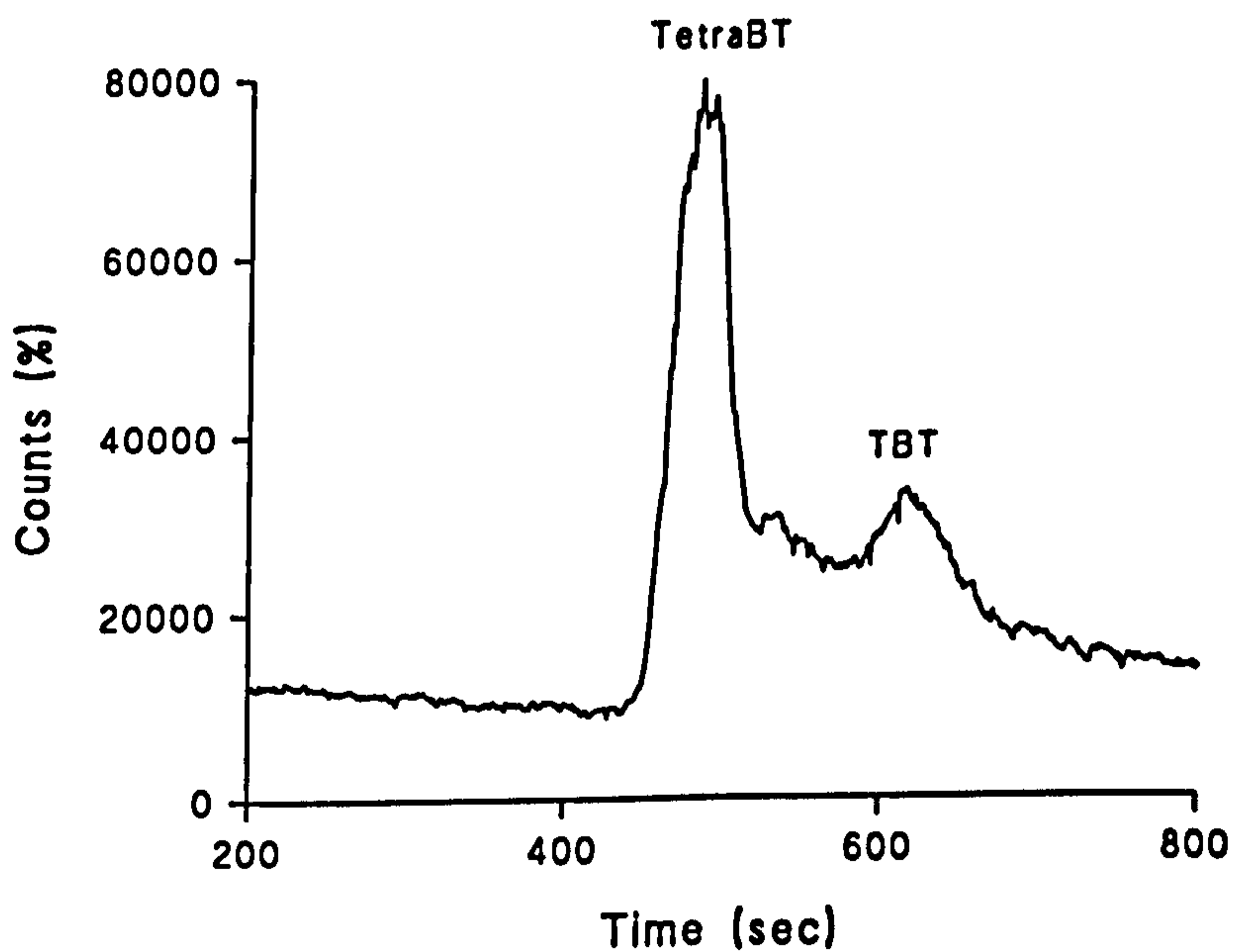


Figure S.1.  $^{13}\text{C}$  spectrum of TBPI and TetraBT isotopically enriched in  $^{116}\text{Sn}$

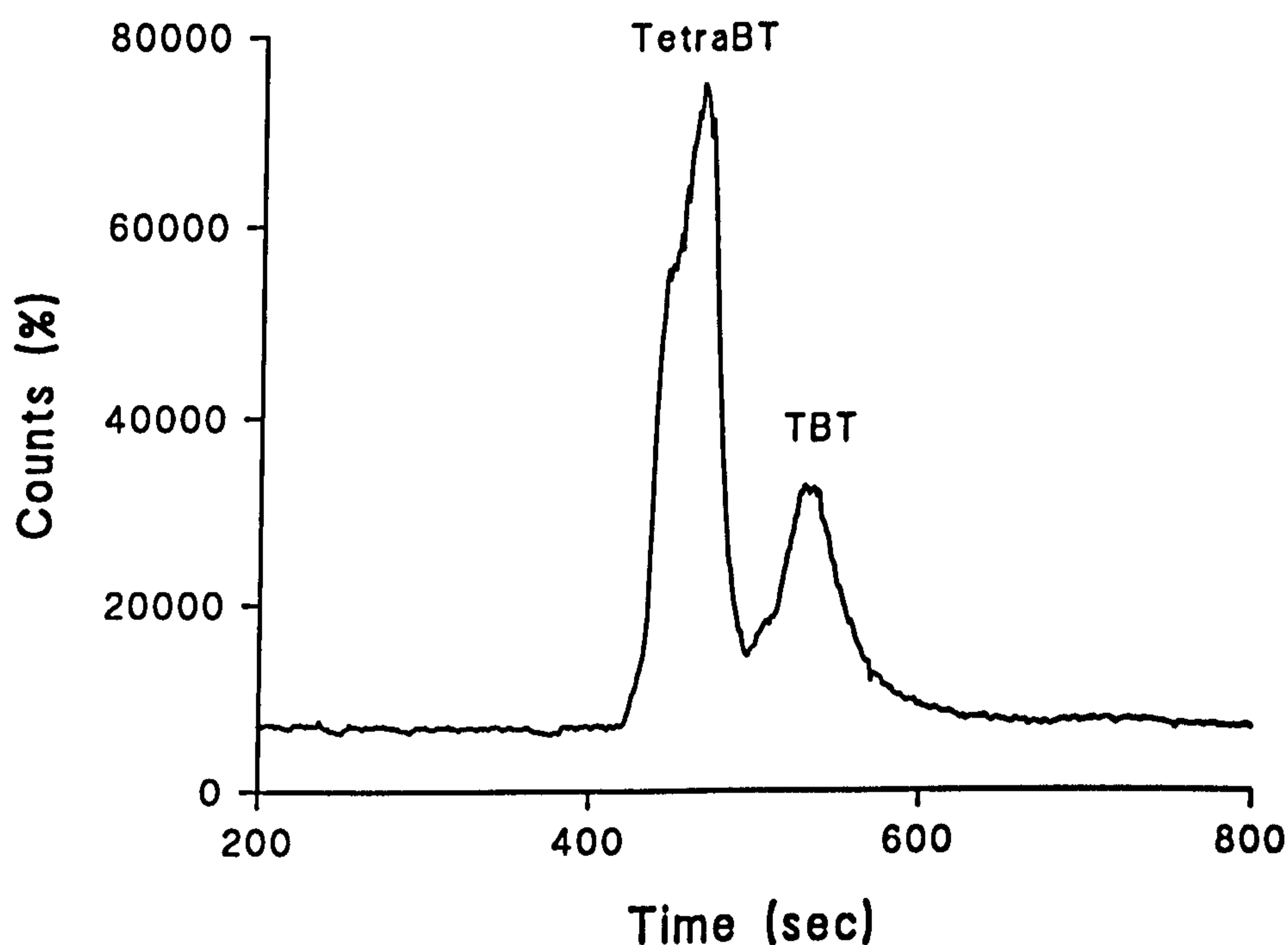


*Figure 5.2. Chromatogram of TBT and TetraBT, isocratic elution, showing coelution. Mobile phase: 70% MeOH, 30% citrate buffer, pH=5.8*



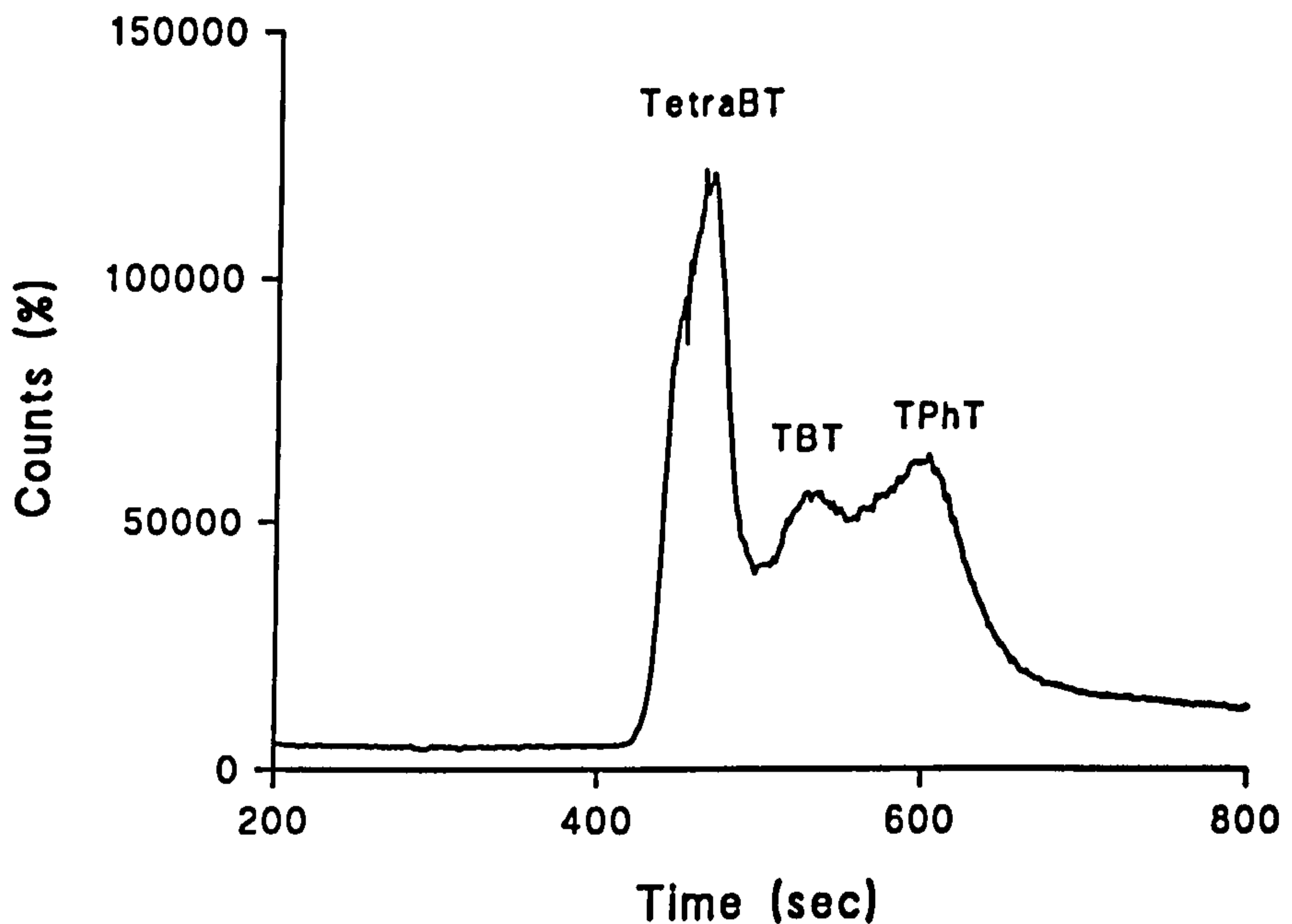
*Figure 5.3. Chromatogram of TBT and TetraBT, isocratic elution. Mobile phase: 60% MeOH, 40% citrate buffer, pH=5.8*

intermediate content in methanol should be used to avoid coelution of TBT and TPhT. Thus, a 65% MeOH and 35% buffer solution, pH=5.8, was tried. Figure 5.4 shows the separation obtained and Figure 5.5 the separation between TetraBT, TBT and TPhT. The peaks are not base line resolved but the separation was accepted as a compromise situation. Peak height would be used for quantification purposes.



*Figure 5.4. Chromatogram of TBT and TetraBT. Mobile phase: 65% MeOH, 35% citrate buffer, pH=5.8. Isocratic elution*





**Figure 5.5.** *Chromatogram of TBT, TPhT and TetraBT. Mobile phase: 65% MeOH, 35% citrate buffer, pH=5.8*

### 5.2.2. Trimethyllead chloride

This material was already available in the laboratory from previous isotope dilution studies<sup>(236)</sup>. A certified reference material SRM NIST 983, enriched in <sup>206</sup>Pb, was purchased as starting material and PbCl<sub>2</sub> was synthesised from this metal. The next step was a Grignard reaction with methylmagnesium iodide (MeMgI). Trimethyllead was purified by recrystallisation from hot ethyl acetate. The final product was a white crystalline solid. The purity of the final trimethyllead chloride compound was checked against commercially available TMLCl by nuclear magnetic resonance of <sup>1</sup>H, <sup>13</sup>C and <sup>207</sup>Pb.

### **5.3. IDA-HPLC-ICP-MS DETERMINATIONS**

#### **5.3.1. Organotin species**

The parameters of the time resolved acquisition part of the software had to be optimised to obtain the best accuracy and precision in the isotope ratio 120/116. The only parameter which had a real effect was the time per slice,  $t_s$ , which is the time that the detector takes on acquiring a data point used to complete the chromatogram. If this time is too low, the counts observed will be greatly affected by the background noise. If too big, the counts may not represent the real counts for the analyte at that elution time since the intensity may be also due to contamination in the eluent.

A mixture of TBT and TPhT of approximately 200 ng.g<sup>-1</sup> each analyte as Sn in the mobile phase was injected and different  $t_s$  studied (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 sec). The isotopic ratio 120/116 at each  $t_s$ , using peak height, was calculated. The results can be seen in Figure 5.6-a for TBT and Figure 5.6-b for TPhT. The X-axis crosses the Y-axis at the expected ratio. In both cases the accuracy is lost for  $t_s$  higher than 1.0 sec.  $t_s=0.8$  or 1.0 sec are the ones which gave the best accuracy.  $t_s=1.0$  sec (the default value in the software) was chosen since there was no significant difference between the two  $t_s$  either in accuracy or precision.

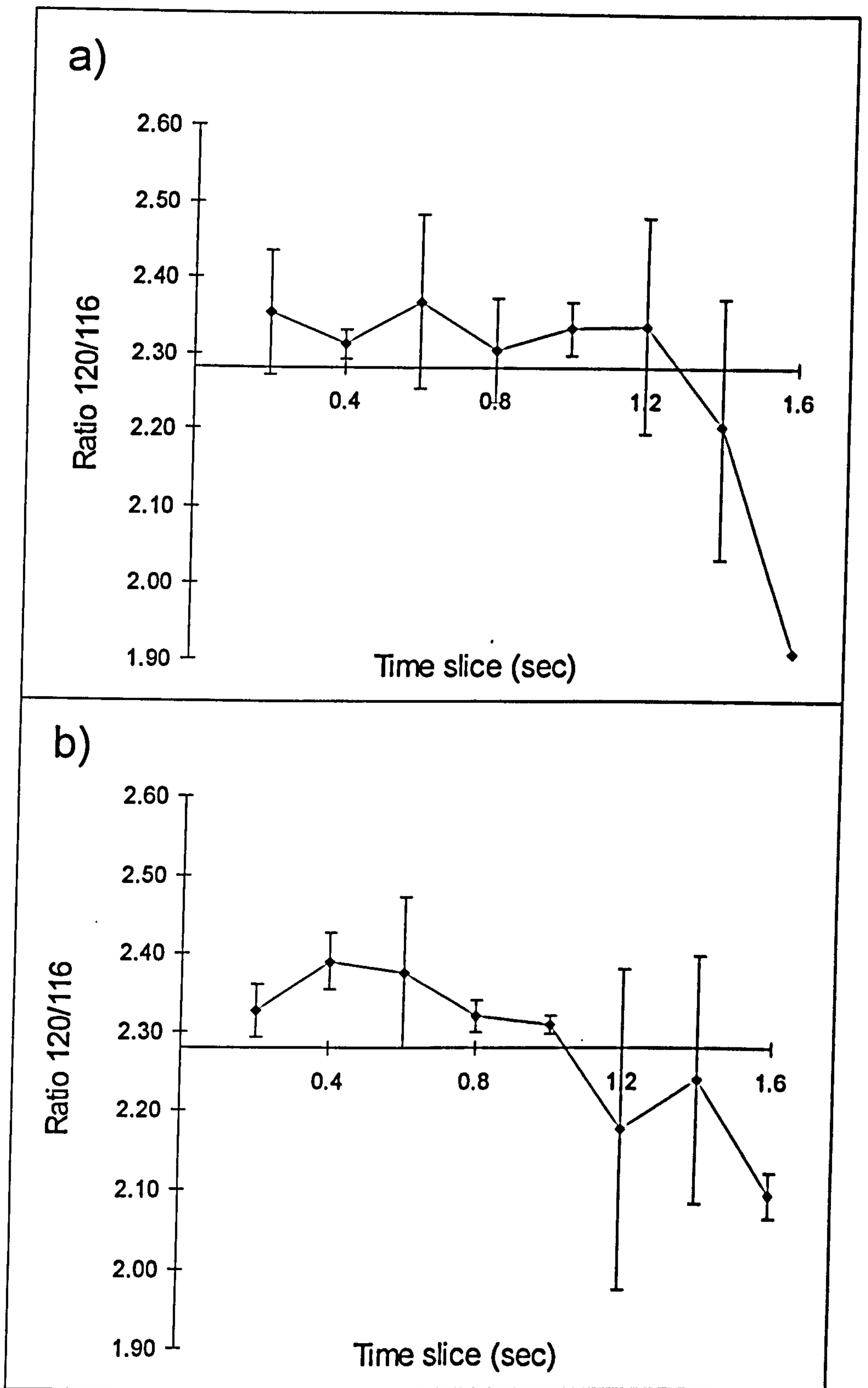
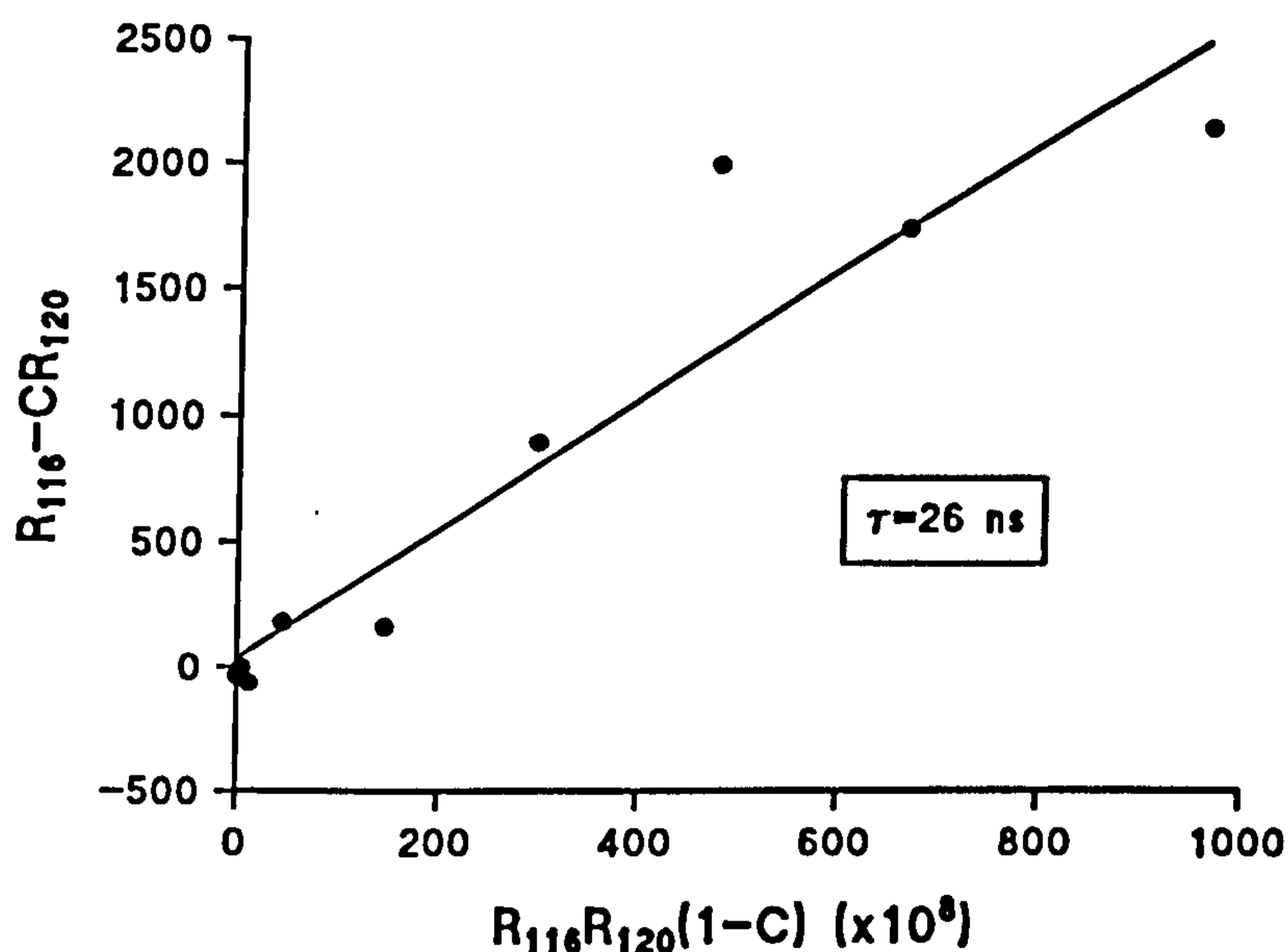


