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"Development of the preconcentration methods for the speciation of toxic metal ions using chromatographic techniques"

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INDEX

1.1 Introduction to metals

In the recent decades, trace metal determination has become recognized and become the major focus for analytical chemists. These metal ions have a variety of pathways by which they move through the environment. The pathway of soil-plant-animal-man has a vast significance to human health concerns [1-3]. Metals are classified according to the different categories according to their composition as pure metals and alloys. There are certain terms used in order to specify the group such as essential, non essential toxic and heavy metals in biological and environmental studies [4]. Some elements can be highly toxic to various life forms; others are considered essential, but can become toxic at higher doses.

1.2 Sources of toxic metal ions

Metals and metalloids play a key role in environmental systems and greatly influence ecosystem functioning and buoyancy. Trace metals are introduced anthropogenically as pollutants into our environment from numerous sources such as agricultural, industrial and domestic wastewater/effluents [5]. Atmospheric loading of heavy metal and metalloid components added to the environment by the coal and oil fired energy generating facilities. Over the years, their transport and pollution in the environment especially the aquatic ecosystems are becoming an environmental problem of concern owing to their eco-toxic properties. Additional contamination occurs from the disposal of toxic wastes from different sources [6-9].

1.3 Metal Speciation

Metal speciation analysis is an important environmental analytical tool for forecasting metal doom in aqueous samples and emerging successful methods for monitoring of water quality. Chemical compounds that differ in isotopic composition, conformation, oxidation or

electronic state, or in the nature of their complexed or covalently bound substituent's can be regarded as distinct chemical species [10]. The metal behaviour may be completely changed by its oxidation state or its association with specific ligands. Exemplified by the contrasting toxicities of methyl mercury and inorganic mercury species or Cr(III) and Cr(VI) species [11]. The importance of oxidation states is demonstrated by the elements chromium and arsenic also. The metalloid tin also shows markedly different health threats depending on its association with specific ligands. Metallic and inorganic form of tin doesn't present any health threats whereas organo tin compounds which are created by human industrial processes are highly toxic in nature (ATSDR 2005c). Just like behavior of chromium Cr(III) is an essential element plays role in glucose and lipid metabolism where as Chromium (VI) is highly toxic. Speciation of trace metal is essential to understand the toxicity and bioaccumulation. In the last decades considerable interest has increased in speciation of metals due to their toxicity and mobility that depend on the chemical form [12]. Metal speciation is achieved by the combination of two different techniques one is providing an efficient and reliable separation procedure and the other is detection and quantification.

Trace elements constitute a minor part of the composition of the human body and inspite of the low concentration of the elements in human body and tissues their function is by far not negligible [13]. Table 2 summarizes the uses, sources, deficiencies and their maximum permissible limit. Therefore, careful regulation of the trace element concentration in body and tissues is required to maintain the homeostasis that means the optimum concentration of each element. Their concentration needs to be reduced to improve its condition.

Table 2: Uses, sources and maximum permissible limit associated with metal concentration.

S. No.	Metal	Source	Accumulation	Biological functions	Other Uses	Excess symptoms	Deficiency symptoms	Maximum permissible limit (MPL)
1	Aluminium (Al)	Bauxite and recycling	Bones, Kidney and Brain	neither required by biological systems nor is it known to participate in any essential biological processes.	Packaging material, transportation, railway cars, food and beverages cans, spacecraft	Morbidity, mortality, excessive fear, panic attacks, emotional trauma, mental illness	-	0.2 mg/L
2	Chromium (Cr)	Leather tanning industries, clay, wood, glass products, AC supplying services	Liver, hair and blood	Involvement in glucose tolerance	Alloying element, as a catalyst in photographic industry, medicinal astringents, antiseptics.	Skin irritation, discoloration of teeth, lung cancer, intense thirst, abdominal pain	Diabetes, metabolic syndromes, blood sugar metabolism, anxiety or fatigue.	0.05 mg/L
3	Cobalt (Co)	Ores as a byproduct of mining and	Lungs	Oxidase, alkyl group transfer, component	Electroplating, dyes for jet engines,	Asthma, Pneumonia, Nausea and	Loss of appetite, weakness, anemia and	1.5 mg/L

		refining		of a cobalamine	gas turbines, magnetic steels	vomiting	emaciation	
4	Nickel (Ni)	Mining and refining, electroplating and electroforming, welding	Kidneys, Liver, Lungs	Hydrogenase, Hydrolase	Coins, reachargeable batteries, wires, ceramics, paints and computer parts	Irritation of nose and sinuses, Anaemia, asthma, bronchitis, cancer of larynx and stomach, chest pain	Urinary tract infection, allergic infections, skin rashes, paralysis, inflammation of liver and lungs.	0.02 mg/L
5	Palladium (Pd)	Earth crust, metal refining	Plant roots	As a catalyst	Dental alloys, jewellary	Fever, Necrosis, Erythema, oedema, skin disorders	-	-
6	Molybdenum (Mo)	Beans, lentils, sunflower seeds, wheat flour	Blood and Urine	Nirogen fixation, oxidase, oxo-transfer	Treatment of anemia, prevention of dental caries, vitamin mineral supplement	Eye irritation, shortening of breathe, wheeze, cough, respiratory symptoms	High blood level of urate and sulfite,	0.07 mg/L
7	Tin	Ores,	Liver and brain	Nil	Toxicity is	Eye	Fatigue,	-

	(Sn)	earth crust			severe just as a cyanide especially organotin compouds	irritation, brain damage, liver damage, chromosomal damage	depression, low cardiac output	
8	Zinc (Zn)	Ores, metallurgy, nuclear fission reactors	Prostate gland	Structure, Hydrolase	Rubber industry, antidandruff shampoo, sunscreens, alloys	Gastroenteritis, nausea and vomiting	Hair loss, skin lesions, diarrhea, eyesight, taste, smell and memory, weight loss, delayed healing of wounds	3 mg/L
9	Cadmium (Cd)	Ores, smelting, refining, Elecroplating, battery manufacturing	kidneys	Transported to blood via metallothionene	Anticorrosive, silver solders, welding pigment and plastic manufacturing	Chronic renal failure, pulmonary disease, cardiovascular disease, mild anemia, yellowing of teeth	Dry skin, loss of hair, loss of body weight, reduced growth, immune suppression.	0.003 mg/L

1.4 Instrumentation and Sample preparation

Instrumentation is required to enable the flow of the mobile phase through the stationary phase and also to convert the separated components into meaningful information. To perform the analysis it is necessary to set up the instrumentation and prepare the appropriate analyte solution following the HPLC analytical method.

