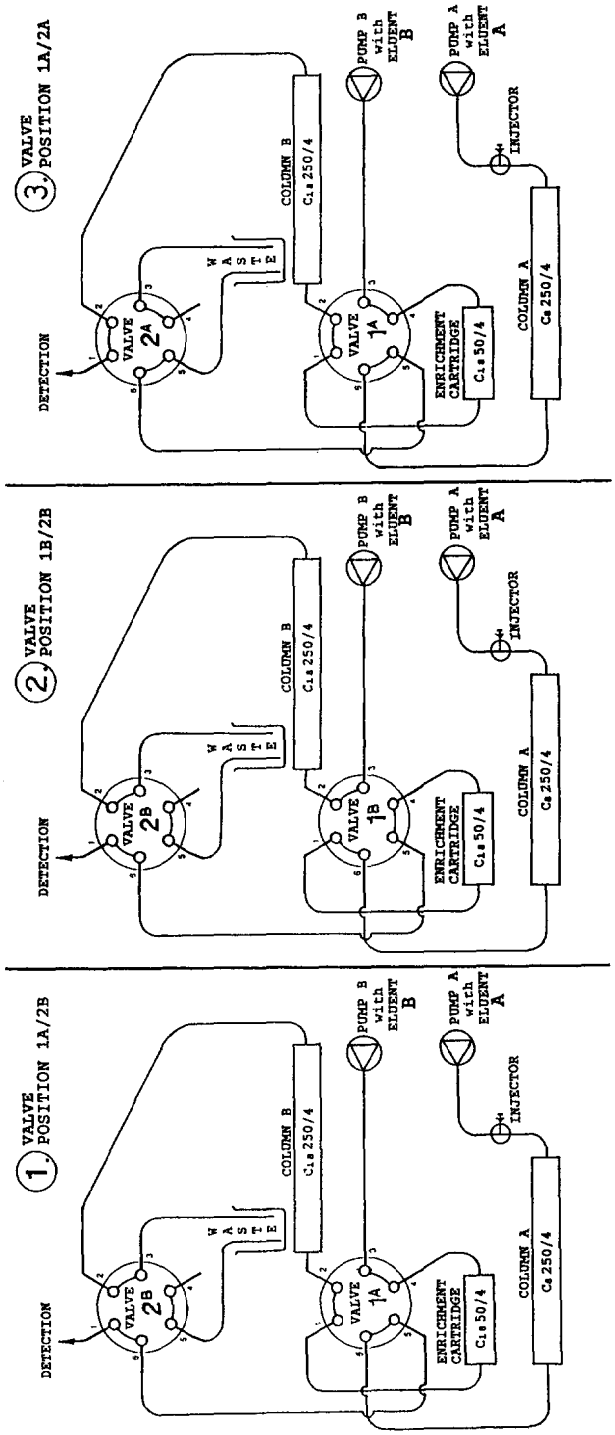

Fig. 13. Column-switching system for HPLC of ADAM derivatives [90].

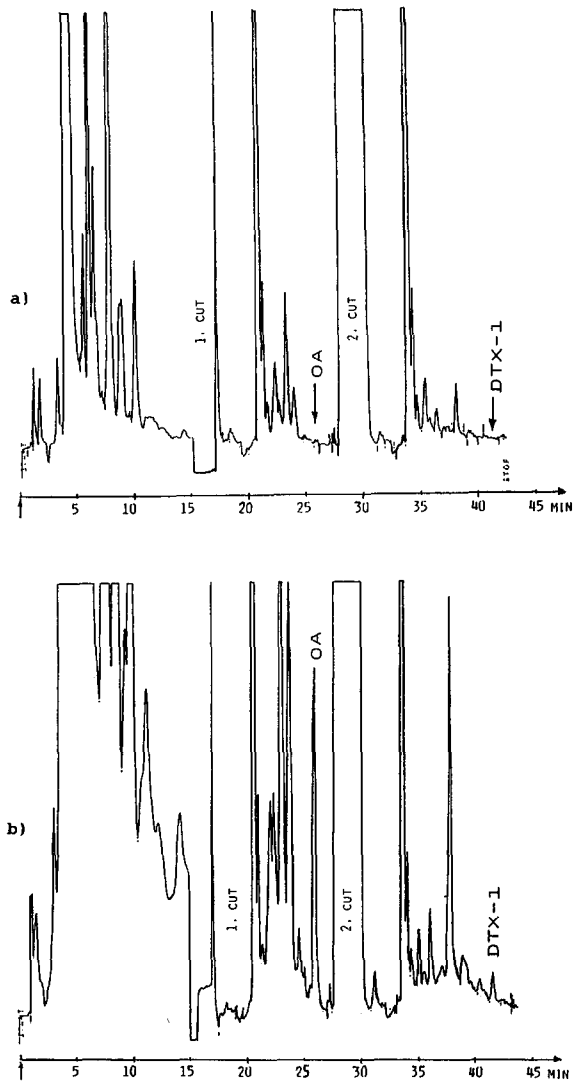

Fig. 14. HPLC of ADAM derivatives [91]. Column-switching system with two cuts. (a) Hepatopancreas (mussels, North Sea, uncontaminated); (b) hepatopancreas (mussels, Limfjord, contaminated).

tion provided by an HPLC–MS analysis is unsurpassed and will facilitate a greater understanding of the chemistry and biochemistry of seafood toxins.

3. CONCLUSIONS

Numerous cases of seafood poisoning occur worldwide each year especially due to the consumption of shellfish contaminated with high levels of toxins produced by marine dinoflagellates. As such incidents present a serious threat to public health and to the economy, there is a need for a better understanding the chemistry and biochemistry of seafood toxins and to develop analytical methods in order to guarantee safe, high-quality seafood products.

The most effective preventive measure is a monitoring programme with control at the source of the harvesting area. Production areas should be closed to harvesting when the toxin level in the shellfish approaches the established guideline or tolerance.