Figure 5.6. Effect of time per slice on isotope ratio 120/116: (a) TBT, (b) TPhT

The detector dead time ( $\tau$ ) (section 1.6) had to be calculated. Solutions of 1, 5, 10, 20, 30, 50, 65, 80 and 100 ng.ml<sup>-1</sup> of Sn normal abundance in 2% HNO<sub>3</sub> were prepared. The ratio 116/120 was calculated (blank subtracted) for all of them and  $\tau$  calculated using Eq. 1.20 (Figure 5.7). The value obtained was  $\tau=26$  ns.



*Figure 5.7. Empirical calculation of the detector dead time ( $\tau$ ) for organotin determinations*

The mass discrimination factor (K) was also calculated (Eq. 1.17) everyday with a solution of known isotopic composition in <sup>120</sup>Sn and <sup>116</sup>Sn. It can be clearly seen in Figure 5.6-a that there is a difference between the true isotope ratio and the experimental one. K was normally calculated using a solution of TBTCI, approximately 100 ng.g<sup>-1</sup> as Sn, normal abundance. This value of K was used to correct the experimental isotope ratios following Eq. 1.18. The isotope ratio corrected for mass discrimination was then used for the isotope dilution analysis calculations.

The mixture of TBTI and TetraBT enriched in  $^{116}\text{Sn}$ , prepared as described above, was dissolved in methanol. In order to know its exact concentration in TBTI, this solution was analysed by reverse isotope dilution. An aliquot of this solution was spiked with TBTCl of normal isotopic abundance ( $^{120}\text{Sn}$ -34.8%,  $^{116}\text{Sn}$ -14.4%) and diluted to a suitable concentration with mobile phase 65% MeOH, 35% buffer, pH=5.8 and five replicates were injected. The concentration found for the original solution of TBTI and TetraBT was  $23.55 \pm 1.08 \mu\text{g.g}^{-1}$ , as Sn, of TBTI isotopically enriched. Appropriate dilutions of this solution were further employed in the isotope dilution analysis.

In order to validate the performance of this methodology, the fish tissue NIES-11 was analysed by ID-HPLC-ICP-MS. The extraction procedure was the same as that previously described in section 4.1.3.3. The tissue had been spiked with the appropriate amount of TBTI and left to equilibrate for 12 h. The isotope ratio obtained for each replicate (n=5) was corrected for the mass discrimination factor and the concentration of TBT as Sn calculated using Eq. 1.16. The certified value for TBT as TBTCl is  $1.3 \pm 0.1 \mu\text{g.g}^{-1}$ . The value obtained using IDA-HPLC-ICP-MS was  $1.2 \pm 0.1 \mu\text{g.g}^{-1}$ . This material had also been analysed with external calibration ( $1.29 \pm 0.05 \mu\text{g.g}^{-1}$ , section 4.1.3) with a good agreement between the certified value and that obtained with normal calibration. The analysis with IDA-HPLC-ICP-MS reduces the risks of losses of analyte. The calculations involved in the isotope dilution analysis are simpler, and thus less prone to errors, than the ones with external calibration. This makes the overall process less time consuming. These results show the suitability of this method for the analysis of this material.

The candidate reference material CRM 477 was also analysed with IDA-HPLC-ICP-MS. Again, the mussel tissue was re-wet and then spiked with the appropriate amount of TBTI and left to equilibrate at least 12 h. The extraction procedure was the same than the previously described (section 4.1.3.3.). As usual, the isotopic ratios were corrected for the mass discrimination value and the concentration obtained following Eq. 1.16. The value obtained (n=6) was  $2.48 \pm 0.19 \mu\text{g}\cdot\text{g}^{-1}$  of TBT. There is a significant improvement in the precision obtained using IDA-HPLC-ICP-MS (RSD=7.7%) and using external calibration ( $2.24 \pm 0.57 \mu\text{g}\cdot\text{g}^{-1}$ , RSD=25%). Both values are within the range of mean of means ( $2.20 \pm 0.35 \mu\text{g}\cdot\text{g}^{-1}$ ) of the different laboratories which results were accepted for the certification of this material. As with the reference material NIES-11, the good agreement between the result with IDA-HPLC-ICP-MS and the value obtained with external calibration proves the suitability of IDA-HPLC-ICP-MS.

### 5.3.2. Organolead species

As for organotin species determinations, the best time per slice for organolead compounds analysis had to be selected. Trimethyllead chloride from the equal-atom lead isotopic material SRM NIST 982 (certified as  $R_{208/206} = 1.00016 \pm 0.00036$ ), prepared as described above, was used for this experiment. Times per slice of 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 sec were tested. The isotope ratio 208/206 was calculated using peak height at each  $t_r$  (Figure 5.8). The X-axis in this representation crosses the Y-axis at the expected isotopic ratio. Figure 5.8 shows that the precision is approximately the same for any  $t_r$  higher than 0.6 sec. 0.8 sec was chosen for further studies since no significant difference can



be observed in accuracy either.

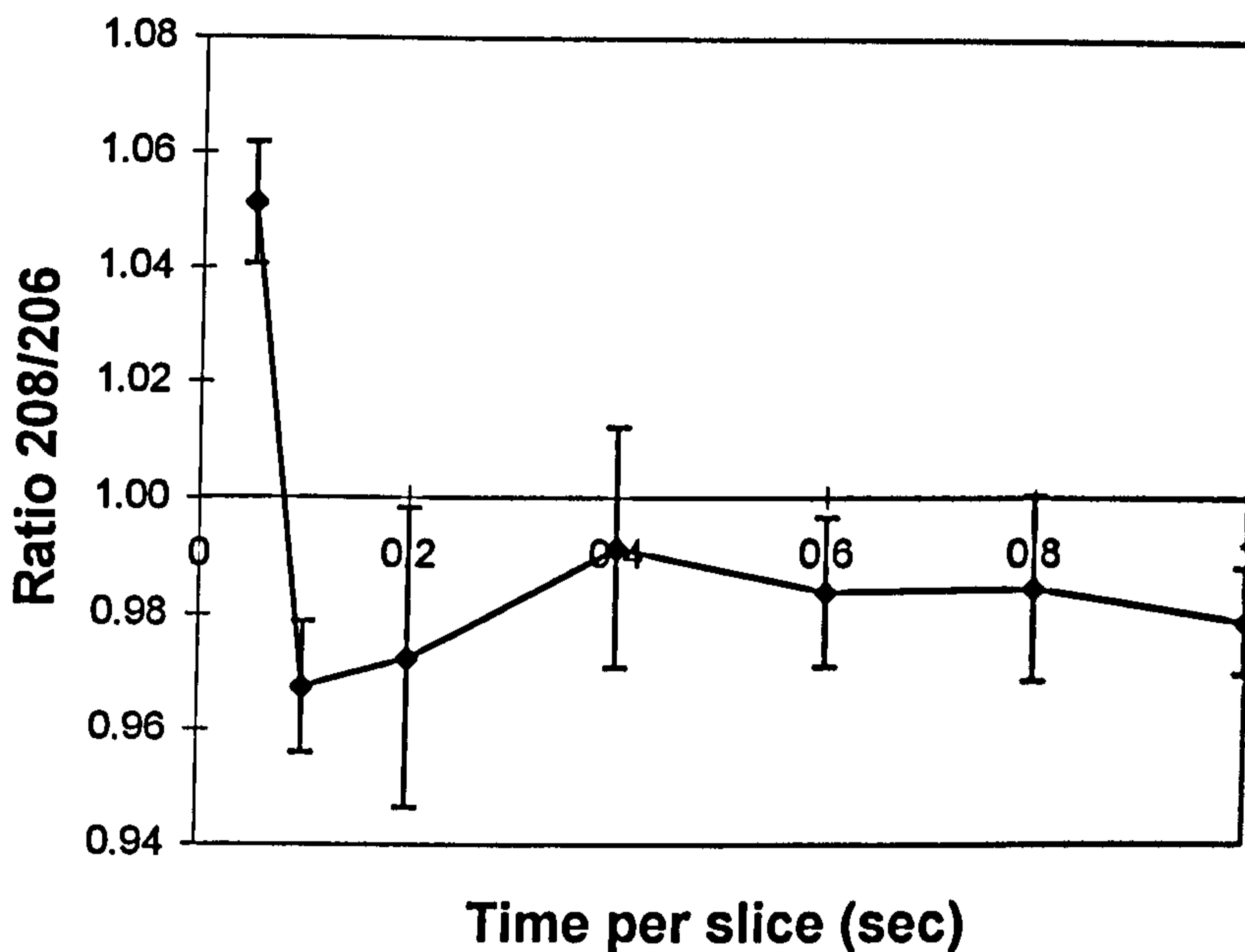
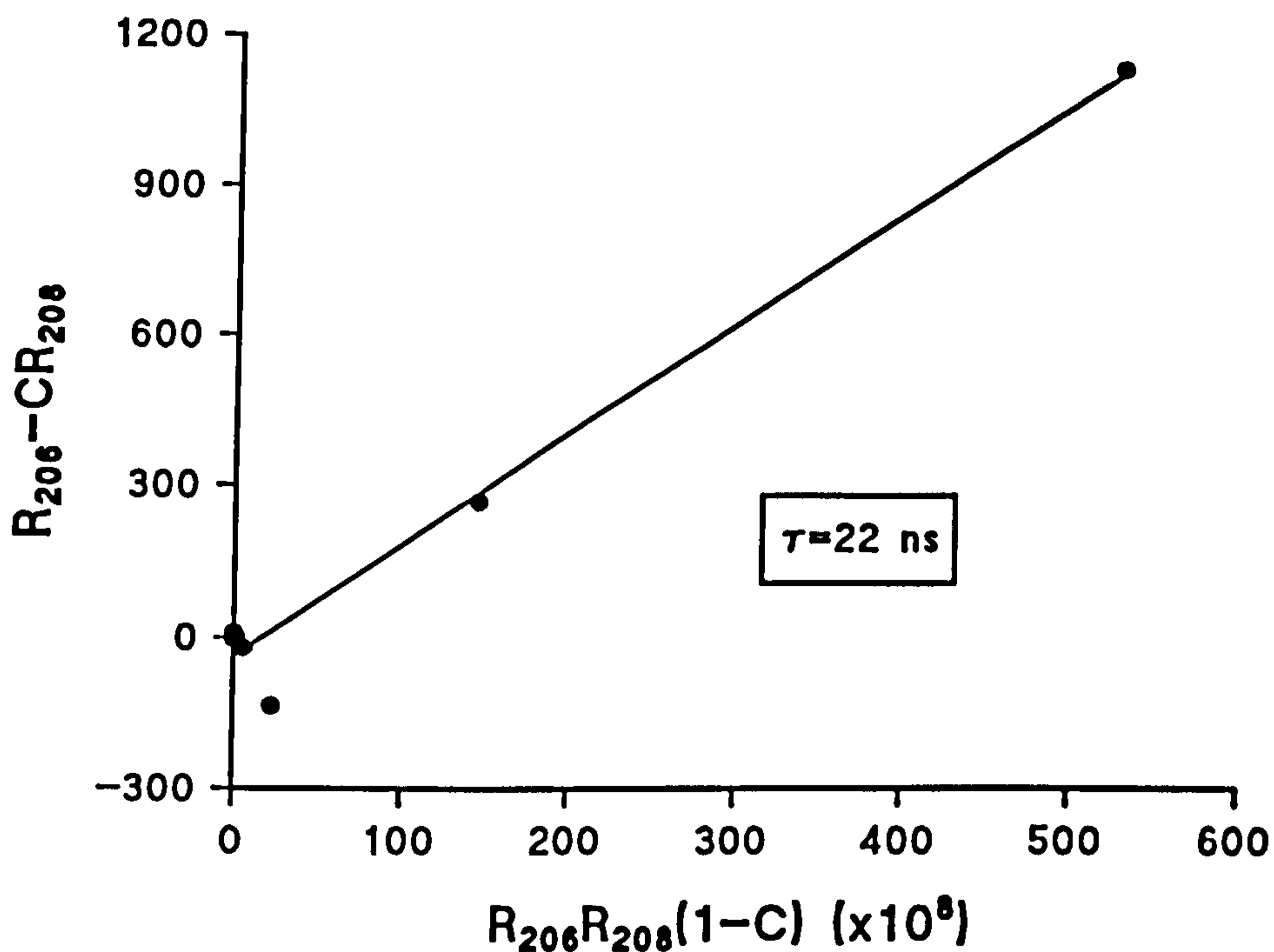


Figure 5.8. Effect of time per slice on isotopic ratio 208/206 for TML

The detector dead time ( $\tau$ ) was calculated for the ratio 206/208 with Eq. 1.20. A solution available in the laboratory of SRM NIST 981 certified reference material of Pb (lead common isotopic abundance (in percentage):  $^{204}\text{Pb}$ -1.4255 $\pm$ 0.0012,  $^{206}\text{Pb}$ -24.1442 $\pm$ 0.0057,  $^{207}\text{Pb}$ -22.0833 $\pm$ 0.0027,  $^{208}\text{Pb}$ -52.3470 $\pm$ 0.0086) was used for this experiment. Solutions of different concentrations (100 and 500  $\mu\text{g}\cdot\text{ml}^{-1}$ , 1, 5, 10, 50 and 100  $\text{ng}\cdot\text{ml}^{-1}$ ) were prepared in 2%  $\text{HNO}_3$ . The isotopic ratio was blank subtracted in all the cases.  $\tau$  was calculated as for organotin determinations following Eq. 1.20 (Figure 5.9). The empirical value thus obtained for  $\tau$  was 22 ns. This value was used thereafter for all IDA-HPLC-ICP-MS determinations of organolead compounds.



**Figure 5.9.** Empirical calculation of the detector dead time ( $\tau$ ) for organolead determinations

The mass discrimination factor (K) was calculated every day with a solution of TMLCl of normal isotopic abundance. It can be seen in Figure 5.8 that not even in the case of the ideal situation to obtain the best accuracy in the isotopic ratio ( $R_{208/206} = 1$ ) was the ratio obtained the true one. This value of K was used to correct the experimental ratio of 208/206 obtained.

In order to know the exact concentration of TML in the trimethyllead chloride solution enriched in  $^{206}\text{Pb}$  available in the laboratory, this solution was analysed by reverse IDA-HPLC-ICP-MS. An aliquot of this solution was spiked with

a known amount of TMLCl of normal isotopic abundance. The isotope ratios were obtained using both peak height and peak area. Peak area determinations lead to better precision and this was used for future determinations. The value obtained was  $49.73 \pm 0.81 \mu\text{g.g}^{-1}$  of TML enriched in  $^{206}\text{Pb}$  as Pb.

The two artificial rain water solutions mentioned in section 4.2.2 were analysed using IDA-HPLC-ICP-MS. The rain water of approximate concentration  $50 \text{ ng.ml}^{-1}$  of TML had previously been analysed for an intercomparison exercise organised by the Standards, Measurements & Testing Programme. The value found as mean of the different determinations was  $42.75 \pm 4.40 \text{ ng.ml}^{-1(216)}$ . After the IDA-HPLC-ICP-MS determination ( $n=6$ ) of this solution the concentration obtained was  $41.23 \pm 2.23 \text{ ng.ml}^{-1}$ . This solution was spiked with TMLCl enriched in  $^{206}\text{Pb}$  on the basis that the concentration was the one found in the analysis of the same material with external calibration (section 4.2.2). Thus, the rain water had been then overspiked ( $R_{208/206} < 1$ ) since this analysis gave a higher value for the concentration of TML than the value obtained as mean of the means for the different laboratories involved in the intercomparison exercise.