1.4.1 Introduction to HPLC

The first use of chromatography in 1906 was credited to a Russian botanist Mikhail Tswett when he separated plant pigments such as chlorophylls and xanthophylls, since then there has been an enormous development of this technique [14]. The separation of analytes is based on differences in rates of migration through the column arising from different partition of the analytes between the stationary and the mobile phase. Depending on the partition behavior of the different types of analytes, these will elute at different times from the column outlet. Those that interact more with the stationary phase will straggle those molecules that partition preferentially with the mobile phase. As a result the analytes that elute last will be the ones that interacted most with the stationary phase and thus moved slowest through the column. The aim of the separation is to obtain the quantitative and qualitative information about the compounds of interest in a sample. It is preferred over other types of chromatography especially for molecules which have high polarity, low vapor pressure, large number of ionic groups, high molecular weight and thermal instability.

1.4.2 Instrumentation

Chromatography can be reported as a mass transfer process involving adsorption using a non-polar stationary phase and a polar mobile phase titrating through the column [15-19]. A typical High performance liquid chromatography consists of following components shown in Figure 1.

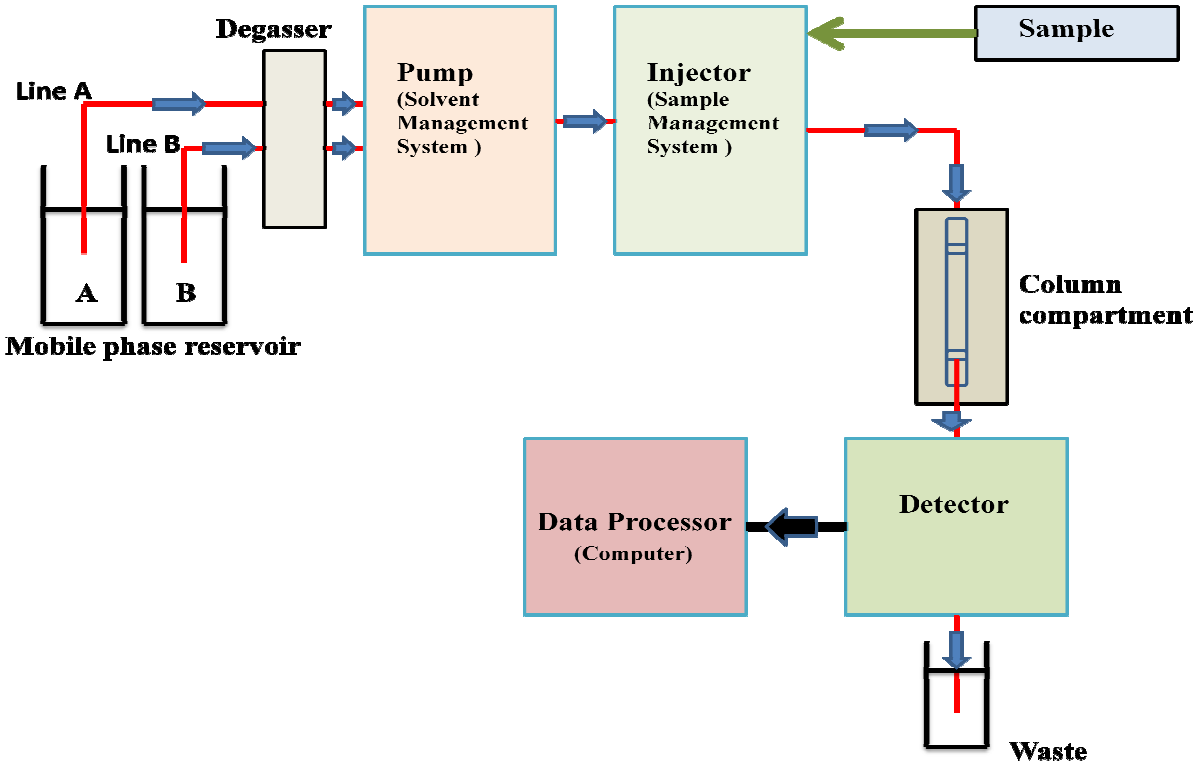

Figure 1: Schematic diagram of HPLC System

Pump

The role of the pump is to force a liquid through the liquid chromatography at a specific flow rate, expressed in milliliters per min (mL/min). Its performance directly affects the retention time, reproducibility and detector sensitivity. The pump delivers a steady stream of solvent from the reservoir to detector through the column.

• Normal flow rates in HPLC are in the 1 to 2 mL/min range.

• Typical pumps can reach pressures in the range of 6000-9000 psi (400 to 600 bar).

A pump can be compared to the human heart which continuously pumps blood throughout the body but though the human heart can withstand changes in blood pressure within specified limit

due to stress and strain the HPLC pump is required to deliver flow of mobile phase at constant pressure and flow rate.

Injector

Samples are injected into HPLC via an injection port. The injection port consists of an injection valve and sample loop. The injector can be a single injection or an automated injection system. An injector for an HPLC system should provide injection of the liquid sample within the range of 0.1-100 mL of volume with high reproducibility. The sample is drawn into a syringe and injected into a loop via sampling valve. A rotation of the valve rotor closes the valve and opens the loop in order to inject the sample into the stream of the mobile phase.

Columns

Columns are usually made of polished stainless steel, are between 50 and 300 mm long and have an internal diameter of between 2 and 5 mm. They are commonly filled with a stationary phase with a particle size of 3-10 µm. The packing consist of small, rigid particles having a narrow particle size distribution. It can be of porous polymeric beads, porous layers beads or silica particles. The packing depends on the mechanical strength. Particles of diameter more than 20 µm can usually be dry packed, whereas for particles with diameter less than 20 µm slurry packing techniques are used.

Mobile Phase

The mobile phase acts as a carrier for the sample solution introduced to the solvent being continuously applied to the stationary phase. The essential property of the solvent is its ability to interact with both stationary phase and analytes in the mixture, resulting in desired separation. An ideal solvent or solvent mixture will be readily available in high purity, economic, safe to use routinely and should be compatible with the entire HPLC machine.

Detectors

The HPLC detector located at the end of the column detect the analytes as they elute from the chromatographic column. HPLC detectors should have an excellent linear response, wide linear dynamic range, sensitivity, reproducibility and high signal to noise ratio. Some of the common detectors include: Refractive index, Ultraviolet, Fluorescent, Radiochemical, Electrochemical, Near-infra red, mass spectroscopy and light scattering. Commonly used detectors for my present research work are Ultraviolet and fluorescence detector.

Recorder

Signals from the detector may be collected on chart recorders or electronic integrators that vary in complexity and in their ability to process, store and reprocess chromatographic data. The computer integrates the response of the detector to each component and places it into a chromatograph that is easy to read and interpret.