The best known hazard is paralytic shellfish poisoning (PSP). The accepted public health guideline recognized by most countries is 80 μg of PSP per 100 g of shellfish meat, using the AOAC mouse bioassay. This mouse bioassay has an acceptable

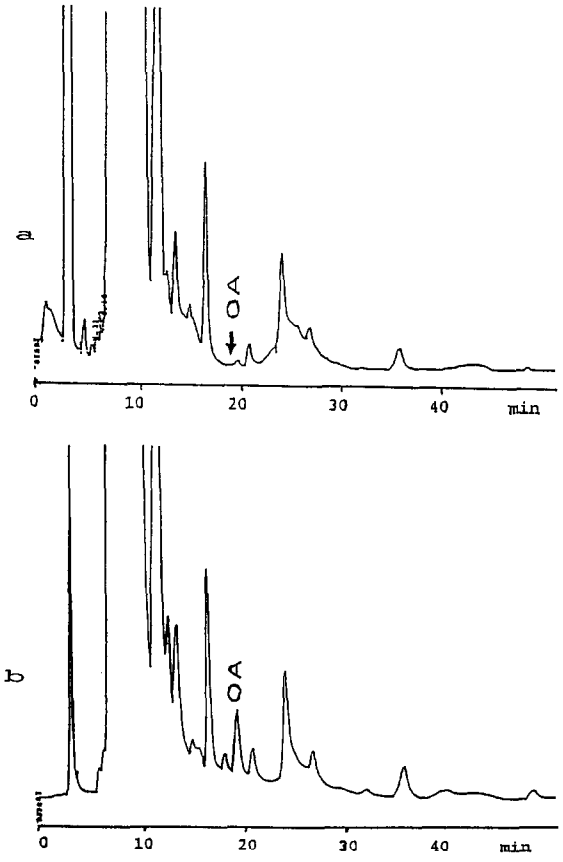

Fig. 15. HPLC of Br-Mmc derivatives [91]. (a) Hepatopancreas (mussels, North Sea, uncontaminated); (b) hepatopancreas (mussels, Limfjord, contaminated).

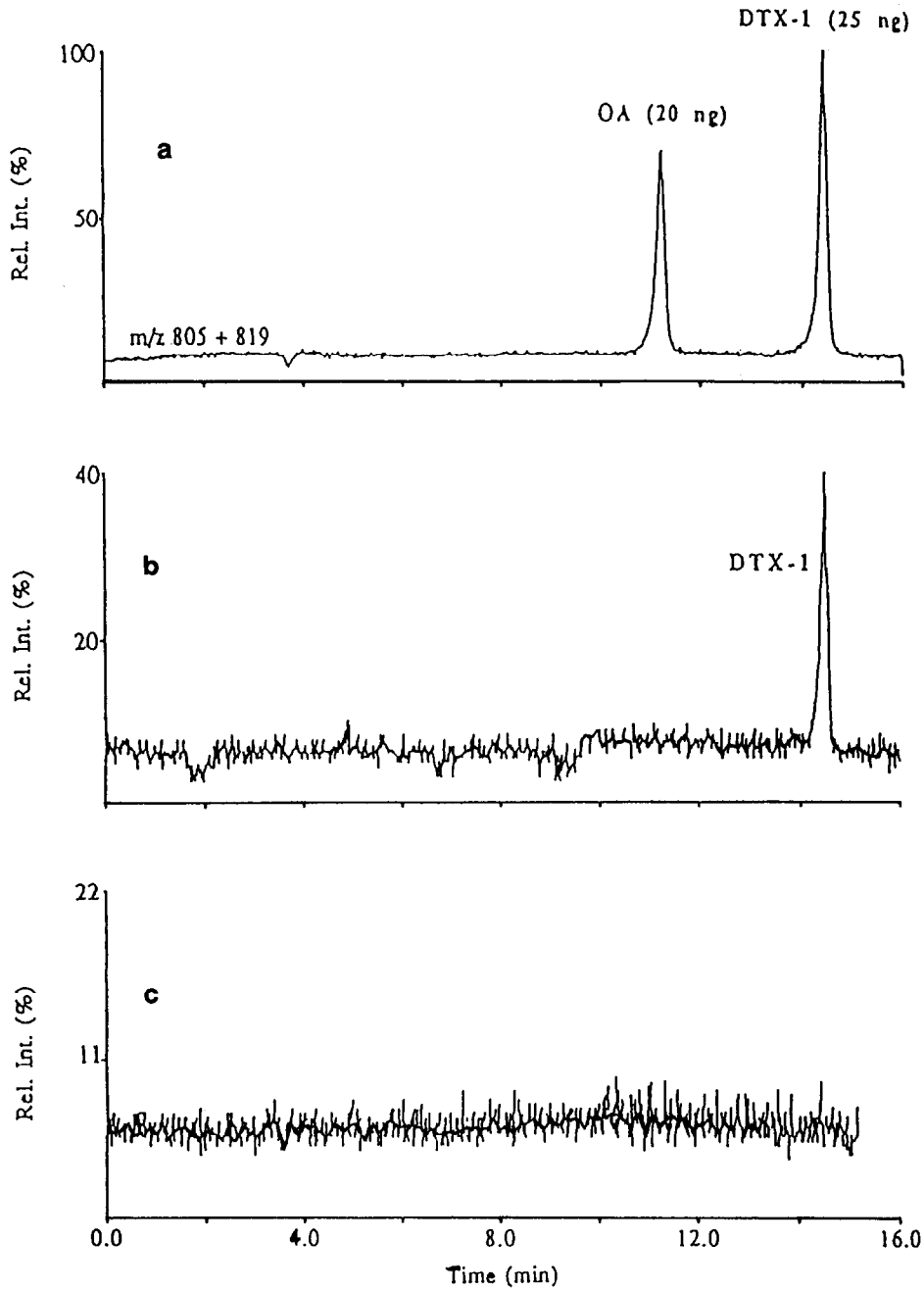

Fig. 16. LC–MS analysis of (a) DSP toxin standards and the extract of (b) suspect and (c) control mussel tissue extracts [83].

precision, but is lacking in sensitivity; 40 μg of PSP per 100 g shellfish meat are detectable. However, HPLC methods have been developed which can detect individual PSP toxins below the 1 μg per 100 g level.

Diarrhetic shellfish poisoning (DSP) is a more recently recognized problem. Okadaic acid (OA) and its derivatives (DTX-1 and DTX-3) are the principal toxins responsible for the diarrhetic symptoms.

The bioassays for DSP determination are not quantitative, the detection limit being 10 μg per rat. Compared with the bioassays, the HPLC method with fluorimetric detection for DSP provide advantages in terms of rapidity, accuracy, specificity and sensitivity.