The second solution of artificial rain water had a concentration of approximately  $5 \text{ ng.ml}^{-1}$  of TML as Pb. Although this value was close to the LOD ( $3 \text{ ng.g}^{-1}$  as lead for TML for the conventional chromatographic method the sample was also analysed. The concentration obtained ( $n=4$ ) was  $5.5 \pm 0.5 \text{ ng.ml}^{-1}$ . This value corroborates well with the approximate concentration of the sample.

#### **5.4. CONCLUSIONS**

IDA-HPLC-ICP-MS has proved to be a suitable method to analyse biological material as shown by the analysis of NIES-11 and the candidate CRM 477. The concentration values obtained for the reference material NIES-11 using either external calibration ( $1.29 \pm 0.05 \mu\text{g.g}^{-1}$ ) or IDA-HPLC-ICP-MS ( $1.2 \pm 0.1 \mu\text{g.g}^{-1}$ ) agree with the certified value ( $1.3 \pm 0.1 \mu\text{g.g}^{-1}$ ). In the case of the candidate CRM 477 the concentration after IDA-HPLC-ICP-MS ( $2.48 \pm 0.19 \mu\text{g.g}^{-1}$  of TBT) matches the value obtained with external calibration ( $2.24 \pm 0.57 \mu\text{g.g}^{-1}$ ). IDA-HPLC-ICP-MS give a better precision in the analysis (7.7% with IDA-HPLC-ICP-MS vs 25.5% obtained with external calibration). Both values are in good agreement with the results obtained by other laboratories in the analysis of this material for its certification and in fact they were included in the final results for the certified value of this material.

Isotope dilution analysis is a way of compensating for different sources of variability, as mentioned above (section 1.6). The concentration obtained with external calibration for the artificial rain water is far from the real value<sup>(216)</sup>. A possible explanation for the difference can be due to matrix effects. These effects are compensated with the use of isotope dilution as calibration. A semiquantitative analysis of the artificial rain water was performed. The other components of the sample, besides lead, were Ca ( $\approx 1500 \text{ ng.ml}^{-1}$ ) and Mg ( $\approx 525 \text{ ng.ml}^{-1}$ ). Although none of these analytes are known to interfere with lead, the "enhancing" effect was observed every time the artificial rain water was analysed with external calibration. No explanation has yet been found for this effect.

Although under and overspiking may lead to worse precision on the analysis of a sample (section 1.6), spiking the sample for its analysis by ID-HPLC-ICP-MS so that the isotopic ratio will be exactly one is not required. The analysis of the artificial rain water, where the ratios were  $< 1$  in all the cases, shows that this is not an essential requirement. This maybe due to the fact that the uncertainty due to the chromatographic separation may be greater than the errors introduced when over or under spiking. IDA-ICP-MS may require, though, a more accurate spiking if better precision is an objective of the analysis, since the best precision should be obtained when the isotope ratio is unity.

The incorporation of isotope dilution analysis also improves the sensitivity of the methodology. The use of IDA removes a number of sources of uncertainty from the measurements, such as percentage recovery and short term instrumental drift. This can be illustrated by the analysis of the certified rain water. The limit of detection ( $3\sigma$ ) by the conventional HPLC-ICP-MS method was  $3 \text{ ng.g}^{-1}$  as lead for TML whereas the estimated limit of detection for TML using IDA-HPLC-ICP-MS was  $1.5 \text{ ng.g}^{-1}$ .

***6. CONCLUSIONS AND  
SUGGESTIONS FOR  
FUTURE WORK***



## **6. CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK**

### **6.1. CONCLUSIONS**

Chromatographic separations have been developed for both organotin and organolead compounds and the analytical characteristics (sensitivity, repeatability and linear range) evaluated. The interface between the HPLC and the ICP-MS for the organotin species separation has been improved. Different types of chromatography separations have been studied for the separation of TBT, TPhT, DBT and MBT and the most suitable system among them selected. This study has taken into consideration possible problems, such as the amount of inorganic tin found in the samples or the difficulty of extraction some of the analytes from the samples. Thus, the effect of complexing agents on the chromatographic system, to help the extraction efficiency, has been evaluated.

Cation-exchange chromatography (2 columns of Partisil-10 SCX, 10  $\mu\text{m}$ , 250x4.6 mm each) lead to the best separation for the organotin species. The use of step gradients helped the elution of some of DBT and MBT. The latter would not be eluted without the pH step gradient applied.

Ion-pairing chromatography proved to be a very effective way of separating TML and TEL and also from the large amounts of inorganic lead that may exist in the samples.

The use of the chromatographic system developed for organotin compounds together with an extraction procedure based on the use of glacial acetic acid showed the suitability of the system to analyse samples of environmental interest, such as marine sediments, and recovery and repeatability studies were performed. The analysis of another type of sample (biological samples) has also been studied. Different extraction procedures were evaluated and the best one, regarding extraction efficiency, simplicity and suitability (to eliminate some matrix effects) for that type of sample was chosen. A certified reference material (fish tissue NIES-11) was analysed to prove the validity of the method for the determination of TBT. The extraction procedure and the chromatographic separation have been used in the analysis of a mussel candidate reference material (CRM 477) from the Standards, Measurements & Testing Programme (formerly BCR). The results obtained for TBT, TPhT and DBT have been included in the certification campaign of this material since they agree with the values obtained by other international laboratories. Unfortunately TPhT will only be an indicative value because of recovery problems. This shows the difficulties found by these expert laboratories in analysing such compound. TBT and TPhT concentrations were calculated using peak height since the peaks are not base line resolved. DBT peak does not coelute with any other and, thus, peak area was used for the calculation.

Artificial rain water has also been analysed for TML. The results showed the suitability of the chromatographic separation for the analysis of organolead species in environmental samples, even in the presence of large amounts of inorganic lead in samples.

Finally, isotope dilution analysis was incorporated to the determination procedure of two of the analytes of interest, TBT and TML, in the analysis of several samples. The parameters related to isotope dilution analysis were optimised and/or calculated. The analysis of real samples (fish tissue NIES-11 and mussel tissue CRM 477 for TBT and two artificial rain waters for TML) were performed using IDA-HPLC-ICP-MS. The results proved the validity of the overall procedure for both determinations. Again, the results obtained for TBT in the analysis of CRM 477 with IDA-HPLC-ICP-MS have been incorporated in the certification campaign of this material. IDA has proved to be an effective and simpler way of analysis. Although this type of analysis may require a rather large initial investment, the cost per analysis is low. The method is less prone to errors since any losses in the extraction procedure and/or drift in the instrumentation will be compensated. Calculations are also more simplified than for routine analysis with external calibration. In general, it can be concluded that the precision obtained with IDA is better than with other calibration methods, especially for the analysis of solid samples (25% for the analysis of TBT with external calibration vs 7.7% with IDA-HPLC-ICP-MS). IDA corrects for recovery and extraction efficiency problems and, thus, the uncertainty in the methodology is lower. The major disadvantage of this approach is the need to synthesise altered isotopic composition analytes, often from milligrams amounts of expensive starting materials. Although the initial cost to do isotope dilution analysis is big, the actual cost per analysis is low since the initial cost is spread during many analyses.

## **6.2 FUTURE WORK**

More work on the separation procedures for organotin compounds could be carried out. The study of other separation systems, such as capillary electrophoresis could be profitable. Capillary liquid chromatography can also be an advantageous system. In these two cases the separation between the different analyte peaks will be improved due to the inherent characteristics of capillary chromatography. The sample volume required will be lower, which will lead to less sample required but major difficulties with detection. More studies could also be done on the mixed properties (ion-exchange and reverse phase) chromatography and/or reverse-phase chromatography. These studies should aim to assess the possibility of employing complexing agents in the extraction procedures. This will be beneficial for the extraction of the organotin analytes from complex matrix samples, especially MBT.

TML can be determined in real samples with the procedure described above. Some of the samples of interest will present low concentration levels of organolead species. Preconcentration procedures should be studied. This may involve the use of solid phase extraction systems. The analysis of solid samples can also be attempted; suitable extraction procedures should be developed for this purpose. Solid phase extraction could also be employed after the extraction of the analytes from the solid sample and thus to preconcentrate or clean up (if necessary) the sample.

The preparation of pure isotopically enriched compounds, such as TPhT, DBT and MBT (or other organometallic species) could be attempted. The increasing demand for the accurate determination of these analytes in several environmental

samples will benefit from this step. Isotope dilution analysis could be then incorporated in the analysis of different species with many other separation techniques (gas chromatography, other liquid chromatographic systems, supercritical fluid extraction/chromatography, flow injection methods, etc.).

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## 7. REFERENCES

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**Speciation of organotin compounds utilising HPLC-ICP-MS: application to the Measurements and Testing (BCR) certification programme**. C. Rivas, L. Ebdon, S.J. Hill. *"Química Analítica"*, 1995, 14, 142.

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## **COURSES ATTENDED**

**1993-1994:** *Atomic methods for trace analysis.* Department of Analytical Chemistry, Faculty of Chemistry, Complutense University of Madrid, Madrid, Spain.

*Metals and their compounds in the environment (speciation).* Department of Analytical Chemistry, Faculty of Chemistry, Complutense University of Madrid, Madrid, Spain.

*Pollution analytical chemistry in the environment.* Department of Analytical Chemistry, Faculty of Chemistry, Complutense University of Madrid, Madrid, Spain.

**1994-1995:** *Chemical sensors based on the use of optical fiber.* Department of Analytical Chemistry, Faculty of Chemistry, Complutense University of Madrid, Madrid, Spain.

*Special molecular luminescence techniques in chemical analysis.* Department of Analytical Chemistry, Faculty of Chemistry, Complutense University of Madrid, Madrid, Spain.

*Planning of experiments and evaluation of results of the analytical method.* Department of Analytical Chemistry, Faculty of Chemistry, Complutense University of Madrid, Madrid, Spain.

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## Speciation of organotin compounds utilising HPLC-ICP-MS: application to the measurements and testing (BCR) certification programme

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**Abstract:** Since initial studies in the early 1980s on the toxicity of the different organotin species in the environment, much work has been done to develop suitable analytical techniques to determine such species at sub-ng.L<sup>-1</sup> levels. Many papers have now been published, most involving a chromatographic separation of analyte species prior to analysis. However, little work has been done to date with an emphasis on quality control, and many workers have suffered due to the lack of suitable reference materials. This area is now of the utmost importance since both the European Community and national legislation in many member states require tin species to be monitored in the aquatic environment. This paper describes work performed as part of an on-going study under the auspices of the Measurements and Testing Programme (formerly Bureau of Community Reference, BCR) to unequivocally determine sub-ng amounts of organotin compounds in environmental matrices with the ultimate aim of producing suitable validated reference materials to aid the determination of organotin compounds in sediments and mussels.

Thus, we report on the development of both the instrumentation and methodology required to fulfil this object with specific reference to tributyltin, dibutyltin, monobutyltin and triphenyltin.

**Key Words:** High performance liquid chromatography, inductively coupled plasma mass spectrometry, organotin speciation, certified reference materials.

### Introduction

Organotin compounds have been widely used now for more than twenty years. They have many applications, each specific on the nature of organic radical attached to the tin atom. Thus for example tributyltin compounds have been used as the active ingredient in antifouling paints, mono- and dibutyltin as stabiliser of polyvinylchlorides and triphenyltins as fungicides [1].

Inorganic tin is relatively non toxic, indeed its major use is in metallic coating for food cans. However the progressive introduction of organic groups greatly influences the physicochemical properties, biological activity, mobility and persistence of organotin species. This leads to an increase in toxicity, which reaches a maximum for trisubstituted compounds such as tributyl, triphenyl and tricyclohexyltin compounds which tend to bind with lipids and thus enable transport across biological membranes [1,2]. Several reviews on the toxic nature of organotin compounds may be found in the literature [3-5]. However trisubstituted organotin compounds are also known to undergo degradation in the environment via both microbial and UV processes which induce dealkylation to inorganic tin. Thus such degradation processes will reduce the environmental threat from the presence of organotin compounds but will be dependant on both the nature of the sample and the prevailing environmental conditions. For example, triorganotin has a life time of days in surface water, whilst the same compound may exist for many years in sediments. In general the environmental persistence and fate of organotins is directly related to the specific characteristics of the aquatic system such as temperature, salinity, pH,

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suspended matter, microbial populations, flushing rates etc.[2].

Many countries have now introduced restrictions on the use of organotin compounds which result in their direct introduction into the marine environment via for example the application of antifouling paints. This is the result of extensive evidence of both environmental and economic damage to non-target organisms such as oysters and mussels following the well documented events in Arcachon Bay France[6]. Such restrictions are also now incorporated in EC legislation[7-9].

It is clear from the discussion above that it is vital to develop analytical methods capable to distinguish between the different species *i.e.* speciate in order to both obtain qualitative and quantitative information to aid environmental studies and increasingly to comply with new legislation which identifies target levels for particular species. The most common way to separate the different analytes is to use a chromatographic method[2]. Principally two types of chromatography have been employed, liquid chromatography[10] and gas chromatography[11]. In the case of the latter, more steps are involved in the treatment of the sample since the analytes often have to be derivatized in order to obtain volatile compounds suitable for gas chromatography. The most common methods of doing this are via the use of Grignard reactions or via the formation of volatile hydrides. Such methods are reviewed elsewhere[12-20].

Liquid chromatography is less commonly employed for organotin speciation, although it does offer some advantages. In liquid chromatography there is no need for a derivatization step and this is clearly a major advantage of this technique, since the sample preparation is easier, less time-consuming and avoids the risks of losses of analyte or contamination of the sample. It also allows the determination of the analytes in the same form in which they are found in the sample. Again several methods have been published employing this kind of chromatography[21-24].

The use of atomic spectroscopy to provide element specific detection in speciation studies employing chromatography has been discussed previously[2,10,11]. More recently the use of inductively coupled plasmas mass spectroscopy (ICP-MS) has been widely adopted[25] since it offers considerable advantages in terms of sensitivity and selectivity. This technique also facilitates the use of isotope dilution analysis which is an acknowledged way to enhance the accuracy of analytical methodology and provides compensation for variability.

During the 1980's, following legislation in several European Community member states to restrict the use

of marine antifouling paints containing organotin compounds (principally tributyltin), the Measurements and Testing Programme (BCR) established a programme to improve analytical methodology for tin speciation and to certify appropriate materials *i.e.* sediments and mussels as certified reference materials (CRMs)[26,27]. This paper discusses initial studies made as part of that programme to develop new analytical methodology, ultimately to provide accurate measurements for the determination of organotin species (tributyltin, triphenyltin, dibutyltin and monobutyltin) employing isotope dilution analysis coupled to high performance liquid chromatography-inductively coupled plasma mass spectrometry (IDA-HPLC-ICP-MS).

## Experimental

### Chemicals

The tributyltin chloride (96%), dibutyltin chloride (96%) and monobutyltin chloride (95%) were obtained from Aldrich Chemical Co. Ltd., Dorset, England. Triphenyltin was purchased from Fluka Chemie, Buchs, Switzerland. Inorganic tin calibrants were prepared from an inorganic tin calibrant solution, 9990 g.ml<sup>-1</sup>, obtained from Aldrich Chemical Co. Ltd., Dorset, England.

Stock solutions (1000 g.g<sup>-1</sup>) were prepared in HPLC-grade methanol (Rathburn Chemicals Ltd., Peebleshire, Scotland) and stored in the dark at 4°C. HPLC grade methanol was also used to prepare the mobile phases for separation studies. Appropriate dilutions of the stocks solutions were made when necessary with the mobile phase employed in the chromatographic separation. Glacial acetic acid was obtained from Merck Ltd., Dorset, U.K., and toluene from Rathburn Chemicals Ltd., Peebleshire, Scotland.

The triammonium citrate and citric acid used as buffers in this study were obtained from Fisons Analytical Reagent, Loughborough, U.K.

Milli-Q deionised water (Millipore, Bedford, MA, U.S.A.) was used throughout the study.

### Instrumentation

The high performance liquid chromatography was based on an inert gradient pump (Varian Ltd., Model 9010, Warrington, U.K.). A 200 l injection loop was fitted to chemically inert injection valve (Cheminer Model C1 valve, Valco Instruments Co. Inc., Houston, Texas, U.S.A.).

The chromatographic column (250x4.6 mm) was packed in house with 10 m Partisil SCX-10 (Thames Chromatography, Maidenhead, Berkshire, U.K.). The mobile phase used throughout the study at a flow rate of 1.0 ml.min<sup>-1</sup> was comprised of a mixture of methanol, water and citrate buffer. In case of isocratic elution, the mobile phase consisted in 70% methanol and 30% water, with 0.03 mol.l<sup>-1</sup> citrate buffer (pH=5.8).

The inductively coupled plasma-mass spectrometer used for the study was a PlasmaQuad 2+ (Fisons Instruments Elemental, Cheshire, U.K.). The operating conditions used are shown in Table 1. The nickel sampler and skimmer cones (Fisons Instruments Elemental, Cheshire, U.K.) had orifices of 1.0 and 0.7 mm, respectively. An



addition of oxygen (1.4%) was made to the argon nebuliser gas to avoid carbon deposition on the cones. The spray chamber was water-cooled to  $-4^{\circ}\text{C}$  with a recirculating chiller (Endocal RTE-100, Neslab Instruments, Inc., New Hampshire, U.K.). The ion lens settings were optimized every day to give the best performance.

Table 1. ICP-MS normal operating conditions

Outer gas flow ( $\text{l}\cdot\text{min}^{-1}$ )	15
Intermediate gas flow ( $\text{l}\cdot\text{min}^{-1}$ )	0.75
Nebulizer gas flow ( $\text{l}\cdot\text{min}^{-1}$ )	0.75
Oxygen bleed (%)	1.4
Forward power (W)	1500
Nebulizer	V-groove type
Spray chamber	Scott-type double-pass

## Results and discussion

### Evaluation of the HPLC-ICP-MS interface

The design of the HPLC-ICP-MS interface is a critical factor when optimising the system to obtain good resolution between peaks and a minimum dead volume in the interface itself. A number of different spray chambers were studied in order to evaluate potential broadening of the peaks and detrimental effects on the chromatographic separation. Three spray chambers were tested: Scott-type double and single pass and a miniaturized home-made double-pass spray chamber. The internal volumes of the spray chambers were 85, 40 and 13 ml, respectively. The chromatograms were obtained using isocratic conditions as shown in (Table 2).