1.5 Sample Preparation

The analysis of trace and ultra-trace level of organic analytes that can be applied to different sample matrices of interest cannot be carried out without proper sampling and sample preparation. Sample preparation may be done on the analytes, on the matrix or both to perform dissolution, cleanup, concentration or chemical modification of the sample for obtaining better analytical results [20]. Therefore, an efficient pre-concentration and extraction technique is needed to be used for the determination of trace and ultra-trace level of organic analytes prior to their analysis. The separation/preconcentration step is the most important step for the purification of samples and determination of trace metal ions [21-23]. Preconcentration of analytes may be defined as collection of the analytes from the large volume of a solvent to a smaller volume of the solvent. In preconcentration, elution or desorption procedure follows the separation step and

proves an effective way for enriching the target analytes. In order to achieve higher PF the eluent volume should be small as possible and the sample solution volume should be as high as possible. Currently heavy metal pollution is one of the serious concerns all over the world due to industrial activities, improper disposal of wastes, pollution of agricultural soils and accumulation along the food chains. These are among the most hazardous anthropogenic pollutants due to their toxicity and bioaccumulation in the environment. Separation of the trace metals from the drinking water, river water, ground water, tap water has been widely applied to the purification of these samples. The main purpose of such studies is to remove the toxic metal ions from the sample. The quality and quantity of the respective element species in a matrix are highly responsible for the mobility, bioavailability and finally the ecotoxicological or toxicological impact of the element. Metal speciation or determination depends on the technique used for the extraction. The analysis of trace amount of metals in complex matrix has been usually complicated, inspite of the various modern analytical instrumentation now available. In such matrices, determination should be preceeded by separation and preconcentration steps prior to HPLC analysis. Several of the off-line techniques can be employed not only to remove the interferences from the complex matrix but also to concentrate the samples. But for the present work a new generation sample preparation technique, Fabric Phase Sorptive Extraction, has been developed to address most of the sample preparation challenges encountered by separation scientists.

1.5.1 Fabric phase Sorptive extraction (FPSE)

Fabric phase sorptive extraction technique was developed by Dr. Abuzar Kabir from South Florida as a method of taking sample from liquid and injecting it into analytical system for the analysis. To be consistent with today's challenges of sustainability and environmental issues

green analytical chemistry (GAC) demands are taken into the consideration which can be met by using modern solvent minimized sample preparation technique. FPSE has emerged as a powerful sample preparation technique for the analysis of trace metals from environmental samples. It is an ideal preparation technique that effectively incorporates most of the advantages of solid-phase microextraction (equilibrium based extraction) and solid phase extraction (exhaustive extraction). It minimizes majority of the shortcomings inherently present in these sample preparation techniques due to the (1) shortcoming in their geometrical configuration; (2) inefficiency originated from the immobilization of sorbent onto the substrate surface. The Sol-gel coating technology applied in FPSE is a highly effective chemical coating process that ensures superior level of batch-to-batch coating reproducibility. It ensures faster, cleaner, highly pre-concentrated target analyte solution without solvent dry-down and reconstitution process, ready for instrumental analysis. It is a sample preparation technology that favors sample preparation directly in the field, thus reduces the cost and errors involved in sampling, sample transportation to the lab, storage, and prepare the sample in the lab followed by instrumental analysis. It also minimizes number of sample preparation steps, thus reduces potential sources of errors in analysis. It has been extensively studied for the analysis of volatile compounds, pharmaceutical drugs, organic pollutants, semi volatile organic compounds and especially for environmental matrices [24-28]. FPSE is relatively insensitive to matrix effects if other standard parameters like pH, mobile phase composition etc are controlled.

1.5.2 Working with FPSE

Cleaning of FPSE media:

Before the extraction, immerse the FPSE media in 2mL Acetonitrile: Methanol (50:50) for 5 minutes then rinse the same in 2 mL Deionized water to remove the organic solvents. Scheme is given in Figure 2.

Extraction:

Transfer a suitable amount of the sample in 10/20 mL vial. Insert a magnetic bar followed by the cleaned FPSE media. Set up the magnetic stirrer at a medium level and then extract for 10-40 minutes depending upon the nature of target analytes.

Back Extraction:

Take 500 µL of the extracting solvent/solvent system in a deactivated glass vial. Now allow it for 5-10 minutes for back extraction. Centrifuge or filter with syringe filter, the back extracting solution if it looks cloudy. Then transfer it to a GC/LC vial for analysis.

Cleaning FPSE media and storing for future use:

After the back extraction is complete, immerse the FPSE media in methanol: water (50:50) for 5 minutes. Dry the FPSE media and store it in air-tight glass container for further use.

