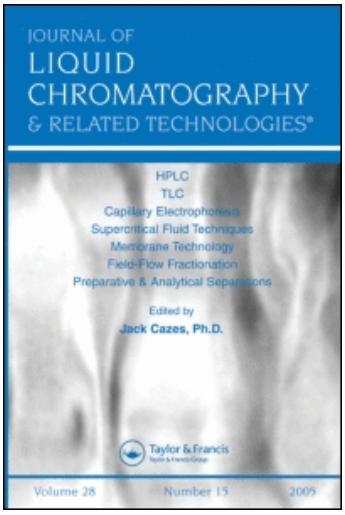

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LIQUID CHROMATOGRAPHY OF ORGANOMETALLIC COMPOUNDS

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LIQUID CHROMATOGRAPHY OF ORGANOMETALLIC COMPOUNDS

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ABSTRACT

The newest achievements in the use of liquid chromatographic techniques employed for the separation and quantitative determination of organometallic compounds in various environments are collected. The methods applied for the analysis of these classes of molecules are surveyed, critically evaluated, and the benefits and drawbacks of the individual chromatographic processes are enumerated in detail.

Key Words: Organometallic compounds; Sample preparation; Thin-layer chromatography; HPLC

INTRODUCTION

Organometallic compounds are of considerable practical and theoretical importance. As they are widely used in various industrial processes (catalysts, stabilizers, biocides, etc.) there are many possibilities for their entry into the environment causing serious environmental pollution.

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Species may markedly differ in their physicochemical characteristics resulting in modified solubility, leakage, bioaccumulation, toxicity, environmental impact, etc.

Gas chromatographic techniques have also been frequently used for the separation and quantitative determination of organometallic compounds. Thus, separation and determination of methyl- and ethylmercury,[1] methylmercury in biological matrices,[2] speciation of dimethylarsinic and monomethylarsonic acid in urine,[3] trimethyllead in rainwater and urban dust,[4] trimethyl and triethyllead in spiked samples,[5] various organotin compounds,[6,7] butyltin species in water and sediment,[8] and lard samples[9] have been recently reported.

The aims of this review are the collection of the newest achievements in the field of the liquid chromatographic separation of organometallic compounds, the description and critical evaluation of the techniques, and the comparison of the benefits and shortcomings of the various chromatographic methods.

PLANAR CHROMATOGRAPHY

The low operation cost, simplicity, numerous possibilities of detection, and simultaneous analysis of a considerable number of samples, made paper chromatography (PC) and various thin-layer chromatographic (TLC) techniques frequently used procedures in the analysis of organometallic compounds. Earlier results in their TLC determination have been previously reviewed.[10]

However, these compounds can be strongly bonded to the other constituents of the accompanying matrices. This binding has to be taken into consideration in the development of an efficient extraction process. The selection of a successful extraction method strongly depends on the character of the accompanying matrix and on the physicochemical parameters of analytes to be extracted.

The successful application of PC for the study of the ethylene dicysteine and ethyl cysteinate dimer complexes of Ga(III) and In(III) has been recently reported.[11] The complexes of ^{67}Ga and ^{111}In have been separated on a Whatman no. 1 paper, employing various mobile phases (85% of methanol; acetone–acetic acid, 3 : 1 v/v; methyl ethyl ketone, and chloroform). After development, the paper was cut into sections of 1 cm and evaluated. It was established that the method can be used for the study of the stability of such complexes.

Both PC and TLC have been employed for the study of the rhenium (^{186}Re) complexes of methylenediphosphonic acid, ethylenediaminotetra acetic acid, and citric acid. PC separations have been carried out on Whatman 1 paper using 0.9% aqueous NaCl. TLC investigations were performed on silica stationary phase, acetone being the mobile phase.[12]

It was found that the planar chromatographic systems separated, effectively, the various complexes and can be employed for the determination of the radiochemical yield of complexation.