The application of HPLC to the determination of the acidic components of DSP complex allows the detection of 10 μg of OA and/or DTX-1 per 100 g of shellfish meat. Therefore, HPLC analysis of these DSP components can act as indicators of DSP contamination, as no case of DSP contamination is known without the presence of at least one of these acidic components.

The acceptable levels for PSP and DSP differ significantly between countries. It is desirable for international organizations to evaluate the hazards caused by marine phycotoxins in order to provide a common basis for risk assessment, *i.e.*, to establish international toxin tolerances. For such an evaluation toxicity data are needed based on reliable analytical methodology. The further development of analytical methods for marine phycotoxins is especially needed as the enforcement of phycotoxin legislation is ultimately based on the ability of analysts to identify and determine these toxins accurately in seafood products. The HPLC methods discussed in this review are appropriate for solving these problems.

4. ABBREVIATIONS

Phycotoxins

PSP	Paralytic shellfish poisoning
B1–2, C1–4	N-Sulphocarbamoyl toxins
GTX I–IV	Gonyautoxin I–IV
dc-GTX I–IV	Decarbamoylgonyautoxin I–IV
NEO	Neosaxitoxin
dc-NEO	Decarbamoylneosaxitoxin
STX	Saxitoxin
dc-STX	Decarbamoylsaxitoxin
DSP	Diarrhetic shellfish poisoning
DTX	Dinophysistoxin
OA	Okadaic acid
PTX	Pectenotoxin
YTX	Yessotoxin

Derivatization reagents

ADAM	9-Anthryldiazomethane
Br-Mmc	4-Bromomethyl-7-methoxycoumarin

Units

MU	Mouse units

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Review

Application of ion chromatography to the determination of inorganic anions in foodstuffs☆

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ABSTRACT

A review on the applications of ion chromatography (IC) to the determination of inorganic anions in foodstuffs is presented. The anions most commonly determined in food, *i.e.*, SO^{2-}_3, NO^-_3 and NO^-_2, and to a lesser extent Cl^-, Br^-, I^-, SO^{2-}_4, IO^-_3, BrO^-_3 and phosphate, are considered. In comparison with standard methods for the determination of anions in food products, chromatographic methods are rapid, sensitive and precise. They also have the advantage of determining several ions simultaneously. The separation may be achieved by conventional IC, by ion interaction chromatography or by ion exclusion chromatography. IC has also been applied to the determination of Br, I, N and S in foods after oxidation or combustion of samples and conversion into anionic forms.

CONTENTS

1. INTRODUCTION

Originally, ion chromatography (IC) was developed as a chromatographic method for inorganic ions, using an ion-exchange resin as stationary phase and conductivity detection [1]. Currently, the definition of IC has been broadened to include the determination of organic ions and other techniques, such as ion interaction chromatography (IIC) and ion exclusion chromatography (IEC), which are based on other separation mechanisms rather than ion exchange, combined with nearly all of the HPLC (electrochemical or optical) detection systems. The stationary phases normally used in the ion-exchange mode are chemically modified polymers or silica gels with ionic functional groups,

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while the reversed phases widely used in HPLC are frequently employed for IIC separations. In IEC the separating material is a high-capacity cation-exchange resin in the hydronium form [2–6].

Ion chromatography has rapidly become a standard technique in water analysis [7,8], and it has also been demonstrated to be a very suitable technique for the solution of environmental problems [9–11] and in food analysis [12–14]. This paper reviews the applications of IC to the determination of inorganic anions in food products. The anions most commonly determined in food are SO_3^{2-}, NO_3^- and NO_2^- and, to a lesser extent, Cl^-, Br^-, I^-, SO_4^{2-}, IO_3^-, BrO_3^- and phosphate.

2. SAMPLE PREPARATION

When developing analytical methods for complex food matrices, sample preparation may be of fundamental importance [6]. Aqueous samples often require very little sample treatment; this also applies more or less to many beverages, but not to solid food samples. The extraction of ionic species from solid samples prior to IC analysis can often be achieved by heating a mixture of the homogenized sample with water, extractant solution or eluent in a blender or an ultrasonic bath for a specified time. After the sample has been dissolved, a further clean-up stage is often essential before an injection can be made into the ion chromatograph. Clean-up methods may be as simple as a filtration step or an adjustment of the pH, or they may be more elaborate such as selective removal of the analyte from the sample or elimination of interfering matrix components.

In this stage of the sample preparation many foods present problems owing to the existence of protein matter in the extract, which makes it difficult to obtain a clear solution. Such interference can be eliminated by boiling the extract to denature the proteins, by adding an organic solvent (such as acetone or methanol) or by employing Carrez I {$K_4[Fe(CN)_6]$ solution} and Carrez II [$Zn(CH_3COO)_2$ in acetic acid] reagents, which are very common in food analysis. The small sample volume required for IC analysis, in comparison with other analytical methods, makes this clarification step easier. Subsequently a separation of particulate matter, by filtration with or without a previous centrifugation step, is normally performed. Ultrafiltration devices in which the sample is forced under pressure through a membrane can also be applied.

Another common interference is due to soluble organic matter. There are several approaches to eliminate organic matter, such as treatment with activated carbon, solvent extraction or even selective removal of the analyte from the sample using ion-exchange resins. Usually, when anions are determined in foodstuffs by IC, the last stage of sample preparation before injection frequently implies removal of organic material by passing the solution through a disposable solid-phase extraction cartridge that contains a hydrophobic stationary phase (*e.g.*, C_{18} or polymeric) [15] in tandem with a disposable 0.45- or 0.20-μm filter in a single operation.

3. SULPHITES

Sulphites have long been used as preservatives in foods. There are three functions performed by sulphiting agents (sulphur dioxide, sulphites, hydrogensulphites and metabisulphites) in food products: antimicrobial agent, antioxidant and browning inhibitor. The last action could be related to the prevention of enzymatic browning in fresh fruits and vegetables or to the control of non-enzymatic browning in processed food. Sulphite is known as one of the most effective inhibitors of non-enzymatic browning, and although its chemical mechanism is not fully understood it possibly involves interactions of hydrogensulphite with carbonyl compounds present in food.

Recently, the addition of sulphites to food has become an important safety subject because of a number of documented adverse reactions in hypersensitive individuals, especially asthmatics. This has caused government agencies to review its use and to issue new regulations. Thus, the US Food and Drug Administration (FDA) requires that the presence of sulphite at or above 10 ppm of SO_2 be declared on the label of food products.