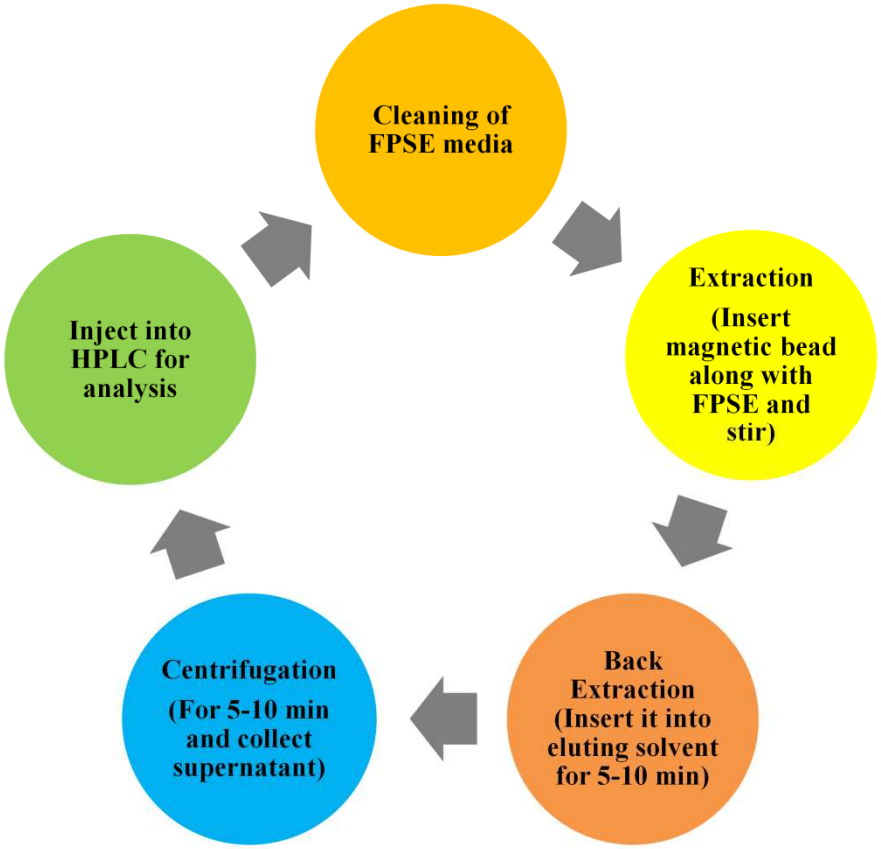

Figure 2: Schematic steps of working of FPSE

The FPSE (Fabric Phase Sorptive Extraction) media utilizes a sol-gel process to create a hybrid organic-inorganic polymeric network and to anchor the polymeric network onto the surface of flexible substrate materials. The chemically bonded sorbent system offers very high specific surface area, and excellent chemical and high thermal stability. The media has been successfully tested in solvent minimized or solvent-less microextraction conditions for the extraction of trace and ultra-trace levels of organic analytes including highly polar analytes from different sample matrices. Sample preparation with this media is equally compatible with gas chromatography, liquid chromatography and other analytical instruments. Hence, the FPSE provides an improved new generation technique for trace and ultra-trace sample preparation for instrumental analysis.

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Review of Literature

2.1 Literature

The development of bioinorganic chemistry has revealed the presence of strong organic metal complexes in biological systems so there is a need of complexing agent in order to make the metal ion UV sensitive so that we can easily analyze the proportion of metal ion present in a particular composition. Apart from their importance in the materials and catalysis areas, metal ions and their complexes play an important role in vital functions of living organisms. The stability, reactivity, synthesis, structure and formation of these compounds are studied by a variety of analytical and physical techniques. Literature reveals that traditional methods of sample preparation are typically time consuming, employ multistep procedures having high risk for loss of analytes and use of extensive amounts of organic solvents [1, 2]. Therefore there is a demand of special attention on the techniques which are characterized by a considerable reduction of organic solvents and overcome the drawbacks of previous ones. A various unconventional LLE methodologies have been reported like single drop microextraction (SDME) [3], solid phase extraction (SPE) [4], cloud point extraction (CPE) [5], solid phase microextraction (SPME) [6], liquid phase microextraction (LPME) [7, 8] and dispersive liquid-liquid microextraction (DLLME) based on solidification of a floating organic drop (DLLME-SFO) [9-11].

From the past year literature, we have concluded the use of most versatile systems i.e High Performance liquid chromatography for the separation and determination of species present in chemical mixtures. This investigation effort provides a review of the articles involving the determination, preconcentration and speciation of metal ions in a various sample matrices via reversed phase ion interaction and Chelation chromatographic separation modes. Regarding future research trends and development in this rapidly expanding field, it can be ensured that

HPLC attains the status of a comprehensive analytical technique for the determination of metal complexes. The process of metal complexation commonly known as chelation is an important which influences the mobility and solubility of heavy metal ions. This process involves the formation of two or more separate coordinate bonds between a polydentate ligand and a central metal atom. Various complexing agents results from chelation process, such as diphenylthiocarbazone (dithizone), dithiocarbamate, cupferron, and 8-quinolinol (oxine), can be used for the speciation of a large variety of metal ions using chromatography in combination with expensive techniques like integrated coupled plasma-mass spectrometry (ICP-MS), ion chromatography-ICP-MS, flame atomic absorption spectrophotometry (AAS), capillary electrophoresis and ICP-AES. Strong metal complexes with organic ligands had been recognized before 1980 [12] and to characterize this complexation the most important parameter is the stability constant, which is thermodynamically unique for a complex between a metal ion and an organic ligand. The stability constant is determined by the chemical properties of both the organic ligands and metal ions. In the field of metal complex analysis, from the past three years, reversed-phase (RP) HPLC has more widely used than normal-phase (NP) HPLC and is rapidly gaining popularity whereas other separation modes, such as ion-exchange HPLC, (HPIEC) and size-exclusion HPLC have also been applied. So there is a need of a complexing agent in order to make the metal ion UV sensitive so that we can easily analyse the proportion of metal ion present in a particular composition. Table 1 summarizes many of the HPLC applications in the analysis of metal complexes and an attempt has nailed for the speciation of different organocompounds such as organomercury, organotin, organoselenium, organochromium by using chromatographic techniques. Summarized form of review of literarture for HPLC applications in analysis of metal complexes is as:

Table 1: Recent applications of HPLC to some metal complexes

S. No.	Chelating Reagent	Metal ion	Mobile phase	Column	Particle size	Wavelength/ detector	PH/ Flow rate	LOD/ LOQ	Marix	Ref
1	8-HQ	Cu(II) and Zn(II)	Methanol: water (75:25) containing 0.03M 8-hydroxy quinoline	Zorbax Eclipse XDB C18 Column	250 mm x 4.6 mm i.d. 5 mm particle size	380 nm/ HPLC-DLLME	6.0/ 1.0 mL/min	3/10 µg/L	Water samples	13
2	Sodium DDTC	Methylmercury ($MeHg^{+}$) and mercury (Hg^{2+})	0.06 mol/L ammonium acetate	XBridge C18 Column	150 x 4.6 mm i.d., 5 µm	HPLC-DLLME	1.0 mL/min	0.0014 , 0.0076 / 0.004, 0.025 ng/mL	Water samples	14
3	8-Hydroxy quinoline- 5 sulfonic acid	Al(III)	Acetonitrile, methanol and water	Ascentis express C18 Column	(10 cm^{-1} 4.6, 2.5 mm)	λ ex 410 λ em 510 nm/HPLC-FLD	8.5/0.50 ml/min	0.05/0.15	Environmental aqueous sample	15
4	DDTC	Cr(III) and Cr(VI)	Methanol, acetonitrile and buffer of pH 3.6	RP-C18 column	(Maxsil ODS 5 m, 25 cm×4.6 mm)	254 nm/HPLC-UV	7.0/1.0 ml/min	3.4-5.2/10.2-15.6 µg/L	Aqueous samples	16
5	MDTC	Co(II),Ni(II), Pd(II)	Acetonitrile and water	RP-C18 column	250 mm×4.6 mm, 5µm	300nm/ HPLC-UV	6.0/1.0 ml/min	0.06-0.17/0.18-0.51 ng/ml	Alloys and aqueous sample	17