The constituents of commercial Fe(III)-chelates (ethylenediaminedi(*o*-hydroxy-*p*-methylphenylacetic) acid and (ethylenediaminedi(*p*-sulfoxy-2-hydroxyphenyl) acetic acid have been separated by PC (stationary phase: Whatman no. 1; mobile phase: *n*-butanol–acetic acid–water, 4 : 1 : 5, v/v) and by TLC on silica and cellulose layers using the same mobile phase. It was stated that the method can be used for the purity control of commercial products.[13]

PC and TLC have been employed simultaneously for the study of the complexes of ^{99m}Tc with N′(butan-2-enyl-3-oximino)ethyl cysteinate and N′(3-oximinobutyl)ethyl cysteinate.[14] PC has been carried out on Whatman 3 paper with acetonitrile–water (1 : 1, v/v) mobile phase. TLC was performed on silica layers using 0.9% NaCl for development. It was established that these systems are not suitable for the study of the Tc complexes. Similar PC (Whatman 1 paper, acetone) and TLC (silica, saline) methods have been applied for the quality control of [^{99m}Tc]DTPA-folate complex.[15]

TLC has been extensively used for the investigation of the various complexes of heavy metals. It has been applied for the investigation of the DNA adducts of *cis*-diamminedichloroplatinum II (cisplatin).[16] HeLa cell DNA was incubated with cisplatin at various molar ratios and incubation times. DNA was extracted, enzymatically digested, and separated by two-dimensional TLC on cellulose layer. The first mobile phase was 1.0 M acetic acid (pH adjusted to 3.5 with NaOH), the second one consisted of 74 g of diammonium sulfate, 0.4 g of ammonium hydrogen sulfate, and 4.0 g EDTA dissolved in 100 mL of distilled water. The results proved that cisplatin form adducts with DNA, and the quantitation of the adducts, may help monitoring the cell response to cisplatin chemotherapy.

The separation of oxaliplatin from impurities was also performed with TLC carried out on silica layers and methanol–diethylamine–water (20 : 2 : 0.5 : 1.25, v/v) as mobile phase.[17] It was alleged that the procedure is sensitive and highly selective.

The separation of methionin and selenomethionin was obtained on octadecylsilica (C_{18}), diatomaceous earth and mixed layers (silica + diatomaceous earth) impregnated with 8% paraffin oil in benzene. Mobile phases consisted of various mixtures of methanol, acetonitrile, water, and phosphate buffer (pH 4.5). The best separation was achieved on C_{18} plates with 0.1 M KH_2PO_4–water–acetonitrile, therefore, this system was proposed for the measurement of methionine selenomethionine ratio in different matrices.[18]

The various complexes of ^{99m}Tc have also been investigated by TLC methods. The bis(*N*-cyclohexyl dithiocarbamato) nitrido ^{99m}Tc complex has been prepared, and its purity has been checked with TLC performed on silica layers

using dichloromethane–methanol (9 : 1, v/v) as mobile phase.[19] It was established that the radiochemical purity of the product is over 90%. The polydentate ligands of ^{99m}Tc [tris(1′,1′-dimethyl-2′-oximinopropyl)2-aminoethyl]amine and tris[(1′-methyl-2′-oximinopropyl)-2-aminoethyl] amine) have also been investigated with TLC,[20] using silica stationary phase and saline and acetonitrile eluents. TLC verified the purity of complexes. The efficiency of *N*-(2-pyridylmethyl)-iminodiacetic acid to form a complex with ^{99m}Tc was measured with TLC on silica stationary phase and acetonitrile–water (7 : 3, v/v) mobile phase.[21] It was found that the complexation resulted in one product.

The simultaneous application of TLC and high-performance liquid chromatography (HPLC) exploiting the advantages of both separation techniques has been demonstrated many times. The various complexes of ^{99m}Tc have also been investigated by TLC and HPLC. The ligands 3,3,9,9-teramethyl-6-R-4,8-diazaundecane-2-dionedioxime (R = benzyl, *p*-nitrobenzyl and *p*-aminobenzyl) were synthesized and complexed with ^{99m}Tc[22]. TLC was performed on silica layers with saline as mobile phase, and reversed-phase HPLC (RP-HPLC) was carried out on a PRP-1 column (150 mm, particle size, 5 μm) using gradient elution with mixtures of acetonitrile and water. The chromatographic patterns proved the adequate purity of complexes. A slightly different TLC and HPLC method has been employed for the purity control of Re(V) 2-(20pyridylmethylthio)-aniline complex.[23] TLC on silica stationary phase with saline and methylethyl ketone, RP-HPLC with PRP-1 column and water–methanol gradient elution demonstrated the purity of complexes.