Sulphite added to food is present in free or bound forms. Bound sulphite consists of reversibly and irreversibly bound forms. The reversibly bound sulphites may be released either by an alkali treatment or more slowly by distillation with acid, whereas irreversibly bound sulphites, which form very stable addition compounds, are not detected by most ana-

lytical techniques. The sum of free and reversibly bound sulphite is referred to as total sulphite.

Over the years, the Monier-Williams (M-W) [91] method became the standard method for the determination of sulphite in food products with which other methods were compared. Although numerous modifications have been made since its first presentation, it consists of an acid distillation of samples, liberating SO_2. The latter is collected in hydrogen peroxide solution and oxidized to H_2SO_4, which is determined either by titration or gravimetrically. The modified M-W method is time consuming, it is not applicable to certain foods containing volatile acids and organic sulphur compounds and is subject to interference at low sulphite levels. Further, it is not very sensitive; in fact, the 10 ppm level was chosen by the FDA because it is considered to be the limit of detection of the method. In the FDA-optimized M-W method [92] as little as 1 ppm of SO_2 in foods can be detected (provided that the interference level is low), but even though the contribution from interferences is minimal, the method is still slow because it requires distillation for 1.75 h and it is not suitable for rapid screening of multiple samples. Further, because the allergic reactions might be to free but not to bound sulphite, a more reliable analytical technique is needed that is able to distinguish between free and bound sulphite (the M-W method cannot).

Recently, rapid and sensitive instrumental methods have been proposed for determining sulphite in food products. Among them, those using flow-injection analysis [16], differential-pulse polarography [17] and especially IC methods must be emphasized. The different procedures described in the literature for determining sulphite by IC have been classified in three groups, as shown in Table 1.

The procedures included under acid distillation in Table 1 combine the time-tested M-W procedure for isolating SO_2 from a complex food sample (in a reduced time) with a sensitive and specific detection system provided by IC. The approach implies a 10-min sample distillation with H_3PO_4, followed by purging with nitrogen and collecting the SO_2 produced in different absorbing solutions that are then analyzed by IC. In order to avoid interferences from other volatile and oxidizable sulphur compounds, the SO_2 released may be collected and stabilized as sulphite in a non-oxidizing alkaline medium of formaldehyde and NaOH [18–20], or in NaOH solution alone although there is a greater risk of oxidation to sulphate [21]. Other methods use formaldehyde in an almost neutral medium of potassium hydrogenphthalate, which forms the hydroxymethylsulphonate adduct (HMS) which is subsequently separated by IC [22]. Procedures that collect the distilled SO_2 in H_2O_2 solution, as in the M-W method, and determine the SO_4^{2-} produced by means of IC are also included within this group [23,24].

Generally, most acid distillation techniques provide a sensitive determination of sulphite in many foodstuffs, the limit of detection being 1 ppm of SO_2. The previous separation of sulphite from the matrix caused by the acidic treatment makes the further chromatographic determination much easier and, therefore, the chromatographic systems described in the literature are all equally efficient. One objection may be the 10-min distillation which is apparently insufficient in some instances. Further, although two of the procedures showed the possibility of distinguishing between free and bound sulphite, the procedures for determining free sulphite were different, one using an identical technique to that for total sulphite (treatment with H_3PO_4), but without heating [19], and the other measuring the free SO_3^{2-} in a mixture of the sample with an alkaline solution (NaOH–HCHO) [18]. The time for the complete analysis is about 25 min.

Other techniques are based on the liberation of bound sulphite from food by an extraction with alkali, which is more rapid than acid distillation. The headspace liquid chromatographic procedure [25,26] uses the headspace technique to liberate SO_2 from the alkaline extract and ion chromatography to determine low ppm levels of sulphite. The technique involves converting free and reversibly bound sulphite to SO_3^{2-} by treating the sample with an extracting solution at pH 11. An aliquot of the extract is acidified with H_3PO_4 to convert the liberated SO_3^{2-} to SO_2, which fills the headspace. Finally, a portion of the headspace is sampled and the SO_2 is converted back to SO_3^{2-} in the sampling syringe, which contains a basic trapping solution that is then analysed by IC. The procedure is very efficient in avoiding matrix interferences, its limit of detection is 1 ppm of SO_2 and the comparison with the M-W method was concordant for most samples,

TABLE 1

ION CHROMATOGRAPHIC DETERMINATION OF TOTAL SULPHITE IN FOODSTUFFS

Flow-rates and retention times are not included because they do not play an important role as a result of the previous sulphite treatment.

Treatment	Absorbing solution	Column	Eluent	Detection	Ref.
Acid distillation	HCHO–NaOH	Dionex AS-3	2.9–3.0 m*M* $NaHCO_3$–	Conductivity and amperometry (Ag, +0.4 V)	19[a]
		Dionex AS-5	2.2–2.4 m*M* Na_2CO_3	Conductivity	20[a]
		Dionex AS-3		Conductivity and amperometry (pulsed, Ag, +0.4 V)	21
	NaOH	Dionex AS-3		Conductivity	22
	HCHO–KHP (pH 6.1)	Nucleosil 10 Anion	10 m*M* phthalate (pH 6.1)	Indirect refractometry	23
	H_2O_2	Chrompack Ionsphere A		Conductivity	24
	H_2O_2		Phthalate (pH 5.7)	Indirect spectrophotometry (280 nm)	25
Alkaline extraction	Na_2HPO_4–mannitol–$FeSO_4$ (pH 11)	Hamilton PRP-X100	30 m*M* methanesulphonic acid (pH 11) (5% ACN)	Amperometry (glassy C, +0.6 V)	26,27[a]
	Na_2HPO_4–mannitol (pH 9)	Wescan anion-exclusion Brownlee Polypore Hion exclusion	5.0–20 m*M* H_2SO_4	Amperometry (Pt, +0.4 V/+0.6 V)	28–32[a]
	Na_2HPO_4–mannitol (pH 9)	Dionex ion-exclusion AS-1	10 m*M* H_2SO_4	Amperometry (pulsed, Pt, +0.7 V)	33
	HCHO–NaOH	Dionex AS-3	2.9 m*M* $NaHCO_3$–2.2 m*M* Na_2CO_3	Conductivity and amperometry (pulsed, Ag, +0.4 V)	21
Non-alkaline extraction	H_2O	Dionex AS-3	2.9 m*M* $NaHCO_3$–2.2 m*M* Na_2CO_3	Conductivity and amperometry (pulsed, Ag, +0.4 V)	21
	10% ethanol	Hamilton PRP-X100	30 m*M* methanesulphonic acid (pH 10.8) (5% ACN)	Amperometry (glassy C, +0.6 V)	34
	HCHO (pH 5.1)	Brownlee Polypore Hion exclusion	10 m*M* H_2SO_4	Amperometry (Pt, +0.7 V)	35
	HCHO (pH 5)	Spherisorb 10 ODS Altex Ultrasil Octyl	52 m*M* TBA–50 m*M* acetate (pH 5.8)	Spectrophotometry after post-column reaction (412 nm)	36
	HCHO (pH 5)	Zorbax ODS	5 m*M* TBA–50 m*M* acetate (pH 4.7)	Spectrophotometry after post-column reaction (412 nm)	37