6	DDTC	Ni(II), Co(II), Hg(II)	Methanol and water	Agilent Eclipse XDB C18 Column	(4.6 mm x 250 mm, 5 µm)	320 nm / HPLC	1.00 ml/min	0.04-0.9/ 0.12-2.7 µg/L	Water samples	18
7	-	TMSe, SeMet, Se(IV), Se(VI), $SeCys_2$	Pyridineformeate and methanol	Agilent C8 column	(150 x 4.6 mm) 5 µm	280 nm/ ICP-MS ion pairing	4.5/0.5ml/min	-	Selenium methyl selenocysteine, fish oyster	19
8	-	Selenocysteine selenomethionine	Water:phosphate buffer (50:50)	Poly styrene DVB exchange Column	(250 x 4.1 mm) 10 µm	253.7 nm/UV	7/ 0.6 ml/min	6.4,12/ 19.2,36 µg/L	Garlic	20
9	-	Selenocysteine, selenomethionine	Ammonium acetate and acetonitrile	C18 column with didodecyldimethylammonium bromide	-	HPLC-UV-HG-AFS	Gradient 0.5-1.00 ml/min	0.31-0.43/ 0.93-1.29 ng/mL	Urine sample	21
10	TiO2 adsorbent	Cr(III) and Cr(VI)	Nitric acid and distilled water	PTFE microcolumn	(20 mm×3.0 mm id)	ICP-MS	5-8.0/0.09 ml/min	0.0075 / 0.022 ng/mL	Tea leaves and infusion	22
11	TAN	Cr(III) and Cr(VI)	A mixture of methanol–water (69:31, v/v) solution and 4.5 $mmolL^{-1}$ 18 CTMAB buffered with 0.03 mol L^{-1} NaAc–HAc	C-18 Column	(30mm x 0.25 mm 0.50 µm)	HPLC-CPE	5.5/0.8 mL/min	7.5, 3.5 and 24.7, 11.5 µg L^{-1}	Sediments	23

			solution							
12	MDTC	Cr(III) and Co(II)	Acetonitrile and water (50:50)	Zorbax Eclipse XDB C18	25 cm x 4.6 mm	HPLC-DLLME	6.0/1.0 mL /min	3 / 9.9 µg L^{-1}	Aqueous environmental samples	24
13	MDTC	Cr(III), Cr(VI)	Acetonitrile and water	Dionex Acclaim 120 C18 Column	4.6 mm (i.d.) × 250 mm	320/HPLC-UV	4.0/1.00 mL/min	0.005, 0.007/ 0.015, 0.021 ng/mL	Drinking water	25
14	-	As(III), As(V), DMA, MMA, AsB, AsC	Ammonium carbonate and methanol	Hamilton PRP-X100 anion exchange column	(4.1 mm i.d. × 250 mm) 10 µm	HPLC-ICP-MS	8.5/0.9 mL/min	0.006-0.015/ 0.018-0.045 µg/L	Seaweeds	26
15	PMDTC	Cu(II), Ni(II), Co(III), Fe(II), Mn(II)	Methanol and acetate buffer	Lichrosorb C18	(15034.6 mm)	260/UV	5/1.2 mL/min	2-6 mg/ mL	Coal samples	27
16	8-HQ	V, Ni, Fe and Cu	Methanol and water	RP-C18	(100 mm×4.6 mm I.D.)	370/UV	3.6/0.5 mL/min	2.5,4.5,2/ 7.5,13.5,6 µg/L	Crude oil	28
17	PDTC	Co(III),Co(II)	Methanol: Phosphate buffer (50:50)	Hypersil C18	(150 mm x 2.1mm) 5µm	330 nm/UV	7.00/ 0.3 ml/min	0.46 / 1.38 µg/L	Human Plasma	29
18	Metalothionine cystosolic ligands	Zn(II), Pb(II), Ni(II), Cd(II), Cu(II)	Tris buffer	Gel filtration column	(7.8 mm id 30 cm) 6 µm	ICP-TOFMS	7.4	0.6-10.8 µg/L	Liver of eels	30
19	Tiron	Al(III) and AlF	Ammonium chloride and	Ion Exchange	(50 mm id) 9	310/ UV	3/1.0 mL/min	4.5-5.78	Soil samples	31

			acidified water	column	µm			µg/L		
20	-	AsB	Ammonium carbonate and water	PRP-X 100 anion exchange		ICP-MS SPME	4/ 1.0 mL/min	-	-	32
21	EDTA	Trimethyl lead Triethyl lead	Trifluoro acetic acid and water	C18 column	(guard column) 5 µm	ES-MS	0.5 mL/min	11.3& 12.6 ng/mL	-	33
22	Sulfonylcalix[4] arene ettrasulfonate	Al(III), Fe(III), Sn(IV)	Methanol and acetate buffer	Reverse phase C18 column	(4.6 mm id. X 100 mm)	330/UV	4.7/2.5 mL/min	0.24-0.80 ng/mL	River and tap water samples	34
23	-	Triphenyltin Tetraphenyltin	Acetonitrile and water	Water spherisorb SSW ODS-2 column	10 µm	254/UV	1.0 ml/min	0.1-0.02 mg/L	Enviromental water samples	35
24	8-Hydroxy quinoline	Cadmium	Acetonitrile and tetra butyl ammonium hydroxide	C18 column	(300 X 3.9 mm)	λ_{ex} 360nm λ_{em} 500nm/FLD	4.8-5.4/ 1.5 mL/min	2 µg/L	River samples	36
25	Penta methylene dithiocarbamate	Fe(II), Cr(III), Mn(II), Cu(II), Ni(II), Co(II)	Methanol and buffer acetate	Li chrosorb ODS Column	(150 X 4.6 mm) 5 µm	260/UV	5/1.2 mL/min	14.2/22.6 µg/L	River Indus sample	37
26	EDTA	Cr(III),Cr(VI)	Orthophosphate buffer and water	Nucleosil-100 C18 ion exchange column	(250 X 4.6 mm) 5 µm	200/UV	4.00/1.00 mL/min	0.02,0.3/0.06, 0.6 µg/L	Aqueous matrix	38
27	-	As and Cr	Acetonitrile and water	Econosil C18 column	(250 X 4.6 mm) 10 µm	194 &360 nm/UV	6.00/1.0 mL/min	0.01,0.4/0.03, 1.2 µg/L	Aqueous matrix	39
28	8-HQ	Mg and Al	Acetonitrile and	ODS C18	(150 X	λ_{ex} 360	1.00	18-16	River, rain	40