The oxovanadium(IV) Schiff base complexes prepared from salicylaldehyde and dipeptides have been separated by TLC and RP-HPLC.[24] The purity control of synthesis products and the preparative separation was performed on silica layers using ethanol–water (7 : 3, v/v) and *n*-butanol–ethanol–propionic acid–water (10 : 10 : 2 : 5, v/v) mobile phases. RP-HPLC separations were carried out on a C_{18} column (250 × 4 mm I.D., particle size, 5 μm) using isocratic elution mode with 9% methanol, 5.2% THF, and 4.2% acetonitrile in 0.1 M aqueous sodium acetate (pH 6.8). It was concluded from the results that liquid chromatographic methods are suitable for the purity control of this class of complexes.

Two series of homologous ferrocyanoyl amides, $Fe(CH_2)_mCONH(CH_2)_nH$ ($m = 1$–3; $n = 4$–8) were analysed with both RP-TLC and RP-HPLC.[25] RP-TLC was carried out on Kieselguhr hand-made plates impregnated with paraffin oil–petroleum ether (30–60°C) 5 : 95, v/v. Development was performed with tetrahydrofuran–acetonitrile–water (0.4 : 0.12 : 0.48, v/v). Analytes were detected by their yellow color. RP-HPLC measurements were performed on a C_8 column (150 × 6 mm I.D., particle size, 5 μm) using various mixtures of tetrahydrofuran–acetonitrile–water as mobile phases. The flow rate was 1.25 mL/min, solutes were detected at 254 nm. The data indicated that the retention of analytes

increases with the increasing number of methylene groups in both RP-TLC and RP-HPLC.

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

Although, supercritical fluid chromatography (SFC) is not a strictly liquid chromatographic method, it has to be mentioned that SFC has also been employed for the separation of metal chelated and organometallic compounds and the results has been recently reviewed.[26]

The high reproducibility and low detection limit made the various HPLC techniques a method of choice in the separation and quantitative determination of organometallic compounds in different accompanying matrices. The newest advances in the application of liquid chromatography with mass selective detection for the analysis of environmental metal speciation[27] and, specially the speciation of platinum anticancer drugs,[28] have been currently discussed.

The overwhelming majority of HPLC separations are carried out in reversed-phase systems. The main advantages of RP-HPLC are the short equilibration time, and the use of environmental friendly mobile phases. The metal (Al and Co) complexes of azo dyes have been investigated with a RP-HPLC/ESI-MS (electrospray ionization mass spectrometric method).[29] Solutes were separated on a C_{18} column (250×4.6 mm I.D., particle size, 5 μm) using 5 mM ammonium acetate in water–acetonitrile (70 : 30, v/v). The flow-rate was set to 1 mL/min, the column was thermo-stated at 40°C. Metal complexes of azo dyes were well separated under the RP-HPLC conditions employed, proving that the method can be applied for their separation and for the study of ligand exchange in aqueous solutions.

Another RP-HPLC procedure has been developed and employed for the measurement of organo siloxanes and silanols from aqueous and biological samples.[30] The recovery of analytes on various SPE supports were determined, and it was established that nonpolar siloxanes with low molecular mass on C_{18} cartridge, siloxane polymers on C_4, and polar silanols on an ENV cartridge were effectively retained. RP-HPLC separation was performed on a C_{18} column (250×4.6 mm I.D., particle size, 5 μm) using gradient elution beginning with 100% of distilled water and finishing with 100% of acetonitrile. The baseline separation of silanols has been demonstrated.

RP-HPLC has also found application in the study of the degradation pathways of a peptide boronic acid derivative, 2-Pyz-(CO)-Phe-Leu-$B(OH)_2$[31] a proposed antitumor agent. The parent compound and its four decomposition products was effectively separated on a C_{18} column (150×4.6 mm I.D., particle size, 5 μm) using isocratic elution mode (acetonitrile–30 mM aqueous

KH_2PO_4/H_3PO_4, pH = 3.0; 40 : 60, v/v). The flow rate was 1 mL/min, the analytes were detected at 280 nm.[31]

The *cis* and *trans* macrocyclic complexes of ruthenium [Ru(1,4,8,11-tetraazocyclotetradecane)Cl_2]Cl have also been separated by RP-HPLC.[32] Analytical separations were carried out on a C_{18} column (250 × 4.6 mm I.D., particle size, 5 μm) using methanol–water (45 : 55, v/v) isocratic mobile phase containing 0.1% HTFA (pH = 3.7). Flow rate was 1 mL/min, solutes were detected at 358 nm. *Cis* and *trans* isomers were well separated with retention times of 9.7, and 11.6 min, respectively. It was stated that the method is suitable for the control of the synthesis of these classes of complexes and for the differentiation of isomers.