[a] Free sulphite may also be determined.

except for some vegetables with a natural sulphite content and other foods for which the alkaline treatment liberates additional sulphite that the acid distillation does not. Nevertheless, the method is laborious and not as simple as other IC techniques which are more direct.

The fastest determination of SO_3^{2-} in the alkaline extract involves ion exclusion chromatography (IEC) with amperometric detection [27–33]. Separation into an anion exclusion column (frequently sulphonated styrene–divinylbenzene) is achieved by a combination of mechanisms such as Donnan exclusion, partitioning and size exclusion. Among the various components of the extract, strong anions are repelled by the negatively charged groups of the resin and the cations are retained on the column. The determination of organic acids (weaks acids) is probably the most common use of IEC. Weak acids are eluted in order of increasing pK_a values, hence the property of H_2SO_3 as a weak acid is utilized in this chromatographic technique. The method is rapid; the whole analysis can be carried out within 10 min, with a limit of detection of 0.1 ppm of SO_2, and it is also selective (see Fig. 1). The only major food component that behaves like sulphite in this system is ascorbic acid, although a baseline separation may be achieved by choosing a suitable mobile phase (6 or 20 m*M* H_2SO_4 is used as eluent). As for the headspace technique, this alkali extraction does not detect naturally occurring sulphite in some foods, and also does not effectively release sulphite bound to certain pigments produced in non-enzymatic browning reactions. The method can determine free sulphite (in this instance the extraction is accomplished in an acidic medium of pH 2) and total sulphite.

A direct alkaline–formaldehyde extraction procedure [20] has also been used in determining total sulphite, but the extract was very viscous and some difficulties also seem to exist in obtaining an adequate separation.

The non-alkaline extraction procedures include the extraction of sulphite in a neutral medium [20,34] or the stabilization of sulphite as the HMS adduct by reaction between SO_3^{2-} and formaldehyde in a slightly acidic medium [35–37]. One of these last techniques [35] is similar to the alkaline extraction–IEC determination described above [27–32], but HMS is very stable at pH 5.1 and, although the pH of the extract is rapidly adjusted by the eluent (10 m*M* H_2SO_4) in the chromatograph, it does not seem likely that under these conditions the sulphite may be completely liberated from the adduct. Water extraction [20] involves homogenization of samples with water, centrifugation and filtration of the supernatant. The aqueous extract is combined with an equal volume of CH_2Cl_2 and centrifuged again. An aliquot of the aqueous phase is injected into the column after a further clean-up stage. In the ethanol extraction procedure the sample is homogenized and extracted with 10% aqueous ethanol solution and injected after a C_{18} clean-up procedure. Both procedures use basic mobile phases that liberate the bound sulphite during the chromatographic run. It is worth emphasizing in this group the technique [36,37] based on the separation of the HMS adduct by IIC and further detection by means of a post-column reaction in two stages: first, SO_3^{2-} is liberated by treatment of HMS with alkali and then it reacts with Ellman's reagent and is detected spectrophotometrically. IIC uses a non-polar column in conjunction with an ion interaction reagent (the ionic modifier) in the mobile phase (in this instance tetrabutylammonium ion). The modifier acts as a movable site for ion exchange

Fig. 1. Chromatogram of sulphite determined by alkali extraction–IEC method. Dehydrated apple sample. The calculated concentration in the extract is 0.56 ppm of SO_2. Conditions: Wescan anion exclusion/HS column, eluent 20 m*M* H_2SO_4; amperometric detection (Pt, +0.6 V *vs.* Ag/AgCl). From ref. 31.

TABLE 2

SIMULTANEOUS CHROMATOGRAPHIC DETERMINATION OF NITRITE AND NITRATE IN FOOD SAMPLES

Column	Eluent	Detection	t_R (min) (NO_2^-/NO_3^-)	Sample	Ref.
Hamilton PRP-1	1.0 m*M* TPA fluoride (33% ACN) (1 ml/min)	Spectrophotometry (Conductivity)	10.5/13.3	Infant food, bacon and beer	38
Vydac 302 IC	11 m*M* CMS (pH 5) (2 ml/min)	Spectrophotometry (214 nm)	7.1/9.9	Cured meats	39
Waters CN Rad-Pak	1% Cetrimide–0.1 *M* KH_2PO_4 35% CH_3OH (1 ml/min)	Spectrophotometry (214 nm)	6.7/10.4	Cured meats	39
Waters NH_2 Rad-Pak	16 m*M* KH_2PO_4 (pH 3) (1 ml/min)	Spectrophotometry (214 nm)	6.5/8.4	Cured meats	39
Hamilton PRP-1	5.0 m*M* TPA bromide (33% ACN) (1 ml/min)	Spectrophotometry (240 nm)	7.4/11.1	Cured meats	40
Nucleosil 10 Anion	25 m*M* salicylate (pH 4.0) (1.4 ml/min)	Refractometry			41
Nucleosil 100 C_{18}	10 m*M* octylamine–H_3PO_4 (pH 4.0) (0.6 ml/min)	Spectrophotometry (210 nm)			41
Waters C_{18} Rad-Pak	Waters UV PIC A reagent (3 ml/min)	Spectrophotometry (214 nm)	2.5/4.0	Cured meats and vegetables	42
Ionosphere A	20–60 m*M* $NaClO_4$ (2 ml/min)	Spectrophotometry (209 nm)	3.6/4.2	Cooked ham	43
YEW SAX-1	15 m*M* $Na_2B_4O_7$–1 m*M* Na_2CO_3 (2 ml/min)	Spectrophotometry (210 nm) (Conductivity)	5.1/12.6	Vegetables	44
Waters IC-Pak A	5 m*M* K_2HPO_4 (pH 9.0) (0.8 ml/min)	Spectrophotometry (215 nm)	5.6/9.2	Cured meats	45
Biotronik Anion	5 m*M* CMS (pH 6.2–6.4) (1 ml/min)	Spectrophotometry (210 nm)	4.4/11.2	Cured meats	46
Vydac 300IC.405	Phosphate (pH 6.0)	Spectrophotometry (214 nm)		Cured meats	47

and it can be retained either on the resin alone (usually is a lipophilic ion) or as an ion pair with an ion of the sample (ion-pair chromatography).