			buffer with oxime	column	4.6 mm)	nm λem 516 nm	mL/min	ng/mL	and tap water	
29	DNTB	Zn(II), Cu(II), Pb(II)	Tris HCl BUFFER	Size exclusion column, Superdex75	10/300 GL, Tricorn ,	417 nm / ICP-MS	7.00/0.7 mL/min	-	Fish Bile	41
30	-	As(III), As(V), DMA, AsB	Aqueous Ammonium carbonate	Anion exchange column, isocratic	-	ICP/MS	-	18.7/5 6.1 µg/L	Carrots	42
31	-	As(III), MMA, DMA, AsB	Aqueous ammonium carbonate, gradient	Micro Mist glass concentric nebulize	-	ICP-MS	/1.15 mL/min	0.3-1/0.9-3 µg/L	Urine	43
32	Curcumin	Boron	TBABr : Methanol	Chrome C18 column	(15 cm X 4.6 mm) 5µm	ICP-MS /AES	0.8 mL/min	0.02-0.5/0.06-1.5 µg/L	Uranium oxide	44
33	Phyto chelatins	Cd(II), Pb(II)	Acetonitrile and formic acid	Alltech Ultima, C18 column	150 ×2.1 mm) 5 µm	HPLC/ ESI-MS	4.5/0.2 mL/min	-	Pea	45
34	Carbon nanotubes	Cr(III) and Cr(VI)	Nitric acid and distilled water	PTFE microcolumn	(20 mm×3.0 mm id)	ICP-MS	3.0/1.2 mL/min	0.024 /0.072 ng/mL	Mineral water, lake water	46
35	-	Sb(III), Sb(V), trimethyl antimony(V)	EDTA and KHP salts in diammonium hydrogen phosphate buffer solution	Anion exchange column	-	217 nm HPLC-(UV)-HG-AFS.	1.00 mL/min	7/21 ng/L	Marine biota sample	47

36	3-(2-amino ethylamino) Propyl trimethoxy silane	Cr(VI), Se(VI), As(III), As(V), Cr(III)	Nitric acid and acetonitrile	PTFE micro-column	(20 mm 2.0 mm i.d.)	ICP-MS	3/ 0.8 mL/min	15,16/ 45,48 ng/L	Enviro mental samples	48
37	-	As(III), As(V), DMA, MMA	Hexanesulfonic acid and citric acid	C18 Alltima,	4.6 mm × 150 mm, 5 µm	HPLC-IP	4.5/0.9 mL/min	40,12/ 120, 36 µg/L	Nut oils	49
38	-	Methylmercury, ethylmercury, mercury	2-mercaptoethanol and methanol	Perkin-Elmer C8	(3 mm i.d. × 30 mm).	CV-ICP-MS	4.7/1.2 mL/min	0.003-0.006/ 0.009-0.018 ng/mL	Cereals	50
39	-	Cr(III) and Cr(VI)	$EDTA_2$(Na) 5 mM pH 7	Reverse phase C8 column Agilent part number G 3268A	150 4.6 mm 3.5 µm	HPLC-ICP-MS	7.0/1.2 ml/min	0.1/ 5.1 µg/L	Animal feed	51
40	-	Bromide, iodate, iodide	Ammonium nitrate and methanol	Ion pack anion-exchange column	(250 mm 4 mm i.d.)	HPLC-AEC	1.5ml/min	0.01-0.12/0.03-0.36 ng/ml	Atmospheric particulate matter	52
41	-	Selenium and arsenic	5 mmol l^{-1} TBAH in 2.5 $mmol^{-1}$ $(NH_4)_3PO_4$ (pH 6.0)	ZORBAX Eclipse XDB-C18 Column	(250 mm x 4.6 cm) 5 µm	ICP-ESI-MS	6.0/1.00 ml/min	19 to 22 ng L^{-1} for As and 312 to 442 ng L^{-1} for Se	River, Plant extract and urine matrices	53

42	Quercetin	Aluminium(III)	Perchloric acid and methanol	Nova-Pak C 18	(150 mm × 3.9 mm i.d.)	415/UV	3.5/1.0ml/min	.014/.042 µg/L	Aqueous	54
43	CHAPS	Al(III)	Tris HNO3 and CHAPS	CHAPS coated C18 Column	(250 mm×4.6 mm i.d.)	220/UV	0.7 ml/min	0.74,0.83/ 2.22, 2.49 ng/mL	Human serum	55
44	Quercetin	Al(III)	Trifluoroacetic acid and water	column Shield RP18	(4.6 ×250 mm) 5 µm	350/ HPLC-PDA	7/0.7 ml/min	-	water	56
45	-	As(III), As(V), Se(IV), Se(VI)	Phosphate buffer and Na_2HO_4	Anion exchange column	(250 mm×4.6 mm) 5 µm	196 nm/ HPLC-HG-AAS	5.5/ 3.0 ml/min	7.8-18/23.4-54 ng/ml	Ground water	57
46	PAR	Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II)	Tris buffer	Ion Pac CG5A guard column.	(250 X 4 mm I.D.)	530/PDA	0.5 ml/min	-	Proteins	58
47	PDTC	Ba(II), Mn(II), Cr(III), Ni(II), Cu(II), Zn(II)	Acetonitrile and water	(Techno pak phenyl 300x3.9 mm i.d.)	(150 x 4mm I.D)	254/UV	0.7mL/ min	0.01-0.08/ 0.03-0.24 mg/L	Coal fly ash	59
48	2-acetylpyridine-4-phenyl-3-thiosemicarbazone	Pt(II), cis platin	Acetonitrile, methanol and tetrabutylammonium bromide	Phenomenex C-18	(150 mm×4.6 mm i.d.)	380/UV	1.0 mL/min	50-90 ng/mL	Serum and urine samples	60
49	Emetine-	Mercury,	Acetonitrile and	L-column	(250	HPLC-	6/0.3	30 ng	Water,	61

	dithiocarbamate	methy, ethyl, phenyl mercury	citrate buffer	ODS2 column	mm × 4.6 mm) 5 µm	CL	mL/min	/L, 21 ng /L	biological	
50	Sodiumdibenzyl dithiocarbamate.	Cd(II), Cu(II), Zn(II),Pb(II), Fe(II)	Methanol and acetonitrile	Alltech C18 column.	(150 mm×4.6 mm i.d.)	254/HPLC-AAS	5.2	0.1-1.8 / 0.3-5.4 ng/mL	Water samples	62
51	-	TMSe, SeMet, Se(IV), Se(VI) SeCys2	Triethylamine and acetic acid	Hamilton PRP-X100	(250 ×4.1 mm, 10 µm)	ICP-MS	5.0	-	Yeast	63
52	-	Soluble proteins	Tris HCl and KCl	BioSep SEC 2000	(300–1 kDa)	HPLC-UV/ICP-MS	6.8		Chicken liver	64
53	2-thiophenaldehyde-3-thiosemicarbazone	Co(II), Pd(II), Ni(II), Cu(II)	Acetonitrile and water	Dionex Acclaim 120 C18 Column	(250mm× 4.6mm) 5 µm	390/HPLC-UV	8.0/0.80 ml/min	0.001-0.009/ 0.003-0.027	Alloys and drinking water	65
54	APDC	Cr(VI), Co(II), Cu(II) and Ni(II)	Acetonitrile and water (70:30)	UPLC BEH C8	100 mm x 2.1 mm i.d. 1.7 µm, waters	256 nm/ HPLC-DLLME	3.0/0.5 mL/min	0.5-1.5/1.6-4.9 µg/L	Tap water	66
55	Salophen	Ni(II), Co(II), Cu(II) and Zn (II)	Methanol: acetonitrile: water	Perfect sil target C18 Column	250 mm x 4.6 mm i.d.	436 nm/ HPLC-DLLME	6.5/ 1.0 mL/min	0.8-2.8/ 2.6-9.2 µg/L	Tap water and mineral water	67
56	Sodium DDTC	Methylmercury ($MeHg^{+}$) and mercury (Hg^{2+})	90% (v/v) methanol-10% (v/v) water	Supelco (USA) Discovery	(4.6 mm i.d. x 15	HPLC-CPE	0.8 mL/min	4 and 10 / 13.2	Environmental and biological	68