RP-HPLC found application in the measurement of an oral iron chelator and its iron complex in plasma.[33] Measurements were performed on a C_{18} column (150 × 4.6 mm I.D., particle size, 5 μm) with a mobile phase composed of 0.05 M Na_2HPO_4 and 0.01 M tetrabutylammonium hydrogen sulfate–acetonitrile–methanol (41 : 9 : 50, v/v). The flow-rate was 1 mL/min, analytes were detected at 295 nm. The results indicated that the method allows the simultaneous measurement of the concentration of free chelator and its iron complex in blood plasma, the dissociation of the complex during the chromatographic procedure being negligible.

A new complexing agent (2-thiophenaldehide-4-phenyl-2-thiosemicarbazone) has been successfully employed for the RP-HPLC separation of Co(II), Co(III), and Fe(II).[34] Metal chelates were analysed on a C_{18} column (250 × 4 mm I.D., particle size, 5 μm) with a mobile phase consisting of methanol–acetonitrile–water–1 mM sodium acetate–1 mM tetrabutyl ammonium bromide (78 : 10 : 10 : 1 : 1, v/v). Flow-rate was 1 mL/min, chelates were detected at 254 nm. It was stated that the method is suitable for the measurement of these ions with detection limits of 0.02–2.5 μg/mL. Organoselenium compounds, such as 4-hydroxyphenyl2-methyl-2-aminoethyl selenide, phenyl 2-methyl-2-aminoethyl selenide, 4-fluorophenyl 2-methyl-2-aminoethyl selenide, and 4-chlorophenyl 2-methyl-2-aminoethyl selenide have also been separated by RP-HPLC using electrospray mass spectrometry and postcolumn crown ether complexation.[35] Organoselenium compounds have been separated on a C_{18} column (100 × 2 mm) with gradient elution (1% aqueous acetic acid–methanol/2-propanol, 90 : 10, v/v). In order to decrease the fragmentation of analyte a crown ether (18-crown-6) was added postcolumn to the system. Solid-phase extraction (SPE) has been employed for the preconcentration of analytes from urine samples. It was assessed that the method can be applied for the analysis of other amine-containing solutes.

RP-HPLC has found application in the analysis of unstable zinc dithiocarbamates in rubber gloves.[36] Analytes were extracted with acetone, the extract was dried and redissolved with acetonitrile. Measurements were performed

on a C_{18} column (150×4.6 mm I.D., particle size, 5 μm) using gradient elution the mobile phase consisting of 10^{-5} M $ZnSO_4$ and acetonitrile. The separation of Zn-dimethyl-, Zn-diethyl-, Zn-dibenzyl-, and Zn-dibutyl dithiocarbamates, mercaptobenzo-thiazole, dibenzothiazole disulfide, *N*,*N*,*N*′,*N*′-tetramethyl-, tetraethyl-, dimethyl-, diphenyl-, tetrabutyl- and tetrabenxyl thiuram disulfides was obtained.

RP-HPLC using microbore column and inductively coupled plasma (ICP) and electrospray mass spectrometric detection (ESI-MS) has been employed for the study of metal complexes with metallothioneins and the results were compared with those of capillary zone electrophoresis.[37] RP-HPLC analyses were made on a C_8 column (150×1 mm, I.D.) using gradient elution of acetate buffer and acetonitrile. It was established that RP-HPLC ES MS is an adequate method for the analysis of metal complexes of metallothioneins.