Some of the non-alkaline extraction procedures are the simplest and may be of interest for screening purposes, but nevertheless none of them allows the determination of both free and total sulphite.

As has already been mentioned, some of the procedures described for the determination of total sulphite may also be used to determine free sulphite by the same chromatographic methods but with some modifications in the process of extraction. Nevertheless, concerning the most suitable pH for extracting free sulphite without liberating bound sulphite at the same time, there are some contradictions among the procedures described.

4. NITRATES AND/OR NITRITES

Nitrate salts are naturally present in many foods, mainly in vegetables, in concentrations that are characteristic of every species, although it also depends on the conditions of fertilization. The accumulation of large amounts of nitrate in plant tissues grown on heavily fertilized soils is of concern, particularly in infant food preparation, because the reduction of nitrate to nitrite in the infant intestine (more vulnerable because of its lower acidity), with subsequent absorption, could lead to cyanosis due to methaemoglobin formation. The potassium and sodium salts of nitrite and nitrate are also commonly used in the food industry, in curing mixtures for meats to develop and fix the colour, to inhibit the growth of microorganisms and to produce characteristic flavours. Recently, NO_2^- has been shown to be involved in the formation of low, but possibly toxic, levels of nitrosamines, many of which are potent carcinogens. Furthermore, NO_3^-, although not very toxic, can, under reducing conditions, be converted into NO_2^-. Therefore, the determination of nitrate and nitrite in foodstuffs has become increasingly important because of concern over their excessive dietary intake.

Traditionally, both ions have been determined in foods by spectrophotometric methods, the most common one involving diazotation of NO_2^- with sulphanilamide followed by coupling with N-(1-naphthyl)ethylenediamine. NO_3^- is previously reduced to NO_2^-, usually with Cd, and is then determined by difference. The spectrophotometric methods are time consuming, not very selective and can be unreliable for some samples. In addition, trace levels of nitrite in foods are often not detectable. In comparison, chromatographic methods are rapid, sensitive and precise. They also have the advantage of determining both NO_3^- and NO_2^- simultaneously [38–47]. This is of special concern in the analysis of meat products (see Table 2).

The literature on the application of IC to the determination of these anions shows nearly all the possibilities of this technique. The different modes may be related to the type of column used, *i.e.*, to the nature of the stationary phase. With respect to conventional IC, applications have been described that use silica- [39,41,43,48–52], poly(styrene–divinylbenzene)- (PSDVB) [46,53–55] or polymethacrylate-based (PM) [45,56] ion exchangers. Nitrates and nitrites can also be determined by IIC, employing octadecylsilyl (C_{18}) [41,42,57], octylsilyl (C_8) [58], PSDVB [38,40] or even cyano (CN) columns [39]. Nitrates, but not nitrites, which are eluted in the back of the solvent front, may be determined in a similar way using an amino-bonded column [59,60].

Ion interaction methods using C_{18}, CN or PSDVB columns give satisfactory results with standard solutions, but when the methods were applied to difficult samples, such as many meat products, poor separations were obtained. Irreproducible retention times and problems due to differences in matrix interferences as a result of variations in raw materials and/or processing methods were often found [39,40,42,45]. In contrast, excellent resolution of NO_2^- and NO_3^- is obtained with IC methods employing silica- or polymer-based columns, and the methods have been shown to be free from interferences (see Figs. 2 and 3). Linear calibration graphs have been obtained for nitrite and nitrate over a wide range, the detection limits being 2–3 ng [39,45].

Concerning detection systems, in most applications UV spectrophotometric detection is preferred [38–49,52,57–59], although other methods have also been reported, *e.g.*, conductivity [38,51,53–56], amperometry (NO_2^- only) [50,61] and refractometry [41]. UV detection is especially indicated in the selective determination of NO_2^- and NO_3^- in cured meats (which contain a large excess of chloride).

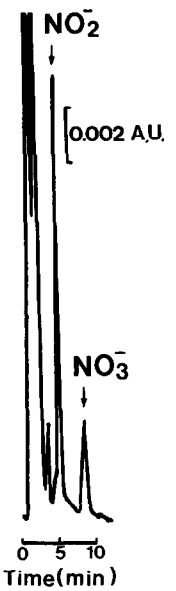

Fig. 2. Separation of nitrite and nitrate. Corned beef sample. Conditions: Waters IC-Pak A column with 5 m*M* dipotassium phosphate at 0.8 ml/min; UV detection (215 nm). The calculated concentration in the aqueous extract corresponds to 57 mg of nitrite and 32 mg of nitrate per kg corned beef. From ref. 45.

Recently, an IEC method with amperometric detection was proposed for the determination of NO_2^- alone [61]. In IEC most anions are eluted in the void volume and do not interfere with NO_2^-. As nitrite has a high pK_a value and is eluted late from the column (it depends on the column employed, but t_R was about 8 min on a Wescan anion-exclusion/HS column), the possibility of interference from other compounds as ascorbic acid or sulphite, which are eluted earlier, is minimal (see Fig. 4). Further, amperometric detection offers additional selectivity by selection of the operating potential. The limit of detection was 0.1 ng, which is one order of magnitude lower than that achieved by IC with UV detection.

Other applications concern the simultaneous determination of NO_3^- and other anions such as Cl^-, Br^-, SO_4^{2-} and PO_4^{3-} (occasionally NO_2^- is also determined), most of them by conventional IC, in different foodstuffs, mainly vegetables [51,52,54–56,60]. In vegetable analysis, interference from organic acids has frequently been observed. Peaks arising from malic, tartaric or glycolic acid may overlap the NO_3^- peak. In some instances this can be overcome by modifying the eluent [56], but in others it is necessary to use more specific procedures. These applications are shown in Table 3.