			containing DDTC 1.0 x 10^{-4} mol/L	C18 column	mm, 5 µm			and 33 ng/L	samples	
57	DDTC	Hg	Triton –X-114 and methanol	Waters C18 ODS Column	(150 cm x 4.6 mm, i.d., 5 µm	HPLC-CPE ICPMS	5.5/0.8 mL/min	8.0-13 / 24.7, 39.5 ng/L	Environmental aqua & biological samples human hair & ocean fish	69
58	DDTC	Cr(III) and Cr(VI)	Methanol: water: acetonitrile (65:21:14)	Waters ODS Column	(150 cm x 4.6 mm, i.d., 5 µm	254 nm/ HPLC-CPE	3.6/1.0 mL/min	3.4-5.2 / 11.2-17.1 µg/L	Snow, river, sea and waste water samples	70
59	-	Tetraphenyltin, triphenultin hydroxide, trihenyltin acetate	Acetonitrile and water	Waters spherisorb ODS guard column	(150 cm x 4.6 mm, i.d., 5 µm	HPLC-UV/254 nm	1.0 mL/min	0.01 µg/mL	Aqueous environmental samples	71
60	PHDT	Arsenobetaine	30Mm $(NH_4)_2CO_3$	Hamilton PRP-X 100 anion exchange column	(250 mm x 4.1mm, i.d.)	HPLC-SPME	0.5 mL/min	14 and 70–1200 ng/ mL,	Water samples	72
61	-	Inorganic mercury and organic mercury	Methanol: acetonitrile : 0.1 M sodium acetate acetic acid (40:38:22)	Spherisorb ODS column Pekin Elmer	22 cm length x 4.6 mm, 5 µm	HPLC-SPE	7.5/ 1.5 mL/min	1.9-2.2 6.2-7.2 ng/L	Water samples	73

					particle size					
62	Mercapto ethanol	Inorganic mercury, methyl mercury and ethyl mercury	(0.4% (v/v) 2-mercapto ethanol, 10% (v/v) methanol)	Kinetex C18 Column	(100 mm length × 2.10 mm i.d., 5.0 µm particle diameter)	HPLC-SPE	2.5/0.7 mL/min	3.3, 2.0, and 3.6/ 11, 6.7, and 12 ng/L	Sea water	74
63	DDTC	Nickel, cobalt and mercury	Methanol and water (80:20)	C18 Column	(4.6 mm x 250 mm, i.d. 5 µm)	320 nm/ HPLC-SPE	1.0 mL/min	0.1-2.7/ 0.33-8.9 µg/L	Environmental samples	75
64	-	Methylmercury, ethyl mercury and phenyl mercury	Methanol and 30% water containing 0.001%2-mercaptoethanol, 20 mmolL^{-1} buffer solution	RP C18 Column (Lichrospher-ODS)	200 mm x 4.6 mm	254 nm/ HPLC-HF-LLME	6.0/ 1.0 mL/min	0.3–3.8 / 0.9-12.5 ng mL^{-1}	Seafood and environmental samples	76
65	o-phenylene diammine	Se(VI)	50 mM ammonium acetate and methanol with gradient program	ODS-3 Column	100 mm x 4.0 mm, with 3 µm	332 nm / HPLC-LPME	4.6	0.1 and 0.02 / 0.3 and 0.06 µg L^{-1}	Urine, natural water and plasma samples	77
66	-	$MeHg^{+}$, $EtHg^{+}$	THF/MeOH/0.1	Phenomnex	150 x	HPLC-	4.0/ 0.8	1.0	Tap, river	78

		and $PhHg^{+}$	M sodium acetate with 50 µm EDTA (36/32/32)	C-18	4.60 mm i.d., 3 µm particle size column	IL-SDME	mL/min	and 22.8 / 3.3 and 75.24 µg L^{-1}	and waste water	
67	Dithizone (DZ)	Mercury species Hg^{2+} methyl mercury (MeHg+) and phenylmercury (PhHg+)	Methanol/ tetrahydrofuran/ (0.1 M acetic/sodium acetate pH 4.0; 100 µM EDTA) (32/35/33,v/v)	Octa dodecyl silica Column	(150 ×4.6m m I.D.)	475 nm/ HPLC-DLLME	4.0/ 1.0 mL/min	0.32, 0.96 and 1.91 µg L^{-1}	Environmental aqueous samples like tap water, lake water and river water	79
68	-	Inorganic mercury Hg^{2+} methyl mercury ($MeHg^{+}$) and phenylmercury ($PhHg^{+}$)	methanol,35% tetrahydrofuran, 35% (0.1 M sodium acetate–acetic acid, pH 4 EDTA), 30%.	Nova-Pak Reverse phase C18 Column	300×3.9 mm i.d. column.	475 nm /HPLC-DLLME	4.00 mL/min	0.58, 0.66 and 0.54/ 1.9,2.2, 1.8 ng/L	Sea Water	80
69	-	Selenium	Phosphate buffer	Multi affinity removal column mark	4.6 x 50 mm	Extraction process/ Multi affinity chromatography	6.0/1.0 mL/min	-	Human serum	81
70	APDC	Cr(III) and Cr(VI)	Acetonitrile: water (2:1)	Non end capped octa-decyl silica	5 µm (15 × 4.6 cm)	540 nm Liquid liquid	4.6/0.6 mL/min	2.2 µg/L for	Industrial waste water	82

				gel		extraction/ HPLC-UV		Cr(VI) and 4.5 µg/L for Cr(III).		