Because of their marked toxicity, much effort has been devoted to the development of HPLC methods suitable for the separation and quantitative determination of mercury and organomercury compounds in water, marine organisms, biological and environmental samples, etc. An extensive review on this field has been recently published.[38]

A flow-injection-liquid chromatography-cold vapour atomic absorption spectrometric method has been developed and employed for the rapid determination of methyl and inorganic mercury.[39] Solutes were separated on a C_{18} column (53×4.5 mm I.D., particle size, 5 μm). Isocratic mobile phase consisted of methanol–water (3 : 1, v/v) containing 1.5 mM of ammonium pyrrolidine–dithiocarbamate. The detection limits were 1.7 and 3.4 pg for methyl and inorganic mercury, respectively. The method was proposed for the monitoring of low amounts of mercury species in environmental samples.

RP-HPLC coupled with atomic fluorescence spectrometer has been used for the study of the transalkylation mechanism between organolead and inorganic mercury in contaminated soils.[40] Organomercury analytes were extracted with a solution of dithizon in chloroform, and extracted again with thiosulfate dissolved in ammonium acetate. Hg(II), methyl-Hg^+, and ethyl-Hg^+ were separated on a C_{18} column with methanol–water (3 : 7, v/v), and the detection limit was found to be 50 pg absolute.

Organotin compounds have also been analysed with RP-HPLC. Thus, the RP-HPLC separation of mon-, di- and tributyltin, mono-, di- and tri-phenyltin has been reported.[41] Measurements have been carried out on C_8 and C_{18} columns (each 250×4.6 mm I.D., particle size, 5 μm). Mixtures of acetonitrile–acetic acid–water and methanol–acetic acid–water containing oxalic acid and triethylamine were the mobile phases. Flow rate was in each instance 1 mL/min. min. It was demonstrated that organotin molecules containing two or three organic ligands can be well separated using gradient elution. Because of the high sensitivity, the method was proposed for environmental analysis of these solutes.

A slightly different RP-HPLC method with atmospheric pressure chemical ionization-mass spectrometry detection has been also employed for the speciation of the same series of organotin compounds.[42] Measurements were performed on a C_{18} column (30×1.2 mm I.D., particle size, 3 μm) using gradient elution with water, aqueous 1% TFA and methanol. Di- and trisubstituted derivatives have been well separated and quantitated by the method, which also was proposed for environmental analysis.

The efficacy of ICP mass spectrometry (ICP-MS) and RP-HPLC has also been used for the analysis of the same set of organotin compounds in sediments.[43] Organotin compounds were extracted from sediment employing pressurized liquid extraction at 100°C. The optimal extraction mixture was 0.5 M acetic acid in methanol and 0.2% of tropolone. Organotin compounds were separated on a C_{18} column (250×4.6 mm I.D.) with the mobile phase methanol–water–acetic acid (72.5 : 21.5 : 6, v/v) containing 0.075% (w/v) tropolone and 0.1% (v/v) triethylamine. Mono-, di- and trisubstituted derivatives have been well separated by the method for the detection limit for tin, varying between 0.7 and 2 ng/g tin.

ION-PAIR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

Because of the ionic nature of many organometallic molecules, the favourable characteristics of ion-pair HPLC has been frequently exploited in their analysis. A semi-quantitative retention model was developed for the description of the retention behaviour of metal chelates in ion-pair and traditional HPLC, and its suitability has been verified using 4-(2-pyridazo)resorcinol as model compound.[44]

The determination of monomethylarsonous acid in human urine has been also performed by ion-pair HPLC.[45,46] The separation of inorganic arsenite and arsenate, mono- and dimethylarsonic acid was achieved on a C_{18} column (150×4.6 mm I.D., particle size, 3 μm) using tetramethylammonium hydroxide as ion-pairing agent and malonic acid buffer. Analytes were detected with hydride generation atomic fluorescence detector. It was established that the method separated the analytes well and can be successfully used for the metabolic and epidemiologic studies of arsenics.

A similar method has been employed for the separation of arsenite As(III), arsenate As(V), monomethylarsonate (MMA), and dimethylarsinate (DMA) in urine samples.[47] Urine samples were centrifuged, filtered, and directly injected into the chromatographic system. Analytes were separated on a C_{18} column (250×4.6 mm I.D., particle size, 5 μm) using isocratic elution with a mobile phase consisting of acetonitrile–buffer, 1 : 100, v/v. Buffer contained 4 mM of

tetrabutylammonium phosphate as ion-pairing agent and 2 mM of Na_2HPO_4 (pH = 7.1). As species have been well separated under these conditions, the limit of quantitation being 36 ± 6, 92 ± 16, 57 ± 9, and 101 ± 14 ng/mL for As(III), DMA, MMA, and As(V), respectively.