As can be seen from the three chromatograms (Figure 1), the Scott-type double-pass spray chamber gives the best signal-to-noise ratio, with no apparent peak broadening, and so it was adopted for use thoroughly this study.

The addition of buffer to the mobile phase originally caused salt deposition in the injector tube on the torch.

Table 2. Chromatographic parameters

Column (and guard column):	Partisil-10 SCX, 10 $\mu\text{m}$ , 250 $\times$ 4.6 mm (guard column 25 $\times$ 4.6 mm)
Mobile phase:	Isocratic elution: Methanol (70%) /water (30%) Gradient elution: Step gradient methanol/water (70% MeOH 1.5 min, 85% MeOH 15 min)
Buffer:	Isocratic elution: Citric acid/ammonium citrate (pH=5.8) Gradient elution: Step gradient citric acid/ ammonium citrate (pH=5.8, 5.5 min, pH=3.4, 10 min)
Flow rate:	1 $\text{ml}\cdot\text{min}^{-1}$
Injection volume:	200 $\mu\text{l}$

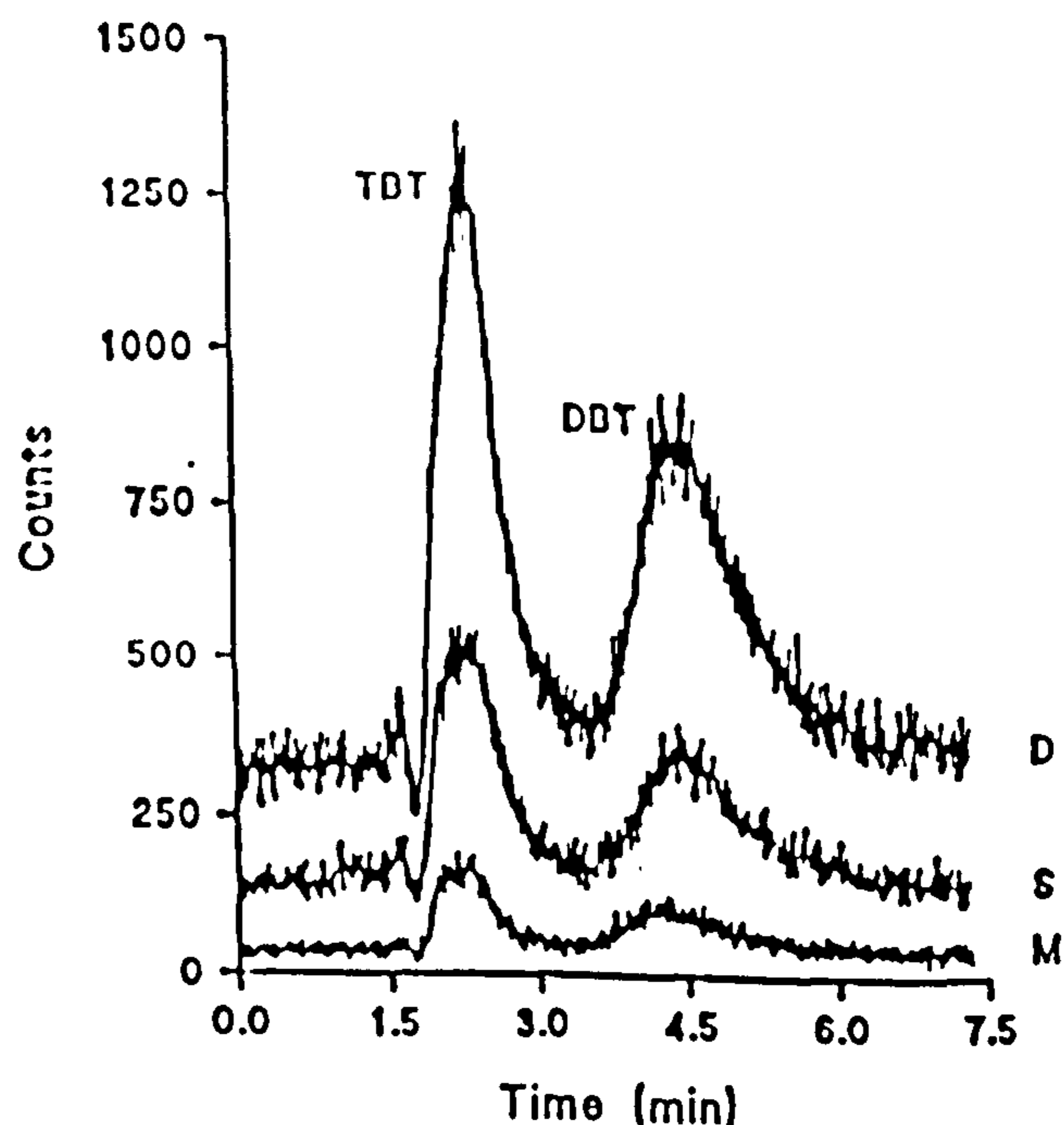


Figure 1. Chromatograms showing the separation of TBT ( $122 \text{ ng}\cdot\text{g}^{-1}$ ) and DBT ( $115 \text{ ng}\cdot\text{g}^{-1}$ ) under isocratic conditions using different spray chambers. Mobile phase: Methanol (70%), water and citrate buffer pH=5.8. Flow rate  $1.0 \text{ ml}\cdot\text{min}^{-1}$ . "D": Scott-type double-pass spray-chamber; "S": Scott-type single-pass spray-chamber; "M": mini double-pass spray-chamber.

On prolonged runs (3 hours) this led to the plasma being extinguished. However, the problem was readily overcome with the use of a demountable torch with a straight injector of 1.5 mm inner diameter.

### Chromatographic studies

Previous studies in this laboratory [18-23] have successfully investigated the separation and quantification of tributyltin and a range of other tin species in various matrices. This paper discusses studies to improve the chromatographic separation of a wider range of organotin, potentially present in the same sample. Thus, although our previous method was successfully utilised in previous certification campaigns, *i.e.* sediment CRM number 462 [26] and RM 424 [27] for tributyltin (TBT) (typical chromatograms are shown in (Figure 1) this work seeks to specifically include monobutyltin (MBT) and triphenyltin (TPhT), the separation being achieved on a single column.

One of the first refinements to be made was to reduce the retention times of the later peaks in the series and to this end a step gradient elution was applied, increasing the amount of methanol from 70% up to 85%. This resulted in an improvement in the elution profile of dibutyltin (DBT), but did not significantly effect the



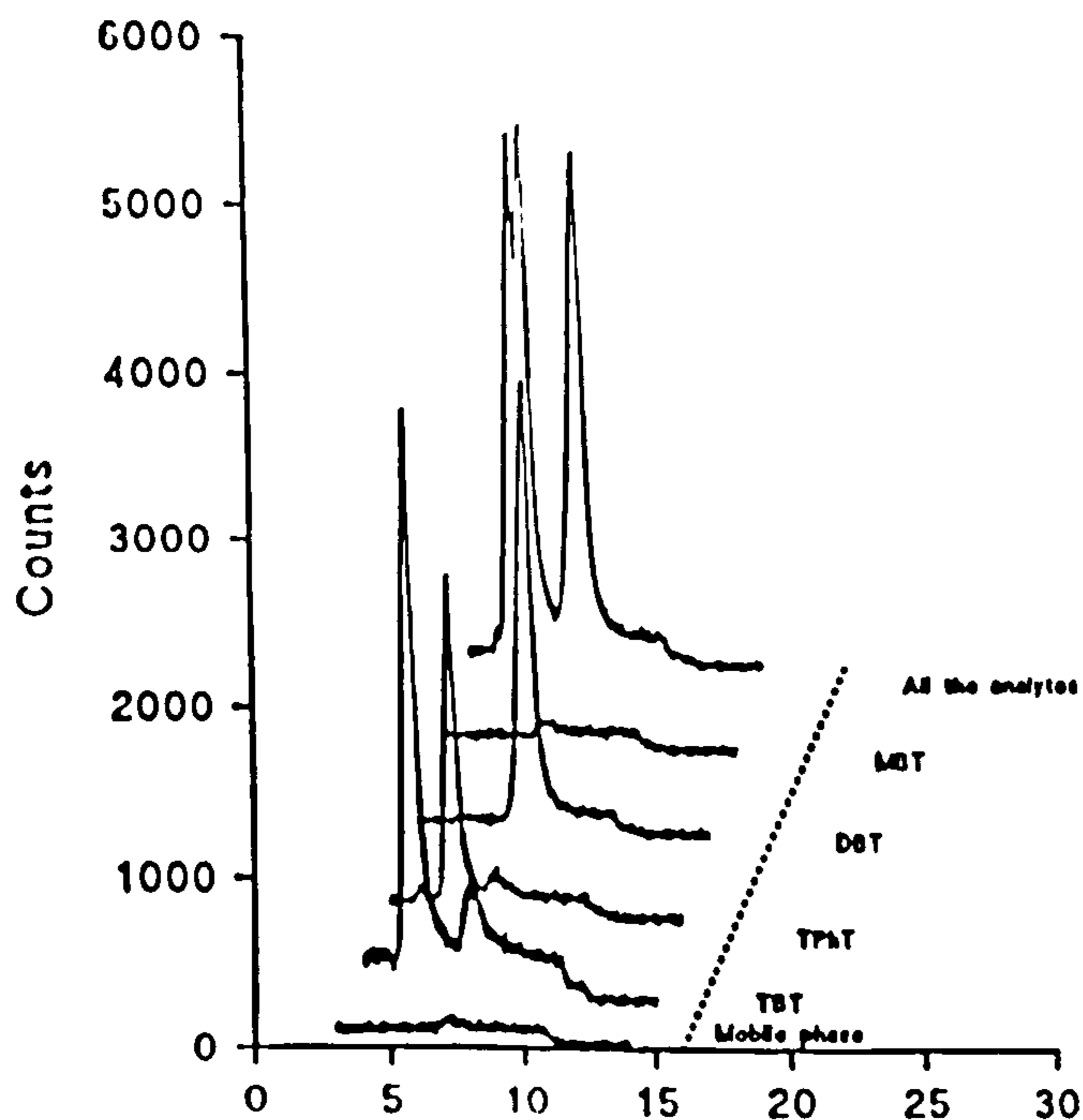


Figure 2. Chromatograms showing the separation of TBT, DBT, MBT and TPhT with step gradient elution (without pH gradient). 125 ng.g<sup>-1</sup> of each analyte, approximately.

elution on MBT (Figure 2). Previous work[29] has suggested a decrease in the pH of the eluent to overcome this problem for MBT. The results obtained can be seen in (Figure 3). The previous study reported a large increase in the base line with the pH gradient, although as can be seen, proposed here gives only minimal background enhancement.

Triphenyltin was found to elute just after TBT without being truly separated although it was fully resolved from DBT. Several attempts were made to improve the separation, including modifying the percentage of methanol (from 60 up to 85%), varying the pH in the range 3.4 to 6.2 (lower or higher pH are precluded due to the type of packing material in the column) and changing the concentration of the buffer in the mobile phase from 0.03 mol.l<sup>-1</sup> down to 0.015 mol.l<sup>-1</sup>. However, it was not possible to significantly enhance the separation over that shown in Figure 3. Increasing the buffer concentration was not evaluated due to the problems of salt loading in the mobile phase causing deposits in the injector of the torch, as discussed above. Thus it was concluded that in order to improve this particular separation, another type of column, or possibly a twin column system should be taken into consideration.

The detection limits for the chromatographic method, based on peak height, are 1 ng.g<sup>-1</sup> for each of TBT, MBT and TPhT and 2 ng.g<sup>-1</sup> for DBT.

The detection limits with the demountable torch of 1.5 mm of inner diameter are comparable with the ones obtained with the normal Fassel type torch (1.2 mm inner diameter), *i.e.* 3.5 ng.g<sup>-1</sup> for TBT and TPhT, 3 ng.g<sup>-1</sup> for DBT and 2 ng.g<sup>-1</sup> for MBT. Although the peaks obtained were less intense, the noise and baseline were also reduced providing detection limits comparable with those previously obtained.

#### Extraction procedure

A brief study was also made to evaluate the extraction efficiency of the different organotin species from sediments. A clean sediment from the Plymouth area was spiked with TBT, TPhT, DBT and MBT as follows. 2 g of sediment was weighed into a centrifuge tube and 250 ng of each analyte (as Sn in methanol) was spiked into the sediments, whilst the same volume of methanol was spiked into the control samples and the blanks. The sediments were then left overnight to equilibrate. After the equilibration period 25 ml of glacial acetic acid was added to each tube. These were then placed on a mechanical shaker for four hours. After this time, the sediment and the acetic acid were centrifuged for 15 min. at 2000 rpm to separate the liquid phase. The extraction with glacial acetic acid was then repeated. The organotin species were then backextracted (three times) into 10 ml of toluene in a separation funnel. Finally, the extracts

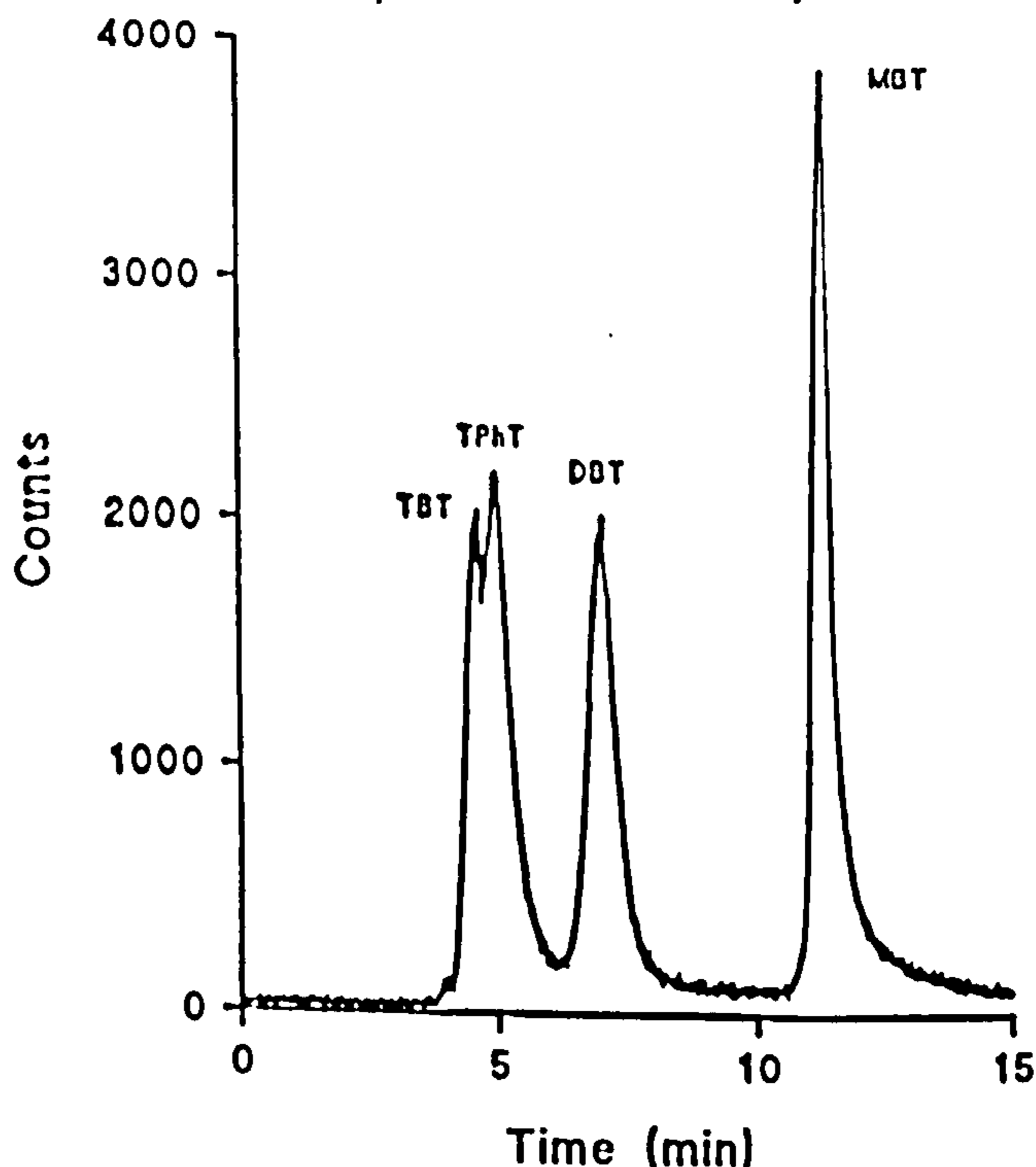


Figure 3. Chromatogram showing the separation of TBT (134 ng.g<sup>-1</sup>), DBT (117 ng.g<sup>-1</sup>), MBT (152 ng.g<sup>-1</sup>) and TPhT (111 ng.g<sup>-1</sup>) with step gradient elution (with pH gradient).

Table 3. Recovery studies

	TBT	TPhT	DBT	MBT
Extraction efficiency (%)	103	61	33	12
RSD (%)	23	9	3	4

were dried in a rotary evaporator and dissolved into the mobile phase prior to analysis. The results obtained are shown in (Table 3).

#### Analysis of certified sediment sample

Following the extraction procedure detailed previously, the Canadian sediment PACS-1, certified for tributyltin, dibutyltin and monobutyltin was analyzed. 0.5 g of sediment were employed in each extraction. The sediment was spiked with TBT, DBT, MBT and TPhT in order to correct the values obtained for the efficiency of the extraction. The results obtained are shown in (Table 4).

Table 4. Analysis of certified reference sediment

	TBT	TPhT	DBT	MBT
Certified value ( $\mu\text{g/g}^{-1}$ as Sn)	$1.27 \pm 0.22$	not certified	$1.16 \pm 0.18$	$0.28 \pm 0.17$
Analysis value ( $\mu\text{g/g}^{-1}$ as Sn)	$1.31 \pm 0.14$	$0.06 \pm 0.01$	$0.64 \pm 0.13$	$0.69 \pm 0.18$

#### Isotope dilution studies

Sample preparation is acknowledged to be one of the major problems in speciation studies. Often the extraction procedures required are long and prone to losses or contamination of the analyte. In addition, the efficiency of such procedures is usually much less than 100% with some of the analyte being retained in the sample matrix. The most promising way of overcoming this problem is the application of isotope dilution analysis, since this methodology provides compensation for the different sources of variability.