5. OTHER ANIONS

The determination of iodine in foods is important because, although it is an essential micronutrient, high levels of iodine in the diet may lead to thyroid-related problems. Because of the low levels at which it may be present and because losses of the element occur during sample digestion, a reliable determination of iodine in foods is very difficult. Milk and dairy products represent the main contribution, in

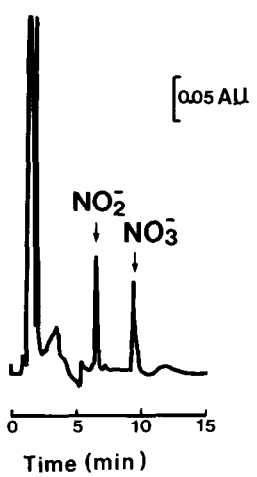

Fig 3. Separation of nitrite and nitrate. Bacon sample. Conditions: Vydac 302 IC column with 11.0 m*M* chloromethanesulphonic acid (pH 5.0) at 2 ml/min; UV detection at 210 nm. From ref. 39.

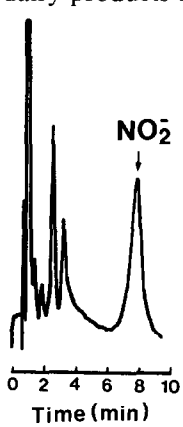

Fig. 4. Ion exclusion chromatographic determination of nitrite in ham. The calculated concentration in the extract is 0.25 ppm of nitrite. Conditions: Wescan anion exclusion/HS column, 20 m*M* H_2SO_4 at 0.8 ml/min; amperometric detection (Pt, + 1.0 V *vs.* Ag/AgCl reference). From ref. 61.

TABLE 3

ION CHROMATOGRAPHIC DETERMINATION OF NITRATE AND OTHER IONS IN FOODSTUFFS

Column	Eluent	Detection	t_R (min)	Other ions: t_R (min)	Sample	Ref.
Waters μBondapak NH_2	10 g/l KH_2PO_4 (pH 3.0) (1 ml/min)	Spectrophotometry (210 nm)	7.9	Br^- (6.4)	Vegetables, rice, cheese and flour	60
Dionex AS-3	2.1 m*M* $NaHCO_3$–1.68 m*M* Na_2CO_3 (3 ml/min)	Conductivity	9.2	NO_2^-, Cl^- (3.2), PO_4^{3-} (6.3)	Meat extract	54
Dionex AS-1	3.0 m*M* CO_3^{2-}–2 m*M* OH^-	Conductivity		SO_4^{2-}, PO_4^{3-}	Meat extract	55
Zipax SAX	0.5 m*M* phthalate	Indirect spectrophotometry (240 nm)	5.9	SO_4^{2-} (13.6), Cl^- (2.4)	Vegetables	52
Wescan 269-001 anion	4.4 m*M* phthalate (pH 3.9) (2 ml/min)	Conductivity	11.0	NO_2^- (7.4), Cl^- (5.8) PO_4^{3-} (pH 3.9) SO_4^{2-} (29.9)	Vegetables and salads	51
BAKC-1 (laboratory packed)	1.5 m*M* gluconic acid–1.5 m*M* boric acid (1.0 ml/min)	Conductivity	2.8	Cl^- (1.4)	Vegetables	56

the iodide form, to the dietary intake of iodine. Another important source of iodine is iodated table salt.

Various instrumental methods have been applied to the determination of iodide in milk, using either differential-pulse polarography [62,63], gas chromatography after conversion into an organic derivative [64,65], or iodide-specific electrodes [66]. The determination of iodide in milk and/or iodated table salt by IC, always with amperometric detection, has also been recommended [14,67,68]. Three different IC systems have been compared for the determination of iodide in milk sample extracts. Although all three columns performed well with standard solutions, there are significant differences when real samples are analysed. The best results were obtained with a Vydac 302 IC column (Separations Group) [68], because it accomplished a better separation of the I^- peak from other sample peaks (see Fig. 5). The method is sensitive, the detection limit for the food studied being about 25 μg/l of I^-. Iodine occurring in table salt may be in the iodide form, but also in the iodate form, and so it can be determined by chromatographic methods. Iodate has been determined either directly by IC [14] or by difference after first reducing iodate to iodide, which is then determined by IIC [69] (see Table 4).

Fig. 5. Ion chromatographic determination of iodide in a whole milk extract. Conditions: Vydac 302 IC column, 6.5 m*M* KH_2PO_4 (pH 6.3) at 2 ml/min; amperometric detection (Ag, +0.155 V). From ref. 68.

Another application relates to the determination of bromate in bakery products [70–73]. BrO_3^- is employed as a flour bleaching agent and dough improver. In all the examples described the interference of Cl^- and procedures for avoiding it are mentioned. Chlorite, another bleaching agent used in the manufacture of candy products, has been determined by IC with UV detection [74] (see Table 4).

Ion chromatographic determinations also have been published for several ions in different beverages [23,33,37,57,75–82], especially brewery products [23,33,75–78]. Other interesting IC applications to the determination of Br^- in vegetables [83] and the determination of Cl^-, HPO_4^{2-} and SO_4^{2-} in various sugar foods have also been proposed [84].

6. ELEMENTS AND ANIONS

IC has been applied to the determination of Br, I, N and S in different foodstuffs after oxidation or combustion of samples and conversion of these elements, normally in organic form, into Br^-, I^-, NO_3^- and SO_4^{2-}, respectively.

Certain vegetable oils contain sulphur (and nitrogen) compounds, probably isothiocyanates and thiocyanates arising from the hydrolysis of other components present in seeds. Fish oils have also been shown to contain sulphur compounds, including sulphides and methylthio esters, considered to be derived from bacterial degradation of methionine. Although these sulphur compounds occur in trace amounts, in many instances they are responsible for characteristic odours and have attracted considerable attention in the recent past, because they are known to inhibit the catalytic hydrogenation reactions of oil hardening processes.