The same arsenic species, selenium(IV) and Se(VI) have been separated and quantitated in one run using gradient elution.[48] Measurements were carried out on C_{18} columns (125×4 mm I.D.; particle size, 5 μm and 250×4 mm I.D., particle size, 10 μm). Didodecyldimethylammonium bromide was employed as ion-pairing component at a concentration of 0.1% (v/v). Gradient elution was performed with NaH_2PO_4 solutions of various concentrations (pH 6.0). It was established that the method separated each species, the absolute detection limit being 1.0, 0.5, 1.0, 0.3, and 0.7 pg for Se(IV), As(III), DMA, MMA, and As(V), respectively.

The separation of 23 selenium compounds was obtained by using perfluorinated carboxylic acid as ion-pairing agent and ICP-MS and electrospray ionization mass spectrometric detection.[49] Selenium compounds were extracted from selenium-enriched yeast, selenium-enriched ramp (*Allium tricoccum*), and selenium-enriched garlic (*Allium sativum*), and analysed on a C_8 column (150×3.9 mm I.D., particle size, 5 μm). Mobile phases consisted of water–methanol (99 : 1, v/v) containing, separately, 0.1% of trifluoroacetic acid, 0.1% of pentafluoropropionic acid, or 0.1% heptafluorobutanoic acid (HFBA); the flow-rate was 1 mL/min. The best separations were achieved by using HFBA and modifying the pH of the mobile phase a half unit lower or higher with HCl or ammonia.

A similar system was employed for the separation of several biologically and environmentally important selenium compounds.[50] Selenium compounds were extracted enzymatically from a selenium nutritional supplement and analysed on a C_{18} column (250×4.6 mm I.D., particle size, 5 μm). Isocratic elution was performed with 2.5 mM sodium 1-butanesulfonate–8 mM tetramethylammonium hydoxide–4 mM malonic acid–0.05% methanol (pH = 4.5). It was found, that the method allows the rapid and baseline separation of organic Se compounds.

Inorganic and organic selenium compounds have been analysed on a silica-based reversed phase column with mixed ion-pairing reagents using flame atomic absorption spectrometry as element specific detector.[51] Good separation of selenic and selenous acids, trimethylselenium iodide, selenocystine, selenomethionine, selenoethionine, and seleno-cystamine was obtained on a C_{18} column (250×4.6 mm I.D., particle size 5 μm). The isocratic mobile phase was 10 mM of 1-butanesulfonic acid–4 mM of tetramethylammonium hydroxyde–4 mM of malonic acid–0.05% of methanol (pH was adjusted to 4.5). The method was proposed for the measurement of selenium compounds in selenium nutritional supplements. Niobium(V) and tantalum(V) have also been separated and

quantitatively determined in geological samples as ternary complexes with citrate and 2-(5-bromo-2-pyridylazo)-5-[*N*-propyl-*N*-(3-sulfopropyl)amino] phenol.[52] Nb and Ta were analysed on a C_{18} column (150×3.9 mm I.D., particle size, 4 μm) mobile phase being methanol–water (52 : 48, v/v) containing 20 mM of tetrabutylammonium bromide and 15 mM citrate buffer at pH 7. It was found that the results of HPLC were commensurable with those of ICP-MS.

Lead nitrate, triethyllead and triphenyl lead were successfully separated with ion-pair chromatography.[53] Baseline separation of solutes was obtained on a C_{18} column (150×3.9 mm I.D.) using 4 mM sodium 1-penta-sulfonate at pH 3 as ion-pairing agent. Gradient elution started at 40 vol% methanol and reached 90 vol% in 10 min. Solutes were detected at 254 nm and with particle beam-hollow cathode glow discharge atomic emission spectrometry. It was stated that this detector is element and ligand specific and can be employed in HPLC.

ION-EXCHANGE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

Because of the capacity of ion-exchange HPLC to separate easily dissociable and highly polar solutes, it has been extensively employed for the speciation of ionic organometallic compounds too.