A range of isotopically enriched organotin species are being prepared for the last stages of this study. Previous work using isotope dilution analysis for the speciation of lead follow HPLC separation was very encouraging [33]. This earlier study identified the practical requirements when using this approach and highlighted the need for suitable software for data handling. Many of the original problems encountered with commercial software packages have now been overcome and this present study on organotins will run parallel to further work (also as part

of the M&T Programme) on the certification of organoleads in candidate reference materials.

## Conclusions

The coupling of high performance liquid chromatography to inductively coupled plasma-mass spectrometry has proved successful for the separation of tributyltin, triphenyltin, dibutyltin and monobutyltin. The sensitivity of ICP-MS readily enables the detection of sub-ng amounts of each analyte. The results presented in this paper once again demonstrate the suitability of our existing method (as used in previous interlaboratory studies under the auspices of the Measurements and Testing Programme) for TBT although also identify a number of weaknesses associated with the original method for the determination of MBT, DBT and TPhT. The results from this study show that the use of gradient programmes for both the mobile phase composition and pH control can overcome many of these problems and greatly improve both resolution and detection limits. However, the brief study on extraction efficiency of organotins from sediments highlights the need for more work in this area. Thus this will be one of the major areas of development in the next stage of this study in line with Measurements and Testing Programme to certify organotins in sediments and mussels.

The added advantage of ICP-MS to performance isotope dilution analysis will be used in the final stages of this study. As discussed earlier the technique overcomes many of the problems associated with extraction, and the coupling of isotope dilution analysis to HPLC-ICP-MS provides great potential for a sensitive, accurate and precise methodology for the determination of organotin species in environmental matrices. This will clearly be of benefit to the BCR certification programme. In addition, work will also continue on the use of IDA-HPLC-ICP-MS for the determination of organoleads and the next stage of this parallel project will be to use isotope dilution techniques in the interlaboratory studies aimed at producing reference materials for trimethyllead in rainwater and urban road dust.

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# Effect of Different Spray Chambers on the Determination of Organotin Compounds by High-performance Liquid Chromatography-Inductively Coupled Plasma Mass Spectrometry

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One of the potential problems to overcome when coupling liquid chromatography with atomic spectrometry is the low sample transport efficiency. Often this is a consequence of the interface design, and in particular the nebulizer and spray chamber configuration. The present study reports on a comparison of several spray chambers (single-pass, double-pass and cyclone types) with respect to both design and performance characteristics. In order to evaluate performance for speciation studies, different configurations have been evaluated utilizing HPLC-ICP-MS. Various organotin compounds (monobutyltin, dibutyltin, tributyltin and triphenyltin chlorides) were coinjected and the effects on resolution, sensitivity and SNR assessed. Of the spray chambers evaluated, a cyclone spray chamber (internal volume 22 ml) with cooling jacket was found to offer the best performance and gave a transport efficiency of 7.5% without loss of chromatographic resolution and sensitivity.

**Keywords:** *Spray chamber design; high-performance liquid chromatography-inductively coupled plasma interface; organotin speciation*

One of the major weaknesses of ICP-MS is the low efficiency of the transportation of the sample to the plasma. The aerosol droplets produced by a nebulizer should be of a diameter of less than 10  $\mu\text{m}$  so that the desolvation, volatilization and atomization are as rapid and efficient as possible. The commonly used pneumatic nebulizers produce a wide distribution of droplet size, usually up to  $10^{-4}$  m in diameter.<sup>1</sup> The main use of a spray chamber is thus to separate the larger droplets from the smaller ones, allowing the latter to reach the plasma. Unfortunately, 98–99% of the sample is lost in this process when employing commonly used spray chamber designs.<sup>2,3</sup> Separation of the droplets occurs as a result of a variety of processes that eliminate the larger drops according to the different trajectories inside the spray chamber and collisions with the walls or with a bead placed inside the chamber. The larger drops go to waste and the smaller droplets are carried into the torch and subsequently to the plasma. Several attempts have been made to improve the efficiency of nebulization systems.<sup>3–9</sup> Most common designs of spray chambers employ flow reversal, cyclones or impact beads. The two first types cause changes in the flow direction as well as impaction on the walls of the chamber. Those employing impact beads operate by placing a device which intercepts the flow of the aerosol and provides an in-line impaction site.

The performance of a nebulizer-spray chamber system can be evaluated through its analytical performance, provided that the operating parameters are fully optimized for each of the

systems. In the present study, seven different types of spray chambers were tested following this procedure. Two spray chambers were Scott-type (double and single pass), one was a miniaturized laboratory-made double pass and the remaining four were of the cyclone type.

The presence of organotin species in the environment has been of great concern in recent years owing to their high toxicity, particularly to marine organisms. Levels as low as  $1 \mu\text{g l}^{-1}$  of tributyltin (TBT) as tin in water can affect the population and mariculture of marine organisms, especially shellfish.<sup>10</sup> Considerable effort has been made to improve the sensitivity and selectivity of the analytical methodology used to detect such species, and in particular to develop techniques capable of determining quantitatively the chemical form of the analyte. One of the more popular approaches used to separate the different species has been HPLC, since it provides a simple and rapid method, without the requirement for derivatization of the analytes prior to the chromatographic separation.<sup>11–17</sup> Thus, the nature of the coupling of the HPLC and the ICP-MS instruments is critical.

Any chromatographic detector should not markedly increase the dead volume of the system, otherwise chromatographic resolution will be lost. Several attempts have been made to couple chromatography with ICP-MS whilst ensuring that there is minimal increase in dead volume, particularly by placing the nebulizer and spray chamber close to the end of the column. A reduced volume spray chamber of the cyclone type has been reported by Wu and Hieftje<sup>4</sup> for ICP-AES. The internal volume of this spray chamber was 40 ml and advantages in transport efficiency were reported. It therefore seemed timely to investigate a number of different spray chambers for directly coupled HPLC-ICP-MS, including a reduced volume cyclone chamber based on the Wu and Hieftje design but modified for ICP-MS.

Four different organotin compounds were separated and the performance of the nebulizer-spray chamber system evaluated, considering both the sensitivity for each analyte and the chromatographic resolution obtained for the organotin species. Various parameters for each spray chamber were evaluated, including transport efficiency, shape and internal volume. The present paper reports the findings of this study.

## EXPERIMENTAL

### Chemicals

The tributyltin chloride (96%), dibutyltin (DBT) chloride (96%), monobutyltin (MBT) chloride (95%) and triphenyltin (TPhT) chloride (95%) were obtained from Aldrich, Gillingham, Dorset, UK.

Stock solutions of each organotin ( $1000 \mu\text{g g}^{-1}$ ) were prepared in HPLC-grade methanol (Rathburn Chemicals,

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Walkerburn, Peeblesshire, UK) and stored in the dark at 4°C. HPLC-grade methanol was also used to prepare the mobile phases for the separation studies. Appropriate dilutions of the stock solutions were made, when necessary, with the mobile phase employed for the chromatographic separation.

The triammonium citrate and citric acid used as buffers were obtained from Fisons, Loughborough, Leicestershire, UK, as analytical-reagent grade.

Lipase and protease enzymes employed in the enzymatic extraction of fish tissue were obtained from Aldrich Chemicals. Sodium dihydrogenphosphate buffer was BDH AristaR grade (BDH, Poole, Dorset, UK). Dichloromethane was HiPerSolv grade obtained from BDH.

Milli-Q de-ionized water (Millipore, Bedford, MA, USA) or equivalent was used throughout the study.

### Instrumentation

The HPLC was carried out using an inert gradient pump (Varian, Model 9010, Warrington, Cheshire, UK). A 200 µl injection loop was fitted to a chemically inert injection valve (Cheminert Model C1 valve, Valco Instruments, Houston, TX, USA).

The two analytical columns (250 × 4.6 mm) were packed in-house with 10 µm Partisil SCX-10 (Thames Chromatography, Maidenhead, Berkshire, UK). The mobile phase used throughout the study at a flow rate of 1.0 ml min<sup>-1</sup> consisted of a mixture of methanol, water and citrate buffer. The chromatographic conditions used are shown in Table 1.

The ICP-MS instrument used for the study was a PlasmaQuad 2+ (Fisons Instruments Elemental, Winsford, Cheshire, UK). The operating conditions used are shown in Table 2. The nickel sampler and skimmer cones (Fisons Instruments Elemental) had orifices of 1.0 and 0.7 mm, respectively. Using a gas blender (Signal Instruments, Camberley, Surrey, UK) an addition of oxygen (1.4%) was made to the argon aerosol carrier gas to avoid deposition of carbon on the cones. The spray chamber was cooled with a recirculating chiller (Endocal RTE-100, Neslab Instruments, Newington, NH, USA). The ion lens settings were optimized every day to give the best performance.

Seven spray chambers (designated as A-G) were investigated. They were conventional Scott double-pass (A) and single-pass (B) spray chambers, a miniaturized laboratory-made double-pass spray chamber<sup>18</sup> (C) and four different cyclone spray

chambers. The first of the cyclone designs had no liquid cooling jacket (D) and the last three (E, F and G) were cooled *via* an integral jacket. These latter spray chambers differed only in the internal volume and the shape of the indentation or 'dimple'. The internal volumes of the various spray chambers are given in Table 3, and the designs and shapes of the spray chambers in Fig. 1. In each case a Meinhard nebulizer was used (Type A, Fisons Instruments Elemental).

## RESULTS AND DISCUSSION

### Transport Efficiency

The silica gel trap method<sup>3,19</sup> was employed in all cases to obtain the transport efficiency. An argon flow rate of 1 l min<sup>-1</sup> was used with a sample uptake rate of 1 ml min<sup>-1</sup> (achieved using a peristaltic pump). Four U-tubes filled with silica gel were connected to the exit of the spray chamber. Each U-tube was weighed before and after the passage of 50 ml of Milli-Q water (also weighed). The spray chamber was cooled (except for types C and D) to -4°C. The experiment was conducted in triplicate. The results obtained are shown in Table 4.

As expected, the cyclone spray chambers gave better transport efficiency, possibly as a result of allowing larger drops to pass through the system. The lowest transport efficiency was found for the miniaturized double-pass spray chamber, in this case probably because of the large ratio of impaction surface to internal volume and the more contorted gas flow necessary to exit this small spray chamber.

It is also interesting to note that although the uncooled cyclone spray chamber D had the highest transport efficiency, the SNR obtained from the chromatogram was poor. Cooling of the spray chamber (*e.g.*, type G), although reducing the transport efficiency, much improved the SNR. The extent of this improvement was influenced by the overall design (Table 4).

### Effect of Internal Volume on Wash-out Time and Resolution

One of the main considerations in the coupling of HPLC with ICP-MS is the dead volume of the interface. This volume affects the resolution of the chromatographic system, potentially losing the separation previously achieved by the column. Clearly, the internal volume of the spray chamber can increase the dead volume of the system. The different internal volumes of the spray chambers used in the present study are shown in Table 3.

The wash-out time of a sample introduction system is the time required to clean the system and is most commonly defined as the time required for the signal to return to 1% of the original maximum. In the present study, this time was calculated by monitoring the decrease in the signal of a solution of 100 ng ml<sup>-1</sup> of <sup>115</sup>In in 70% methanol after being replaced by a 70% methanol solution without In.

The wash-out curves obtained for both the Scott-type double pass (A) and the new cyclone spray chamber (G) can be seen in Fig. 2. Surprisingly the wash-out time in the cyclone spray chamber is slightly longer (24 s) than in the much larger

Table 1 Chromatographic parameters

Column (and guard column)	Partisil SCX-10, 10 µm, 250 × 4.6 mm (guard column 25 × 4.6 mm)
Mobile phase (gradient elution)	Step gradient methanol-water (70% methanol 1.5 min; 85% methanol 9.5 min)
Buffer (gradient elution)	Step gradient citric acid-ammonium citrate (pH 5.8, 4.0 min; pH 3.4, 7 min)
Flow rate	1 ml min <sup>-1</sup>
Injection volume	200 µl

Table 2 Operating conditions for the ICP-MS system

Outer gas flow/l min <sup>-1</sup>	15
Intermediate gas flow/l min <sup>-1</sup>	0.75
Aerosol carrier gas flow/l min <sup>-1</sup>	0.85
Oxygen bleed (%)	1.4
Forward power/W	1500
Nebulizer	Meinhard
Spray chamber	Various
Signal monitored ( <i>m/z</i> )	120

Table 3 Internal volumes of the spray chambers

Spray chamber	Internal volume/ml
(A) Scott-type double pass	88
(B) Scott-type single pass	40
(C) Miniaturized double pass	13
(D) Cyclone (no jacket)	20
(E) Cyclone (jacketed)	40
(F) Cyclone (jacketed)	27
(G) Cyclone (jacketed)	22



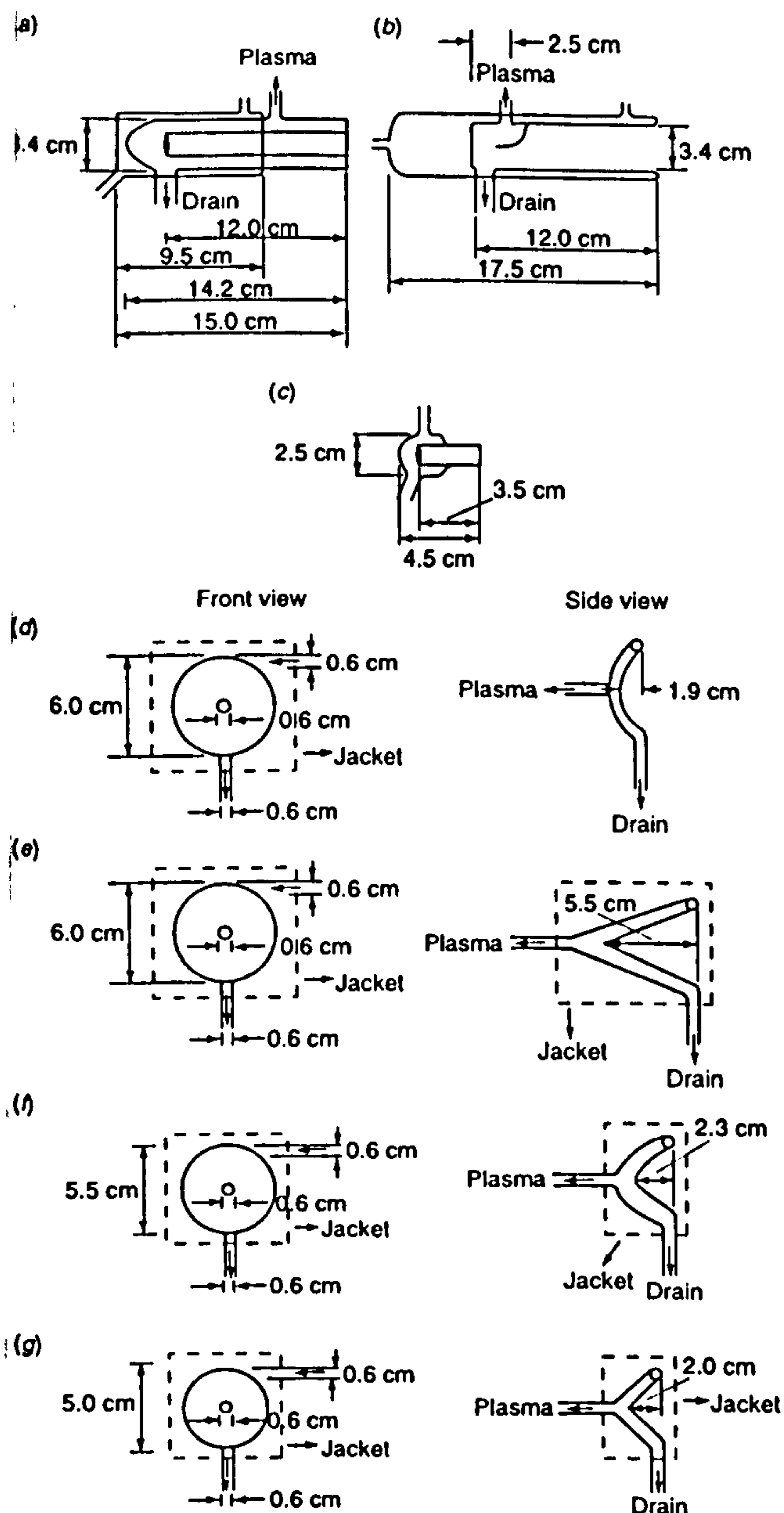


Fig. 1 Types of spray chamber used: A, Scott-type double pass; B, Scott-type single pass; C, miniaturized double pass; D, cyclone without jacket; E, F and G, cyclones with jackets

Table 4 Transport efficiency and SNR values for TBT with each spray chamber

Spray chamber	Transport efficiency $\pm s$ (%)	SNR
(A) Scott-type double pass	2.45 $\pm$ 0.07	39
(B) Scott-type single pass	2.92 $\pm$ 0.20	59
(C) Miniaturized double pass	1.74 $\pm$ 0.05	41
(D) Cyclone (no jacket)	8.05 $\pm$ 0.12	50
(E) Cyclone (jacketed)	3.95 $\pm$ 0.07	56
(F) Cyclone (jacketed)	5.85 $\pm$ 0.10	56
(G) Cyclone (jacketed)	7.53 $\pm$ 0.04	108

internal volume double-pass spray chamber (15 s). This would indicate that there are stagnant pockets within the cyclone spray chamber which are not being efficiently removed. Optimizing the size of the dimple is intended to minimize this problem.