Chromatographic procedures for the determination of sulphur in oil and grease involve the combustion of samples in an oxygen bomb in such a way that all forms of sulphur are converted into SO_4^{2-}. After the combustion, the interior surfaces of the bomb were rinsed with the chromatographic eluent and, after a further clean-up stage, the purified solution was injected into a Waters IC Pak A column and detected by conductivity [85] or indirect UV spectrophotometric detection [86]. The method is rapid and sensitive, the detection limit being 0.1 ppm of S with spectrophotometric detection (phthalate eluent, pH 6.5) or 0.5 ppm with con-

TABLE 4

ION CHROMATOGRAPHIC DETERMINATION OF LESS COMMON IONS IN FOODSTUFFS

Ion	Column	Eluent	Detection	t_R (min)	Sample	Ref.
I^-	Dionex AS-2	40 m*M* $NaNO_3$–4 m*M* HNO_3 (2.6 ml/min)	Amperometry (Pt, +0.8 V)	2.0	Table salt	67
	Nucleosil 10 CN	CTMABr–phosphate (CH_3OH)	Amperometry (glassy C, +1.0 V)	13	Milk	14
	Vydac 302 IC	6.5 m*M* KH_2PO_4 (pH 6.3) (2 ml/min)	Amperometry (Ag, +0.155 V)	6.2	Dairy products and table salt	68
	Partisil 10 SAX	6.5 m*M* KH_2PO_4 (pH 6.2) (2 ml/min)	Amperometry (Ag, +0.155 V)	6.0	Dairy products and table salt	68
	Waters IC Pak Anion	5 m*M* *p*-hydroxybenzoic acid (pH 10.5) (0.9 ml/min)	Amperometry (Ag, + 0.155 V)	6.1	Dairy products and table salt	68
IO_3^-	Nucleosil 7 C_{18}	1.66 ml/l octylamine–0.4 g/l Na_2SO_4 (pH 6.0) (1.1 ml/min)	Spectrophotometry (230 nm)		Table salt	69
	LiChrosorb-NH_2	3.6 g/l NaCl–HCl (pH 3.9)	Spectrophotometry (205 nm)	6.42	Table salt	14
	LiChrosorb RP-18	4 g/l HDTMACl–1 g/l NaCl	Spectrophotometry (215 nm)	8.52	Table salt	14
BrO_3^-	Dionex AS-1	3.5 m*M* tetraborate (117 ml/h)	Conductivity	28	Bread	70,71
	Bio-Gel TSK IC anion PW	7 m*M* CMS (pH 5.5) (1.4 ml/min)	Spectrophotometry (205 nm) and conductivity	2.7	Bakery products	72
	Zipax SAX	0.25 m*M* phthalate (pH 4.3) (0.8 ml/min)	Indirect spectrophotometry (230 nm)	5.8	Bread	73
ClO_2^-	Oyobunko ASA400	Phthalate–borate (pH 6.7)	Spectrophotometry (250 nm)		Candy products	74

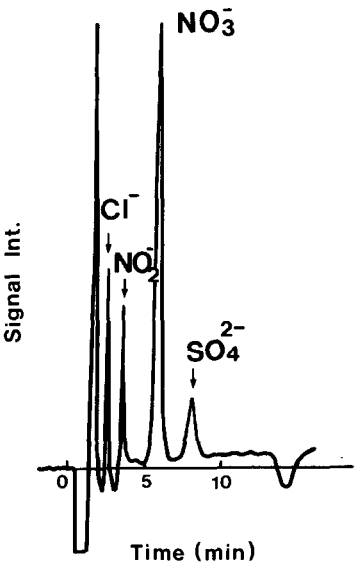

Fig. 6. Chromatogram of a fish oil sample after combustion. Conditions: IC-Pak A column, 1.0 m*M* potassium hydrogenphthalate at pH 6.5; UV detection at 290 nm. From ref. 85.

ductivity detection (borate–gluconate eluent, pH 8.5).

The same technique has also been proposed for the determination of nitrogen, which is oxidized mainly to NO_3^- (and some NO_2^-) and is detected by indirect UV spectrophotometry; hence one can simultaneously obtain the S and N contents of an oil sample by IC (see Fig. 6). In this instance it is necessary to degas the sample before analysis in order to avoid interference from dissolved nitrogen [87].

Similarly, combustion methods for milk and milk chocolate samples [88] (conversion of iodine into I^-) and for cocoa powder samples, after extraction with CH_2Cl_2 and ashing of the extract to convert Br into Br^- [89], have been published. Both ions were then determined by IIC. Total Br has also been determined in crops, after ashing, by IC with UV detection [90].

7. CONCLUSIONS

Among the anion chromatographic determinations described, those giving the best results may be emphasized. Thus, the IEC determination of SO_3^{2-} in food with amperometric detection seems especially suitable and it may be recommended for most foods and beverages at all sulphite levels. A rapid, sensitive and selective analysis can be accomplished, also allowing the separation between free and reversible forms of sulphite. This does not apply to darkly coloured foods.

Regarding NO_3^- and/or NO_2^- determinations, the simultaneous determination of both ions, which can be performed by means of several chromatographic systems, should be emphasized. Nevertheless, more reproducible and better results are obtained for the determination of NO_3^- and NO_2^- in difficult samples (*e.g.*, meat products) by means of conventional IC rather than ICC. The determination of nitrate in food products, especially vegetables, may be achieved with excellent results using different separation and detection systems. Although with many of these nitrate determination methods the interference of organic acids, which give peaks near the NO_3^- peak was mentioned, this interference can often be eliminated without difficulty. If the objective is the selective and sensitive determination of NO_2^- alone, then IEC with amperometric detection is superior and more convenient than conventional IC with either conductivity or UV spectrophotometric detection.

Finally, the very sensitive IC determination of iodide in foodstuffs, mainly dairy products, should be noted.

LIST OF ABBREVIATIONS

ACN	Acetonitrile
CMS	Chloromethanesulphonic acid
CTMABr	Cetyltrimethylammonium bromide
FDA	Food and Drug Administration
HDTMACl	Hexadecyltrimethylammonium chloride
HMS	Hydroxymethylsulphonate
HPLC	High-performance liquid chromatography
IC	Ion chromatography
IEC	Ion exclusion chromatography
IIC	Ion interaction chromatography
K_a	Acid dissociation constant
KHP	Potassium hydrogenphthalate
M-W	Monier-Williams

PM	Polymethacrylate
PSDVB	Poly(styrene–divinylbenzene)
PIC	Paired-ion chromatography
TBA	Tetrabutylammonium
TPA	Tetrapentylammonium
t_R	Retention time
UV	Ultraviolet

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