The retention characteristics and the optimization of the separation of inorganic and organic arsenic compounds have been thoroughly investigated. Thus, the efficacy of three ion exchange columns for the separation of arsenite, arsenate, monomethylarsonic, and dimethylarsinic acids has been compared.[54] It was established that pH and the concentration of EDTA in the mobile phase exert a considerable influence on the separation. Good separation of analytes was achieved on silica-based columns at pH 5.9 and 0.1 mM EDTA. The procedure has been proposed for the analysis of water, soil, and sediment.

The efficacy of pressurized liquid extraction using water was studied for the extraction of arsenic species from freshly harvested plants. Anion exchange column coupled to ICP-MS was employed for the separation; stepwise gradient elution began with 0.4 mM HNO_3 and finished with 50 mM HNO_3, the concentration of benzenesulfonic acid always being 0.05 mM. The successful separation of As(III), dimethylarsinic acid, methylarsonic acid, arsenobetain, arsenocholine, tetramethylarsonium bromide, and trimethylarsine oxide was demonstrated.[55]

A similar set of arsenic species have been measured in seafood.[56] Analytes were extracted three times with methanol–water (1 : 1, v/v), the combined extract was dried at 44°C and redissolved in water. Separation was performed on a cation-exchange column (250×4.1 mm I.D.) using gradient elution between 100 mM ammonium dihydrogen phosphate (pH 4.3) and water. Species were

detected by hydride generation atomic fluorescence spectrometry. As the method well separated arsenobetaine, arsenoocholine, trimethylarsine oxide, and tetramethylarsonium ion it was proposed for the routine analysis and monitoring studies of these species in seafood.

Inorganic and organic arsenic compounds have also been measured in Slovenian mushrooms, and the efficacy of three arsenic specific detectors was compared.[57] Freeze-dried and powdered samples were extracted with boiling water and were injected into an anion exchange column (250 × 4.1 mm I.D., particle size 10 μm). Solutions of potassium hydrogen phthalate and sodium phosphate were used as mobile phases. Arsenite, dimethylarsinic acid, methylarsonic acid, and arsenate were separated, the most sensitive mode of detection being ICP-MS.

Ion exchange HPLC has found application in the study of the *N*-acetylation of 4-aminobenzenearsanilic acid by mammalian enzymes.[58] Measurements were performed on a weak organic acid column (100 × 6.5 mm I.D.) thermostated at 45°C. Isocratic mobile phase consisted of 5.0 mM aqueous sulfuric acid at the flow-rate of 0.6 mL/min. The retention times of various derivatives of arsanilic acid were 13 (4-nitrobenzenearsonic acid), 14 (4-hydroxy-3-nitrobenzenearsonic acid), 19 (4-hydroxy-3-*N*-acetylbenzenearsonic acid), and 33 min (4-*N*-acetylbenzenearsonic acid). It was stated that the method could be used for testing biological systems for acetylation capacity to arsanilate.

Selenium speciation has been obtained by coupling anion exchange and C_{18} columns through a six-port switching valve.[59] Selenite (Se^{IV}) and selenate (Se^{VI}) were well separated on the anion-exchange guard column with gradient elution using acetate buffer, and selenocysteine, selenome–thionine, and selenoethionine were separated on the C_{18} column (150 × 4.6 mm I.D., particle size, 5 μm) using water as mobile phase. Because of the relatively short separation time (15 min), good sensitivity, and repeatability the method was proposed for the analysis of environmental samples.

Another study employed one anion exchange column (120 mm × 4.6 mm I.D.) and isocratic mobile phase consisting of 5 mM sodium salicylate (pH 10)–methanol (98 : 2, v/v) for selenium speciation.[60] Analytes were detected with ICP-MS. The baseline separation of Se^{IV}, Se^{VI}, selenomethionine and trimethylselenonium ion was obtained. It was demonstrated that the sensitivity of the ultrasonic nebulizer is higher for these selenium species than that of a cross-flow nebulizer.

The stability of copper complexes has also been investigated by ion-exchange HPLC.[61] The column was equilibrated with the sample, using it as mobile phase. The interaction of metal species with the cation-exchange column was visualized by using a high specific activity of radiotracer. The dissociation rate constants of complexes Cu-EDTA, Cu-nitriloacetic acid, and Cu-citrate have been determined and found to be $(6.2 \pm 0.3) \times 10^{-3}$; $(1.0 \pm 0.04) \times 10^{-2}$, and $(3.1 \pm 0.2) \times 10^{-2}$, respectively.