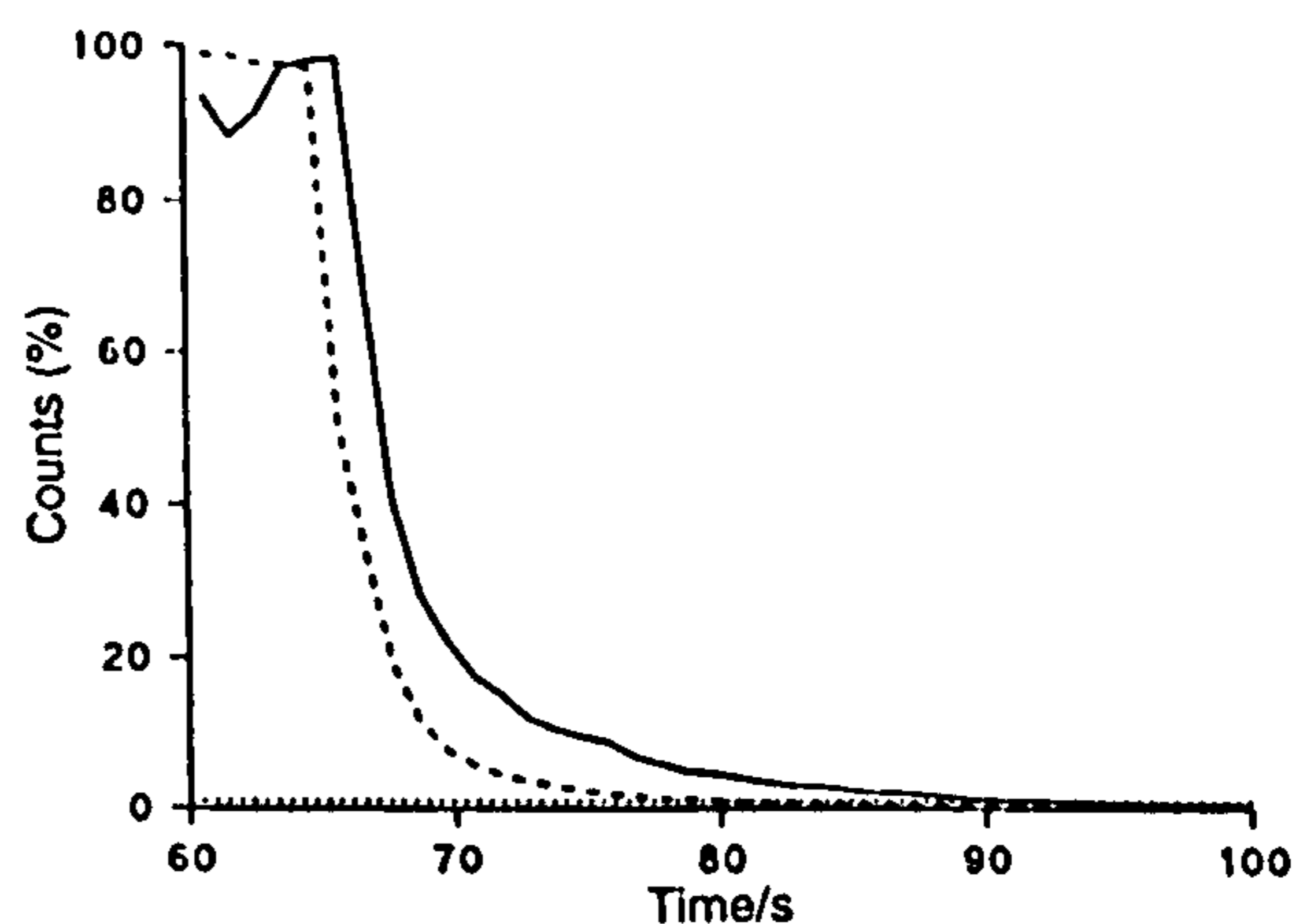


Fig. 2 Wash-out times for 100 ng ml<sup>-1</sup> of <sup>115</sup>In in 70% methanol for two spray chambers. Solid line, new cyclone spray chamber G; and broken line, Scott-type double-pass spray chamber A

Using the present chromatographic system, TBT and TPhT are not base-line resolved.<sup>12</sup> Any loss of resolution in the system will adversely affect the separation of species. The resolution ( $R_s$ ) between these two species was calculated for each of the spray chambers using the equation:<sup>20</sup>

$$R_s = 1.18 \frac{t_2 - t_1}{w_1 + w_2} \quad (1)$$

where  $t_1$  and  $t_2$  are the retention times of the peaks for TBT and TPhT, respectively, and  $w_1$  and  $w_2$  are the peak width at half-height for TBT and TPhT, respectively.

The ICP-MS experimental parameters (*i.e.*, flow rates, torch position and lens settings) were sequentially optimized for each system in order to obtain the best signal (counts s<sup>-1</sup>) using a 100 ng ml<sup>-1</sup> solution of <sup>115</sup>In, made up in the mobile phase (70% methanol, 30% water). The results obtained are shown in Table 5. As can be seen, all of the spray chambers gave similar resolution, although the spray chambers with the smallest internal volumes tended to give slightly better results.

#### Figures of Merit

The system giving the best performance using the above parameters was selected for further study. The cyclone spray chamber with cooling jacket G was one of the three spray chambers giving the best resolution between TBT and TPhT and the transport efficiency was also second highest of those tested. Using the cyclone spray chamber D, although better transport efficiency was achieved, the resolution was not so good as with spray chamber G. The miniaturized double-pass spray chamber C gave good resolution but the sensitivity obtained was inferior to that for spray chamber G. This was attributed to the lower transport efficiency. An additional disadvantage of spray chamber C was the noisy signal obtained as a result of the small internal volume.

Following these considerations, the cyclone spray chamber G was chosen as the best for use in the present application. Examples of the chromatograms obtained with both the

Table 5 Resolution between TBT and TPhT for each spray chamber

Spray chamber	Resolution
(A) Scott-type double pass	0.81
(B) Scott-type single pass	0.83
(C) Miniaturized double pass	0.84
(D) Cyclone (no jacket)	0.74
(E) Cyclone (jacketed)	0.76
(F) Cyclone (jacketed)	0.75
(G) Cyclone (jacketed)	0.83



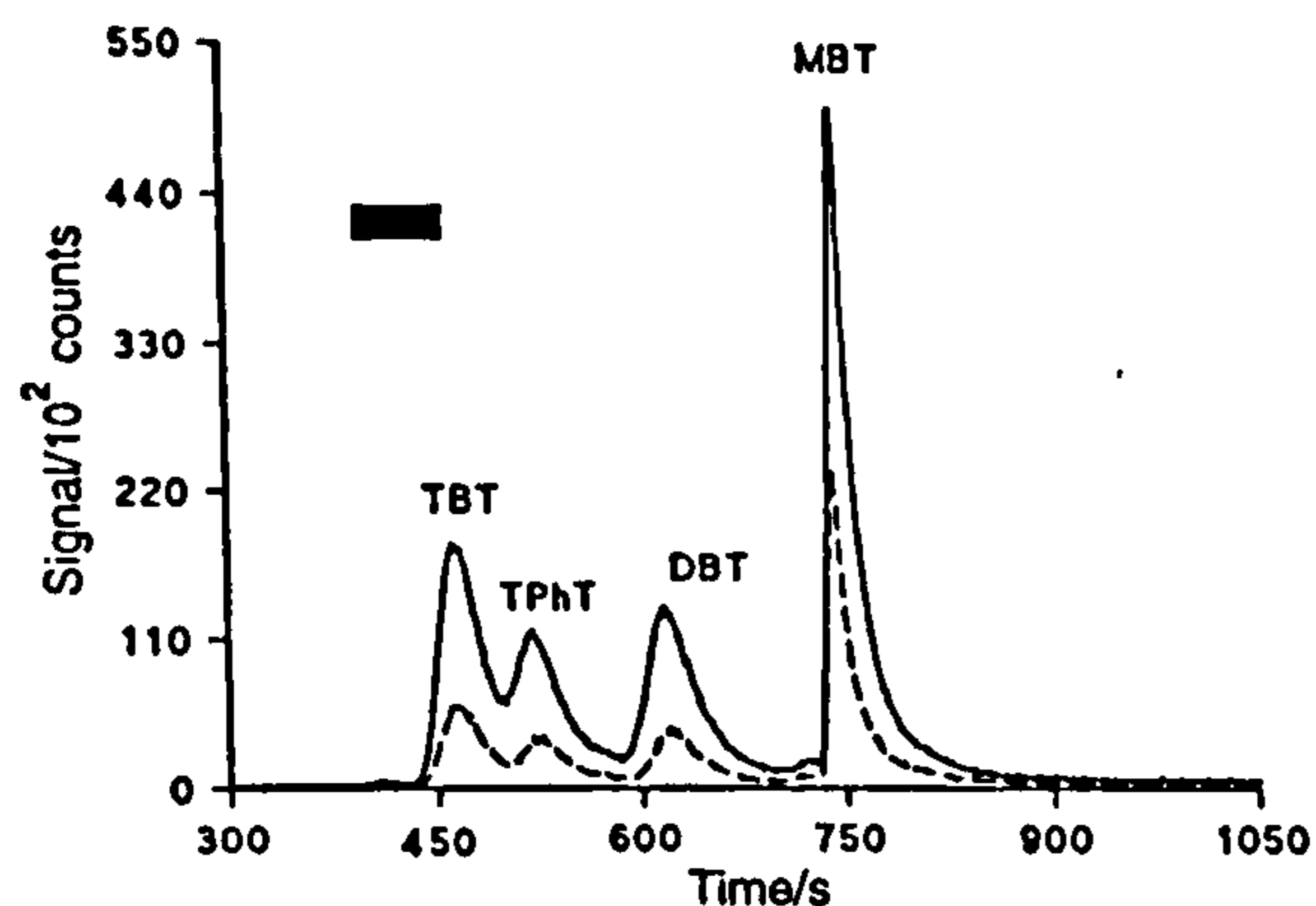


Fig. 3 Chromatograms obtained with Scott-type double pass A and new cyclone spray chamber G. Solid line, new cyclone spray chamber G; and broken line, Scott-type double-pass spray chamber A

Table 6 LOD for TBT, TPhT, DBT and MBT with Scott-type double pass spray chamber A and the new cyclone spray chamber G

Spray chamber	LOD/ng g <sup>-1</sup>			
	TBT	TPhT	DBT	MBT
(A) Scott-type double pass	1	1	2	1
(G) Cyclone	0.44	0.26	1.40	0.23

Table 7 Repeatability (RSD) for cyclone spray chamber G using three chromatographic runs at each of the concentrations

Analyte	RSD (%)		
	Nominal concentration investigated/ng g <sup>-1</sup>		
	10	100	500
TBT	8.3	4.2	2.7
TPhT	8.0	4.5	4.0
DBT	6.5	3.6	2.0
MBT	7.3	10.5	7.6

Scott-type double pass spray chamber A and spray chamber G can be seen in Fig. 3. The detection limits (3σ of the baseline noise) obtained for the four analytes with this new spray chamber are presented in Table 6. The repeatability for spray chamber G was also tested by use of triplicate chromatographic injections of the four analytes, using peak height, at three concentrations (approximately 10, 100 and 500 ng g<sup>-1</sup> as tin). The results obtained expressed in terms of the RSD are shown in Table 7.

#### Analysis of Fish Tissue CRM NIES-11

A certified reference material (fish tissue NIES-11) from the National Institute of Environmental Studies of Japan (NIES) was analysed. This material is certified for TBT (1.3 ± 0.1 μg g<sup>-1</sup> as TBT) and has an indicative value for TPhT (6.3 μg g<sup>-1</sup> as TPhT).

The extraction procedure employed, based on a recently published method by Ceulemans *et al.*,<sup>21</sup> was as follows. Approximately 1 g of fish tissue was accurately weighed. Lipase and protease (0.05 g of each) and 40 ml of citric-phosphate buffer (prepared by dissolving 21.0 g of citric acid, 14 g of dihydrogen sodium phosphate and 64 ml of ethanol in 1 l of water, followed by adjustment of the pH with ammonia solution to pH 7.5) were added. The mixture was left overnight in a shaker at 37°C. The following day, the aqueous phase

was extracted into 10 ml of dichloromethane three times. The organic phase was then rotary evaporated until dryness. The dry extracts were redissolved into a mixture of 66% methanol and 33% mobile phase prior to the analysis.

The separation of the analytes was performed using the chromatographic conditions described previously, utilizing the new cyclone spray chamber, type G.

The method proved to be adequate for the analysis of this material. The result obtained for TBT ( $n=6$ ) following this procedure ( $1.29 \pm 0.05 \mu\text{g g}^{-1}$  as TBT) is in good agreement with the certified value ( $1.3 \pm 0.1 \mu\text{g g}^{-1}$ ).

#### CONCLUSIONS

A modified spray chamber has been designed and characterized for use with HPLC-ICP-MS. The main parameters for its construction and its analytical characteristics have been evaluated. This spray chamber gave improved detection limits for all organotin compounds used in the evaluation. These improvements can be attributed to a higher transport efficiency (7.5%), without loss of resolution and proved to be superior to the conventional Scott-type double pass spray chamber and other designs used in this study.

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# An Evaluation of Reversed-Phase and Ion-Exchange Chromatography for Use with Inductively Coupled Plasma-Mass Spectrometry for the Determination of Organotin Compounds

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An evaluation of reversed-phase high-performance liquid chromatography (HPLC) employing mobile phases compatible with direct coupling to inductively coupled plasma-mass spectrometry (ICP-MS) is described for the selective and sensitive detection of organotin species. The findings of this study are compared with established methods, employing ion-exchange chromatography.

In order to achieve optimum performance, both the HPLC and ICP-MS were optimized for speciation work. The results from studies using various mobile phases for the separation of a range of tin compounds (inorganic tin, tributyltin, dibutyltin and monobutyltin) are discussed both in terms of resolution and compatibility with ICP-MS instrumentation. Tropolone, a commonly used complexing agent for organotin species, is also discussed with reference to the chromatographic separation of tin species.

Finally, the role of isotope dilution analysis in conjunction with HPLC-ICP-MS for organotin speciation is described with respect to the European Community Standards, Measurements and Testing (BCR) certified water programme.

**Keywords:** Organotin compounds; speciation; high-performance liquid chromatography; inductively coupled plasma-mass spectrometry

## INTRODUCTION

The environmental impact of organotin species is now well established.<sup>1,7</sup> It is known that the organic species of tin are more toxic than the

inorganic forms, following the general trend monobutyltin (MBT) < dibutyltin < (DBT) < tributyltin (TBT). It is also recognized that levels as low as 1 ng ml<sup>-1</sup> in water of TBT may cause deformation in shellfish,<sup>8</sup> which in turn may affect the fertility, mariculture and population of shellfish in affected areas. The preconcentration of organotins in shellfish may also lead to toxic effects in man, the nature of which is related to the exact chemical form of the analyte.

One of the major applications of tributyltin has been its use as the active ingredient in antifouling paints, but other organotin compounds have also been used as bactericides, fungicides, insecticides, wood preservatives and poly(vinyl chloride) polymer stabilizers. Toxic levels of these compounds are present in aquatic and sedimentary environments in many areas. Consequently, analytical methods for the precise and accurate determination of mono-, di- and tri-substituted organotin compounds (speciation) are of clear importance.

The aim of this work is therefore to develop an accurate and precise analytical method for the determination of organotin species in environmental samples. To achieve this goal, one of the most promising approaches is to separate the species of interest by high-performance liquid chromatography (HPLC) and then introduce them into a highly sensitive element-specific detector such as the inductively coupled plasma-mass spectrometer (ICP-MS).<sup>9-15</sup> This route has been reviewed and selected as the most appropriate methodology for this study since it avoids derivatization procedures which may alter speciation and be adversely affected by matrix components. The results presented in this paper were obtained using both ion-exchange and reversed-phase chromatographic systems to separate inor-

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ganic tin, TBT, DBT and MBT. In the case of the reversed-phase system, two different procedures were used. In the first, a methanol, water and acetic acid–ammonium acetate mobile phase (in varying ratios) was used in both isocratic and gradient mode. In the second procedure, the complexation of the different organotins with tropolone was investigated.

An additional approach to enhance the accuracy of the analytical methodology is to utilize isotope dilution analysis (IDA), since it provides compensation for several sources of variability. Although this technique has been successfully used in lead speciation studies,<sup>16</sup> it has not yet been reported for use with organotin compounds. Thus in later stages of this study isotope dilution analysis will be used, following the synthesis of organotin compounds with altered but known isotopic composition. Environmental matrices will then be spiked with the synthesized organotin species to allow the accuracy of an analytical methodology to be fully evaluated as part of a European Community Measurements and Testing certification programme. Thus a fundamental objective of this present study is to identify chromatographic conditions compatible with this technique.

## EXPERIMENTAL

### Chemicals

The organotin standards, 96% tributyltin chloride (TBT), 96% dibutyltin chloride (DBT) and 95% monobutyltin chloride (MBT), were obtained from Aldrich Chemical Co. Ltd, Dorset, UK.

Stock solutions ( $1000 \mu\text{g g}^{-1}$ ) of these were prepared in HPLC-grade methanol (Rathburn Chemicals Ltd, Peebleshire, Scotland, UK) and stored in darkness at  $4^\circ\text{C}$ . The same methanol was used to prepare the mobile phases for HPLC studies.

Inorganic tin calibrant was prepared from an inorganic tin ICP/DCP standard solution in hydrochloric acid,  $9990 \mu\text{g ml}^{-1}$ , obtained from Aldrich Chemical Co. Ltd.

The triammonium citrate, citric acid and ammonium acetate used as buffers in this work were purchased from Fisons Analytical Reagents, Loughborough, UK. The tropolone was obtained from Aldrich Chemical Co. Ltd. Acetic acid and nitric acid, AnalAR grade, were obtained from BDH, Poole, UK.

Milli-Q deionized water (Millipore, Bedford, MA, USA) was used throughout the study.

### Instrumentation and operating conditions

The high-performance liquid chromatography system consisted of an inert HPLC gradient pump (Varian Ltd, Model 9010, Warrington, UK), with an injection valve fitted with a 200- $\mu\text{l}$  sample loop (Anachem, Model Rheodyne No. 7010, Bedfordshire, UK).

Ion-exchange chromatography was performed using a Partisil 10-SCX column of 25 cm length and 4.6 mm inner diameter. The mobile phase used when isocratic elution was employed was methanol/water (70:30, v/v) with ammonium citrate–citric acid buffer at pH 5.8. In the case of gradient elution, two step gradients were applied, varying the mobile phase from methanol/water (70:30, v/v), pH 5.8, to methanol/water 85:15, v/v, pH 5.8, and finally to methanol/water (85:15, v/v), pH 3.4.

The reversed-phase chromatography system employed a 5- $\mu\text{m}$  Hamilton PRP-1 column of 15 cm length and 4.1 mm i.d. Various compositions of mobile phases were used with this column, employing an ammonium acetate–acetic acid buffer at pH 6.5. The flow rate used for both the ion-exchange and reversed-phase systems was  $1 \text{ ml min}^{-1}$ .

The inductively coupled plasma–atomic emission spectrometer used was a Varian Liberty 200 (Varian Ltd, Warrington, UK). The flow rates of coolant, auxiliary and nebulizer gases were 15, 1.5 and  $0.75 \text{ l min}^{-1}$  of argon, respectively. The rf power was 1500 W.

The inductively coupled plasma–mass spectrometer employed was a VG Plasmaquad II (Fisons Instruments Elemental, Cheshire, UK). The ICP was operated at 1400 W. Coolant, auxiliary and nebulizer flow gases were at 15, 0.75 and  $0.75 \text{ l min}^{-1}$ , respectively. An oxygen bleed (2.4%) into the nebulizer gas was used to negate the effects of the organic solvent. The nebulizer employed was a V-groove type (Fisons Instruments Elemental, Cheshire, UK) coupled to a Scott-type double-pass spray chamber. An auxiliary chiller unit (Techne Ltd, Cambridge, UK) using 2-propanol was connected to the spray chamber to reduce the temperature to  $-15^\circ\text{C}$ . The sampler–skimmer distance was 1 mm. The mass spectrometer ion lenses were optimized every day in order to obtain the maximal signal

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a solution of 100 ng ml<sup>-1</sup> of <sup>115</sup>In in 2% HNO<sub>3</sub>, the mobile phase employed.

The HPLC-ICP-MS interface consisted of a 1 m length of PTFE tubing (0.3 mm i.d.) to connect the end of the HPLC column to the nebulizer. No further refinements were required for this study as there was no need for desolvation or additional sheath gases.

**RESULTS**

**Evaluation of separation methods utilizing reversed-phase chromatography**

Separation of the different organotin species (inorganic tin, TBT, DBT and MBT) was first evaluated using the reversed-phase column. There are several methods reported in the literature to separate these compounds using this form of chromatography.<sup>14, 15, 17, 22</sup> The first attempts were made using various polar mobile phases with different concentrations of methanol and buffer (acetic acid-ammonium acetate) at pH 6.5. The compositions of the mobile phase ranged from 98% methanol and 2% buffer, down to 90% methanol, 2% buffer and 8% water. Using the 98% methanol/2% buffer mobile phase system, the first species to elute was MBT, followed by TBT, with DBT eluting last. The inorganic tin was thought to be coeluting with the MBT, a small shoulder being observed on the peak profile. A typical chromatogram obtained using this composition of mobile phase is shown in Fig. 1(a).

Decreasing the methanol content of the mobile phase to 90% did not help the resolution of MBT and inorganic tin, both again coeluting as a single peak. The elution of the other species was retarded however, with a degradation in peak shape.

A range of intermediate mobile phases with an ethanol/water ratio between 90 and 98% were also investigated. The best performance was obtained with 93-95% methanol. However, the differences between these and the chromatogram in Fig. 1(a) are small, the TBT and DBT being slightly better resolved although the retention times were longer (Fig. 1(b)). The use of gradient elution was also evaluated in order to separate the organotin and MBT species. Various compositions of the mobile phase were used employing a range of flow rates and gradient steps. Examples

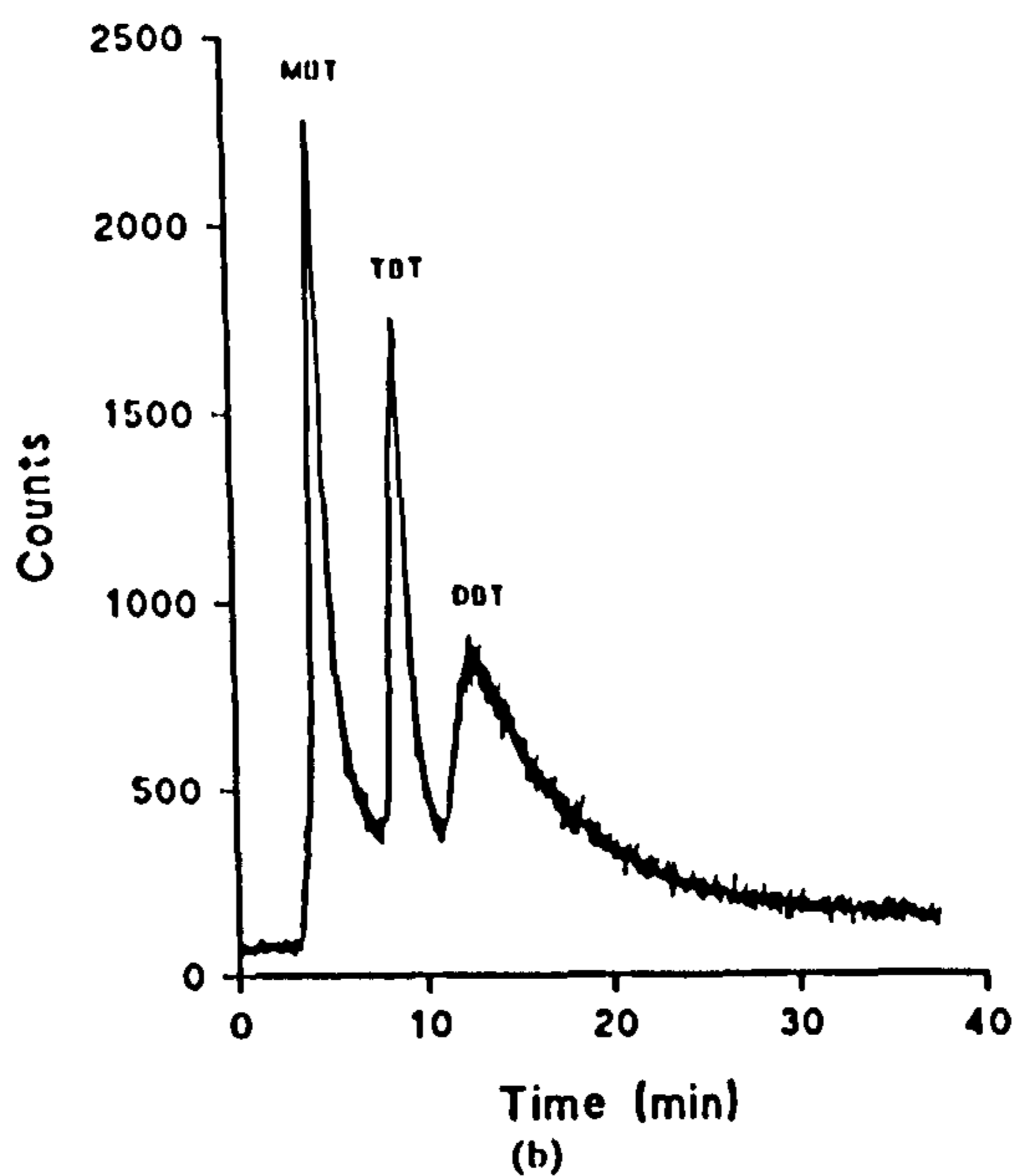
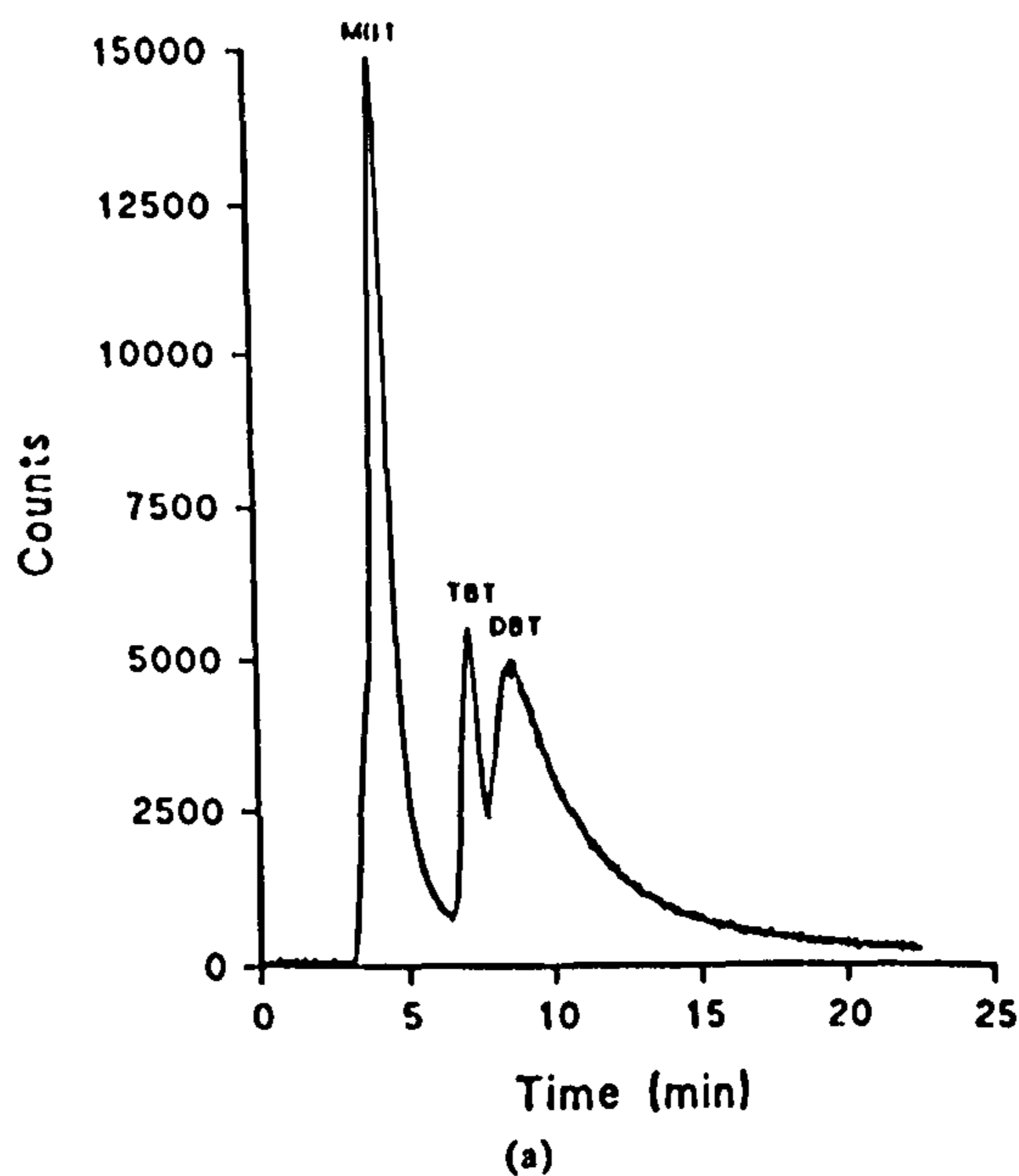


Figure 1 Reversed-phase chromatograms of inorganic tin, MBT, DBT and TBT. Mobile phase: (a) 98% methanol/water, acetate buffer, pH 6.5; (b) 95% methanol/water, acetate buffer, pH 6.5.

of the chromatograms obtained are shown in Figs 2(a) and 2(b). As can be seen, little success was achieved in the separation of the inorganic tin and MBT species employing gradients.



One of the most common problems in speciation studies on organotin compounds is the low extraction efficiency of the species from the original sample. Several workers have proposed the use of a complexing agent at some stage of the extraction procedure to improve the efficiency.

One of the best known of these complexing agents for organotin species is tropolone, which has been used in several methods.<sup>12,14,23,24</sup> In order to study the effect of tropolone on the chromatography, a range of organotin calibrants were complexed with different amounts of tropo-

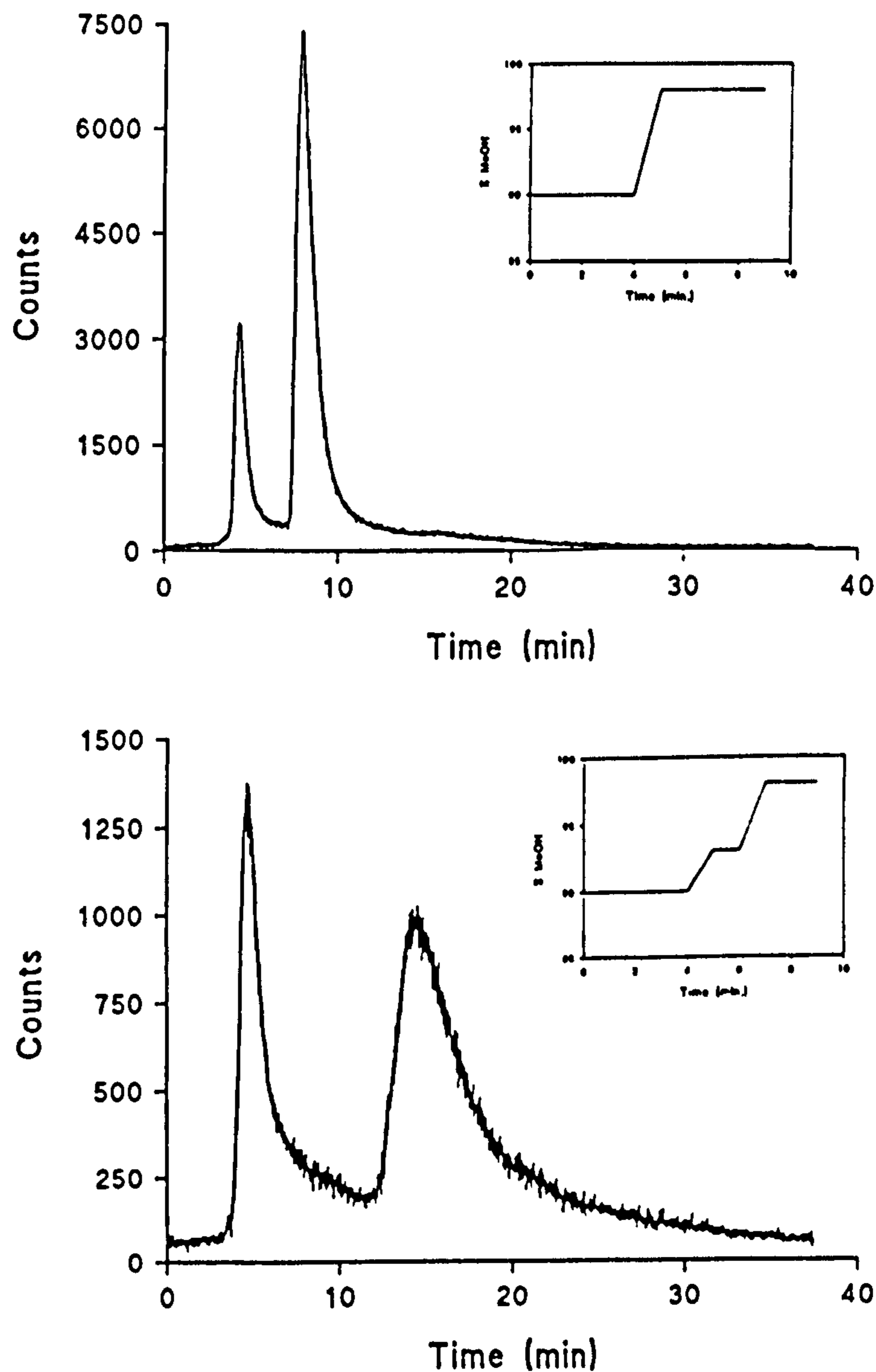


Figure 2 Reversed-phase chromatograms of inorganic tin, MBT, DBT and TBT. Mobile phase: methanol/water, acetate buffer, gradient elution, pH 6.5. The gradient elution profiles are shown in the insets.

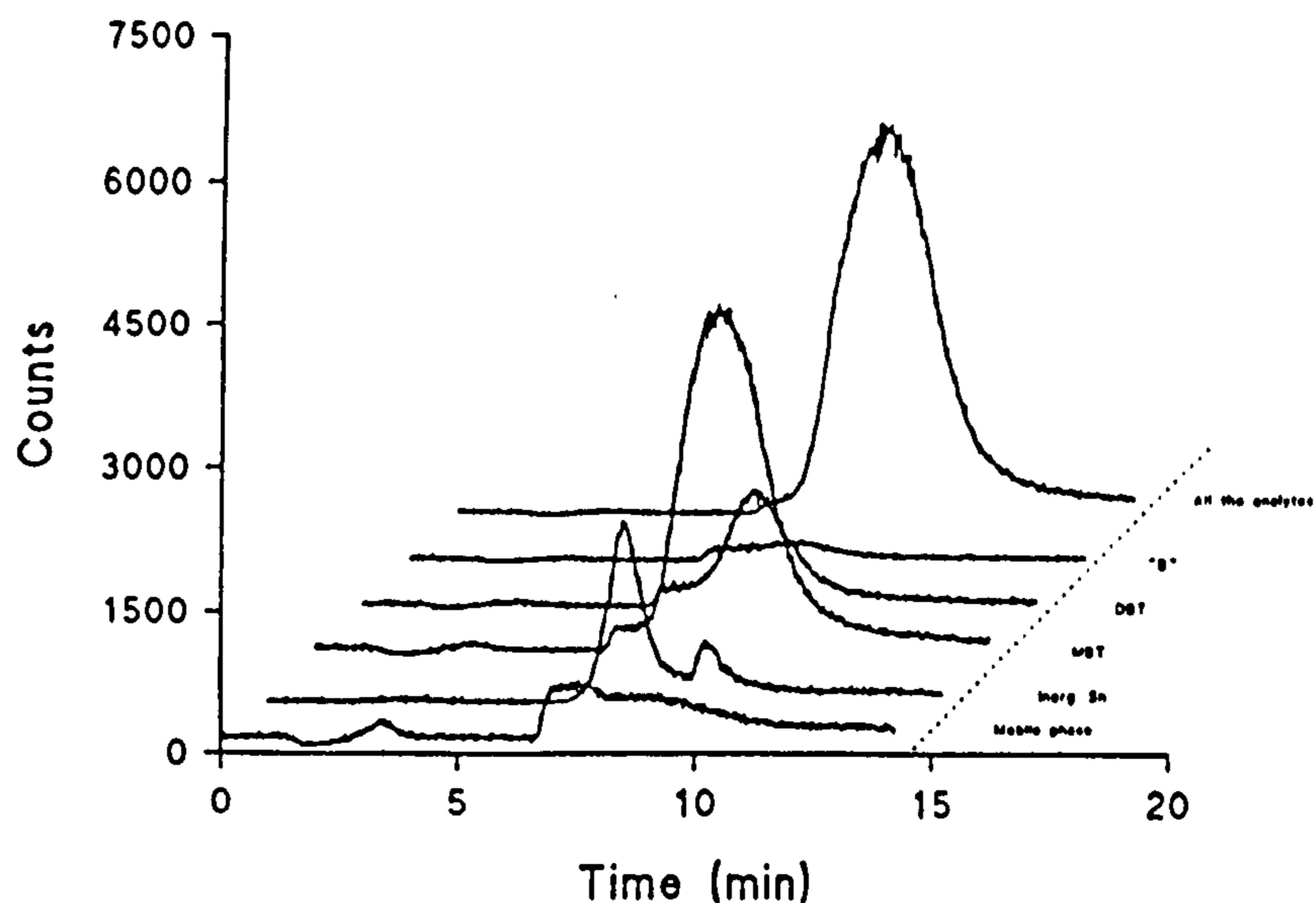


Fig. 3 Reversed-phase chromatogram of inorganic tin, MBT, DBT and TBT complexed with 0.005% (m/v) of tropolone. Mobile phase: 95% methanol/water.

e. Two experiments were carried out with the same calibrants; the first involved a study of the effect of adding the complexing agent to the sample and the second included the tropolone as a component of the mobile phase. Two concentrations of tropolone in two different mobile phases were tried, i.e. 0.0050% and 0.0075% (m/v) tropolone in mobile phases of 85 and 95% methanol/water. In both cases a single peak was obtained with all species coeluting.