Organic tin compounds such as butyltin and phenyltin species have also been analysed by ion-exchange HPLC. Thus, the determination of tri-, di- and monobutyltin from sediment has been reported.[62] Samples of 0.5 g weight were sonicated three times with 15 mL of methanol containing 0.05% of tropolone, 90 μL of acetic acid, and 125 μL of HCl. After centrifugation, the collected supernatants were mixed with 100 mL of deionized water and 30 mL of dichloromethane. The organic phase was separated, dried, and redissolved in 1.5 mL of methanol. Solutes were separated on an ion-exchange column (250 × 4.6 mm I.D., particle size, 10 μm) using 0.1 M ammonium acetate in 80 vol% methanol containing 0.1% of tropolone, and were detected by an inductively coupled plasma atomic emission detector. The method separated, well, the butyltin species and allowed the quantitative determination of butyltins in Venice Lagoon.

The separation capacities of a silica-based (250 × 4.6 mm I.D.) and a polymer-based cation-exchange column (250 × 4.1 mm I.D.) for the analysis of phenyltin and butyltin species were compared.[63] Analytes were detected after post-column derivatization with a spectrofluorimeter. Best separation was obtained with the isocratic mobile phase, 0.05 M malic acid, 0.003 M oxalic acid, and 0.035 m LiOH in methanol. Except for monobutyltin and monophenyltin, the tri- and di-substituted butyl and phenyl derivatives were well separated.

OTHER HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHODS

Although the overwhelming majority of separations of organometallic compounds has been carried out with the HPLC methods discussed above, other techniques, such as size-exclusion, gradient polymer elution, and chiral chromatography have also found application in their analysis. Thus, a two-dimensional HPLC method using size exclusion and anion exchange separation modes, has been developed for the identification of dimethylarsinoyl-riboside derivatives in seaweed.[64] Dried algae was sonicated firstly with water–methanol (1 : 1, v/v) then with water–methanol (1 : 9, v/v), centrifuged, the combined extract was evaporated to dryness, and redissolved in water. Size exclusion chromatography was carried out on a (300 × 10 mm I.D., particle size 13 μm) column using 1% of aqueous acetic acid as mobile phase. The fractions were collected and further analysed on an anion exchange column with 5 mM phosphate buffer. Dimethylarsinoyl-riboside derivatives (arsenosugars) were detected with pneumatically assisted electrospray tandem mass spectrometry. The method allowed the separation and identification of four arsenosugars.

Organosilicone copolymers have been characterised by gradient polymer elution chromatography using evaporative light scattering detection.[65]

Separations were carried out on CN and Si guard columns (20 × 3.9 mm I.D., particle size, 4 μm). Gradient profiles were always: 2 min with 100% non-solvent, 5–10 or 15 min linear to 100% solvent, 5 min with 100% solvent, and 2 min to 100% non-solvent. Solvents were 2-propanol and tetrahydrofuran–methanol (80 : 20, v/v). It was established that the method is suitable for the separation of grafted and block organosilicone copolymers.

Asymmetric organosilicon compounds have been successfully separated on chiral stationary phases.[66] The best separations were achieved on cellulose carbamate stationary phase using hexane as mobile phase.

Chiral *tris*-diimine ruthenium complexes have been separated on a silica-based chiral phase prepared by covalently bonding the glycopaptide antibiotic teicoplatin to isocyanate activated silica.[67] Analyses were performed on a column of 250 × 4 mm I.D. using various isocratic mobile phases (acetonitrile–methanol–0.1 M ammonium acetate, 60 : 20 : 20, v/v; 30 : 50 : 20, v/v; 40 : 40 : 20, v/v and 60 : 20 : 20, v/v). Ruthenium complexes with ligands such as 2,2′-bypyridine, 1,10-phenanthroline, 4,7-diphenyl-1d,10-phenthroline, 3-(pyrazin-2-yl)-1,2,4-triazole, and 3-(pyridin-2-yl)-1,2,4-triazole have been successfully separated.

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