The same methanol/water mobile phases (without tropolone addition) were then used to investigate the effect of adding the tropolone (0.005 and 0.01%, m/v) to the calibrant rather than to the mobile phase. In the case of the 95% methanol with 0.005% of tropolone as complexing agent in the calibrant, again all the peaks coeluted, as shown in Fig. 3. The same was found for the same mobile phase and 0.01% of tropolone in the calibrant.

For the mobile phase of 85% methanol with 0.005 and 0.01% (m/v) of tropolone in the calibrants, the chromatograms were similar. The chromatogram obtained for the inorganic tin species showed three peaks, the first being the solvent peak. The other two may correspond to two different eluted species, one of them inorganic tin non-complexed, the second one a more polar tropolone-complexed species, i.e.  $\text{Sn}(\text{trop})_n$  complex. This species was found to elute for both concentrations

of tropolone. The chromatograms obtained are shown in Figs 4(a) and 4(b).

An alternative mobile phase system was also evaluated. The use of tetrahydrofuran, water, acetic acid and tropolone (54, 38, 8 and 0.2%, respectively) has been reported for the separation of several organotin species: triphenyltin (TPhT), diphenyltin (DPhT), TBT, DBT, MBT, trimethyltin (TMT), dimethyltin (DMT) and monomethyltin (MMT). Here a mobile phase of tetrahydrofuran, water, acetic acid and tropolone (54, 38, 8 and 0.2%, respectively) was employed with atomic absorption spectrometric detection.<sup>17</sup> Reversed-phase chromatography was again employed, using a TSK gel ODS-8TM column (250 mm  $\times$  4.6 mm). Kadokami *et al.*<sup>17</sup> also suggested that the separation could be achieved with 54% methanol in the mobile phase instead of tetrahydrofuran (THF), although no experimental results were given to support this. Since this option offers advantages when the chromatography is coupled to ICP-MS detection (inductively coupled plasmas only tolerate low amounts of THF without desolvation), and additionally offers the possibility of extending the method to the separation of inorganic tin and other species of interest, the substitution of methanol for THF was evaluated. Inorganic tin, MBT, DBT and TBT were injected both as single calibrants and as a mixed calibrant. The suggested flow rate in the

original paper could not be used, due to a very high back-pressure on the column, so it was reduced until a normal back-pressure (approx.  $14 \times 10^6$  Pa) was achieved. Unfortunately, the chromatograms obtained were again disappointing, with all the peaks coeluting at or near the solvent front. The percentage of methanol in the mobile phase was both increased in steps up to

90%, and decreased to 30%. In all cases the organotin species coeluted, and no improvement was obtained.

#### Ion-exchange chromatography

Following the disappointing results obtained utilizing reversed-phase chromatography as described above, the use of ion-exchange chromato-

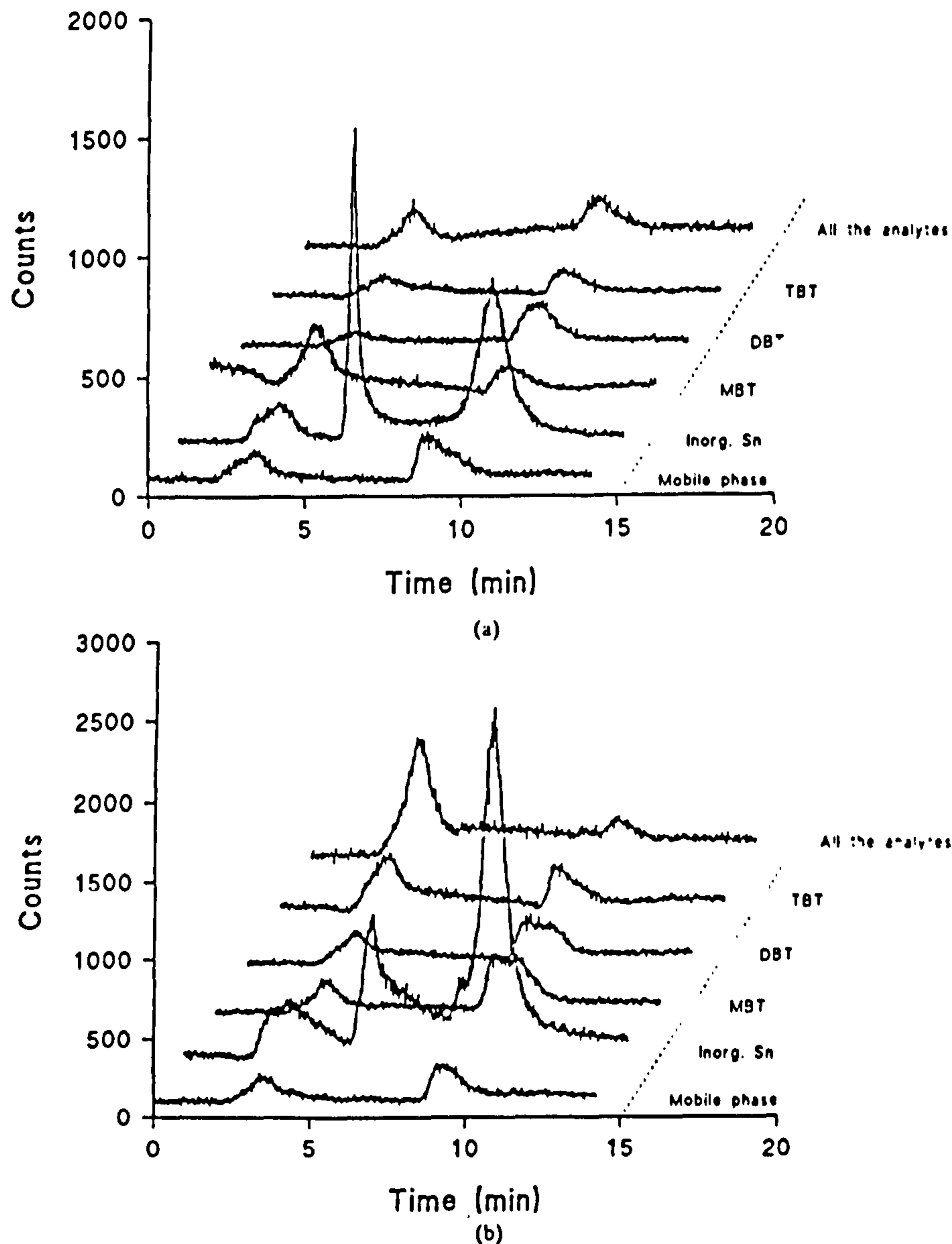


Figure 4 Reversed-phase chromatograms of inorganic tin, MBT, DBT and TBT complexed with (a) 0.015% (m/v) and (b) 0.01% (m/v) of troplone. Mobile phase: 85% methanol/water.



phy was evaluated.<sup>19,26</sup> This approach has already been used for water<sup>1,27</sup> and shellfish<sup>2</sup> and the aim of this study was to evaluate further the chromatography for potential use in later isotope dilution studies on potential candidate BCR reference materials, such as sediments. The organotin species were extracted from an organotin-enriched sediment spiked with MBT, DBT and TBT (10 ng as Sn of each analyte), using acetic acid in the following manner: 25 ml of acetic acid was added to 2 g of sediment and the analytes extracted for 4 h; shaking the samples helped the extraction. The sediments were centrifuged at 4000 rpm for 15 min. The extraction with acetic acid and centrifugation were then repeated. The supernatant was washed twice with 5 ml of acetic acid. The extract was diluted to 50 ml with Milli-Q water. The organotins were back-extracted into diethyl ether (3 x 10 ml). A few drops of sodium hydroxide (4 mol l<sup>-1</sup>) were added. Finally, the extracts were rotary-evaporated to dryness. The sediments were dissolved in the mobile phase prior to the analysis. Extraction efficiencies of 31 ± 25% for TBT, 33 ± 3% for DBT and 12 ± 5% for MBT were obtained using this method. As in previous studies,<sup>1,27</sup> a 25-cm Partisil 10 µm SCX column was used with a methanol/water (70:30, v/v) mobile phase employing ammonium citrate/citric acid buffer at pH 5.8. An example of a chromatogram obtained following this procedure is shown in Fig. 5(a). To improve resolution for DBT and MBT, gradient elution (methanol/water, 70:30, v/v, pH 5.8, to methanol/water, 85:15, v/v, pH 5.8, and finally methanol/water, 85:15, v/v, pH 3.4) has been employed, as shown in Fig. 5(b).

**CONCLUSIONS**

The detailed study presented here of the use of reversed-phase chromatography for the separation of organotin species demonstrates the limitations of this approach when employing methanol/water mobile phases. The restriction on mobile phase composition is imposed in this study due to the need to develop a separation system compatible with the ICP-MS detection system to be used in later isotope dilution studies. Although good resolution was obtained for MBT, DBT and TBT using methanol/water (95:5, v/v) mobile phase, the inorganic tin remained unresolved from the MBT and thus remains a problem when

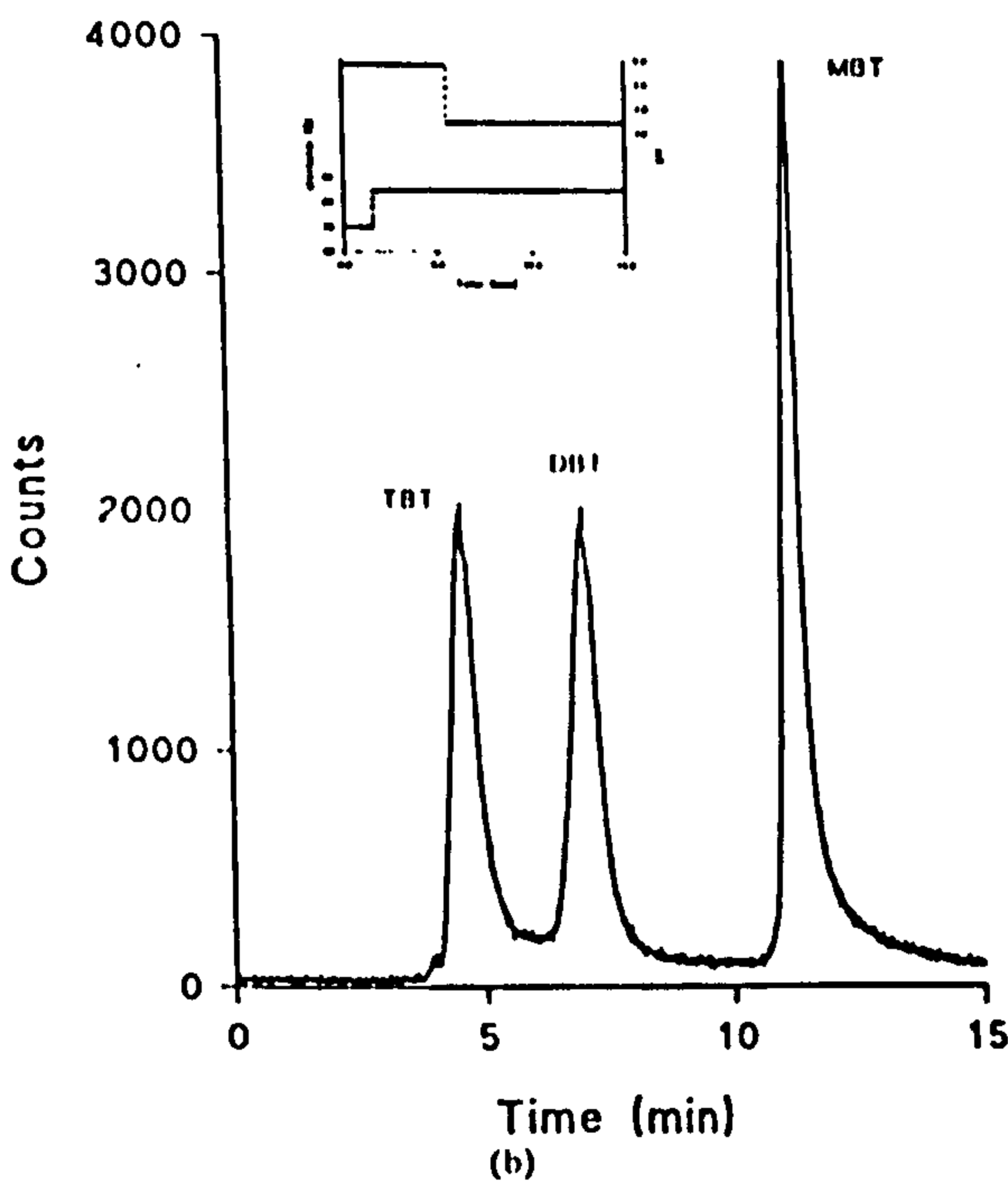
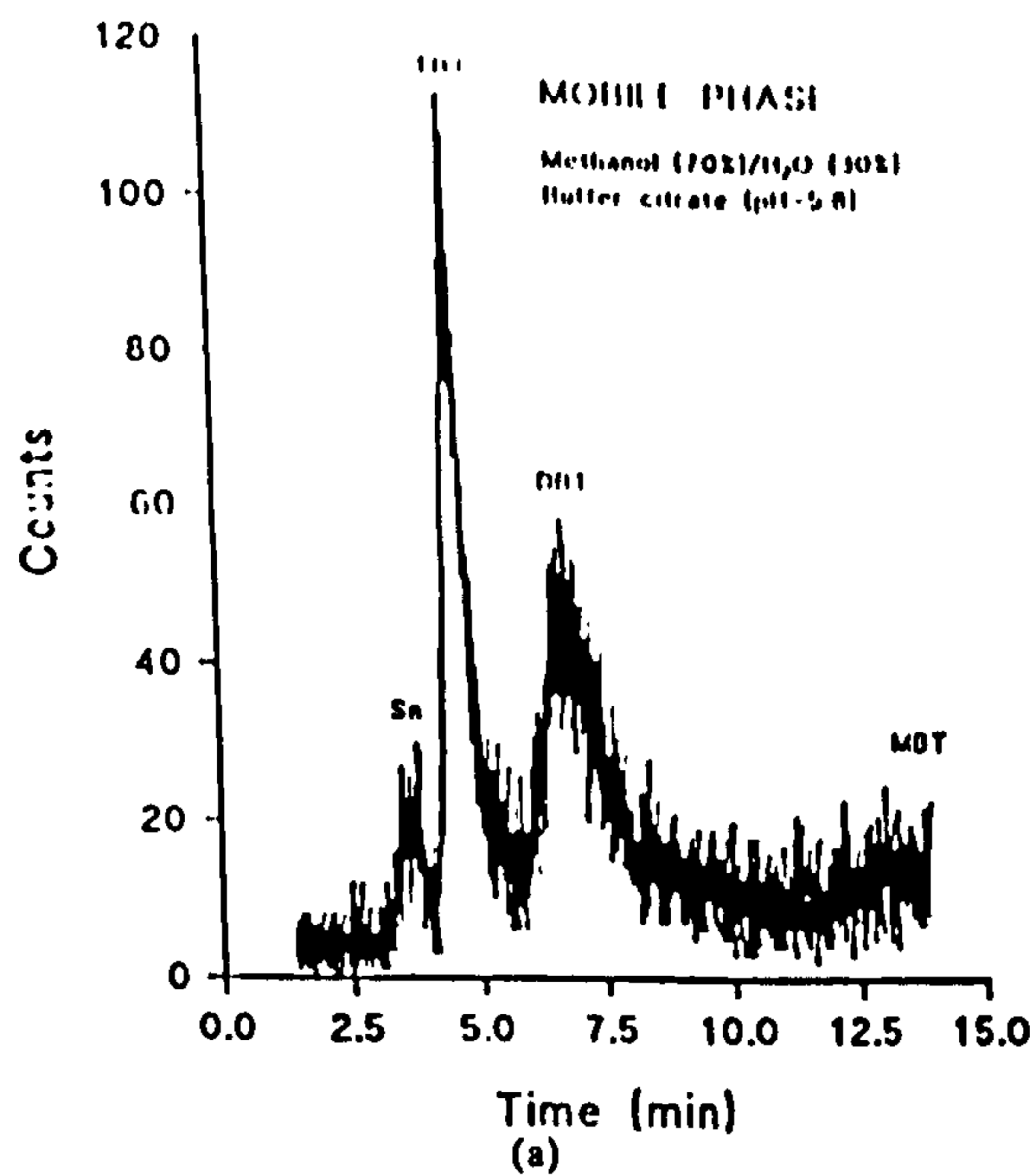


Figure 5 (a) Ion-exchange chromatogram using isocratic elution of inorganic tin, TBT, DBT and MBT. Mobile phase: 70% methanol/water, citrate buffer, pH 5.8. (b) Ion-exchange chromatogram using gradient elution of inorganic tin, TBT, DBT and MBT. Mobile phase: 70% methanol/water, citrate buffer, pH 5.8; 85% methanol/water, citrate buffer, pH 5.8; 85% methanol/water, citrate buffer, pH 3.4.

dealing with real samples. The use of tropolone was found to offer no advantage, and if present in the sample following extraction it may totally degrade the chromatography, possibly due to the formation of Sn(trop)<sub>n</sub> complexes. A similar approach based on a methanol/water/acetic acid/tropolone mobile phase as published in the literature was also unsuccessful, although a reversed-phase column from a different manufacturer was used for this study.

The use of ion-exchange chromatography still seems to offer the most potential for use in future work. This approach has already been used for natural waters<sup>1, 27</sup> and biological samples<sup>2</sup> and has successfully been used to determine TBT in a sediment candidate reference material.

The next stage of this work with respect to the BCR programme is to utilize the advantages of isotope dilution analysis as a highly accurate and precise analytical method to determine organotin species in environmental samples. The chromatography identified as optimal above will be used with organotin compounds of altered but known isotopic composition. Environmental matrices will then be spiked with the synthesized organotin species and determined by isotope dilution analysis—high-performance liquid chromatography—inductively coupled plasma-mass spectrometry (IDA-HPLC-ICP-MS) to assist in the validation of future certified reference materials.

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