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Speciation analysis of chromium ions using Fabric Phase Sorptive Extraction for their quantification via HPLC with UV detection

3.1 Introduction

Currently heavy metal pollution is one of the serious concerns all over the world due to industrial activities, improper disposal of wastes, pollution of agricultural soils and accumulation along the food chains. These are among the most hazardous anthropogenic pollutants due to their toxicity and bioaccumulation in the environment. With the increasing population and advancement in technology metal pollution is a quickly growing problem for oceans, lakes and rivers which is great threat to human health, plants and animals. Industries, mining sites, untreated sewage sludge, combustion of products from coal burning power stations, fertilizers, and traffic are the major hot spots introducing metals to the environment. Unlike the other pollutants, these cannot be eliminated easily from environment thus resulting in serious damage to human body. Therefore, interest and demand is continuously rising for simple, sensitive, economic and rapid metal determination method in biological and environmental samples.

Metal speciation is achieved by the combination of two different techniques one is providing an efficient and reliable separation procedure and the other is detection and quantification. There are two main reasons for studying the speciation of elements one is the biological which includes the bioaccumulation, bioconcentration, bioavailability, toxicity and the other is geochemical which includes transport, adsorption and the precipitation of element [1]. Trace metal speciation analysis is an important environmental analytical tool for forecasting metal doom in aqueous samples and emerging successful methods for monitoring of water quality. It is essential to understand the toxicity and bioaccumulation. Metal ions exist in variable oxidation states and have different impacts on health and environment.

Determination of trace metals are often made possible by the addition of chelating agent. Dithiocarbamtes has been widely used as chelating agent for both analysis and separation of metal ions. Diethyldithiocarbamate [2] and ammounium pyrrolidine dithiocarbamate [3] are the reported complexing reagents for HPLC-UV determination. In the developed method morpholino dithiocarbamate (MDTC) is used as a preferred chelating agent due to its hydrophilic nature.

Heavy metal analysis in environment cannot be conducted directly for their determination because the concentrations of heavy metal ions in environment samples are always at very low levels [4]. So separation and preconcentration procedures are always used before the determination of heavy metal ions such as liquid-liquid extraction [5], cloud point extraction [6], solid phase microextraction [7], solid phase extraction [8] and fabric phase sorptive extraction [9]. Among these methods, fabric phase sorptive extraction (FPSE), developed by Kabir and Furton [10], utilizes sol-gel coating technology developed by Malik et al [11] to create an inherently porous hybrid inorganic-organic sorbent material chemically bonded to the permeable and flexible substrate matrix, is one of the important preconcentration methods in environmental analysis. FPSE exploits the surface chemistry of the substrates (*i.e.,* hydrophobicity, hydrophilicity, hydrophobicity-hydrophilicity mixed) along with adsorption properties of sol-gel precursor and organic ligands to determine the ultimate selectivity and specificity of FPSE media. This opens up the possibility of custom design of the selectivity parameter by a judicious selection of the three selectivity contributors to meet the specific analytical need. For the analysis of chromium ions, various techniques are used frequently but high performance liquid chromatography (HPLC) meets most of the analytical requirements of metal determination. HPLC provides several advantages over other methods for separation and quantitation of Cr ions

down to the trace level concentration [12]. A number of HPLC methods have been evaluated [13-14] and a selective determination of both species can be achieved successfully by using HPLC coupled to inductively coupled plasma mass spectrometry [15] ion chromatography-ICP-MS [16], flame atomic absorption spectrophotometry [17] and capillary electrophoresis [18]. The aim of this study is to evaluate the speciation of Cr(III) and Cr (IV) ions by chelating it with MDTC followed by preconcentration with FPSE media and HPLC-UV determination.

3.2 Experimental

3.2.1 Apparatus

The Dionex HPLC unit consists of a P680 solvent delivery pump, a UVD 170 detector capable of detecting four wavelengths was interfaced to a computer loaded with Chromeleon software (version 6.70). A Supelco Ascentis Express reversed phase column of size 10 cm × 4.6 mm filled with C18 material (2.7 µm) was used for separation. A digital pH meter-101 (Delux, India) was used for all the pH measurements. Elico SL-164 double beam UV-visible spectrophotometer loaded with Spectra Treatz software was used to record the absorption spectra. The IR spectra were recorded on FTIR (PerkinElmer). Gaussian 03 was used for the optimization of the structure of metal complexes. A Barnstead Nano Pure Diamond (Model D11911) deionized water system was used to obtain ultra-pure deionized water (18.2 MΩ cm) for sol-gel synthesis. A digital vortex mixer was employed for thoroughly mixing of sol solutions. Centrifugation of sol solution to obtain particle free solution was carried out in an eppendorf centrifuge model 5415 R.

3.2.2 Materials and Reagents

Standards of Cr(III) nitrate nonahydrate, potassium dichromate, sodium acetate trihydrate, glacial acetic acid, and sodium hydroxide were obtained from Merck.

3.3 Synthesis of Morpholine dithiocarbamate (MDTC)

MDTC was used as a chelating agent and prepared with the method reported by Macrotrigiano *et al* [19].

In round bottom flask Morpholine (0.1 mole, 8.7 mL) was taken along with 150 mL of dry diethyl ether. The stoichiometric amount of 6 mL of CS_2 was added dropwise with constant stirring having magnetic bead and then reaction mixture was cooled. To this dithiocarbamic acid solution a stoichiometric quantity of sodium hydroxide (0.1 mole, 4 g) was added with constant stirring for 3-4 h. The crude product was filtered over suction and washed 3 times with ether. The residue was then recrystallized from isopropyl alcohol. White needle shaped crystals were obtained with melting point 170-174°C and was soluble in water. IR spectra showed the presence of characteristic C=S stretching bands at 1083 cm^{-1} and two bands at 1437 and 1164 cm^{-1} due to C-N and C-O stretching respectively confirmed the formation of MDTC ligand. It was standardized titrimetrically by preparing a solution of morpholino dithiocarbamate using mercuric acetate as titrant and diphenycarbazone as an internal indicator [20].

3.4 Preparation of metal complex

A solution containing Cr(III) and Cr(VI) ions (2 mM and 7 mM respectively) was transferred to a well stoppered bottle. To this, 1 mL (0.4 moles) of MDTC solution (10% w/v) was added. The total volume of solution was made up to 25 mL with water and acetate buffer solution (0.1 M) such that the pH of the resulting solution was 4.0. The prepared solution was heated on water bath at 55°C temperature. The complex obtained was then dissolved in 10 mL acetonitrile solvent to obtain the stock solution. The stock solution was then diluted further as per requirement. The solution was injected into the rheodyne loop and separated by HPLC on a reverse phase C18 column with acetonitrile: water (65:35; v/v) as the mobile phase at a

wavelength of 320 nm. The chromatograms for Cr(III), Cr(VI) and their mixture are shown in **Figure 3.1 a-c.**

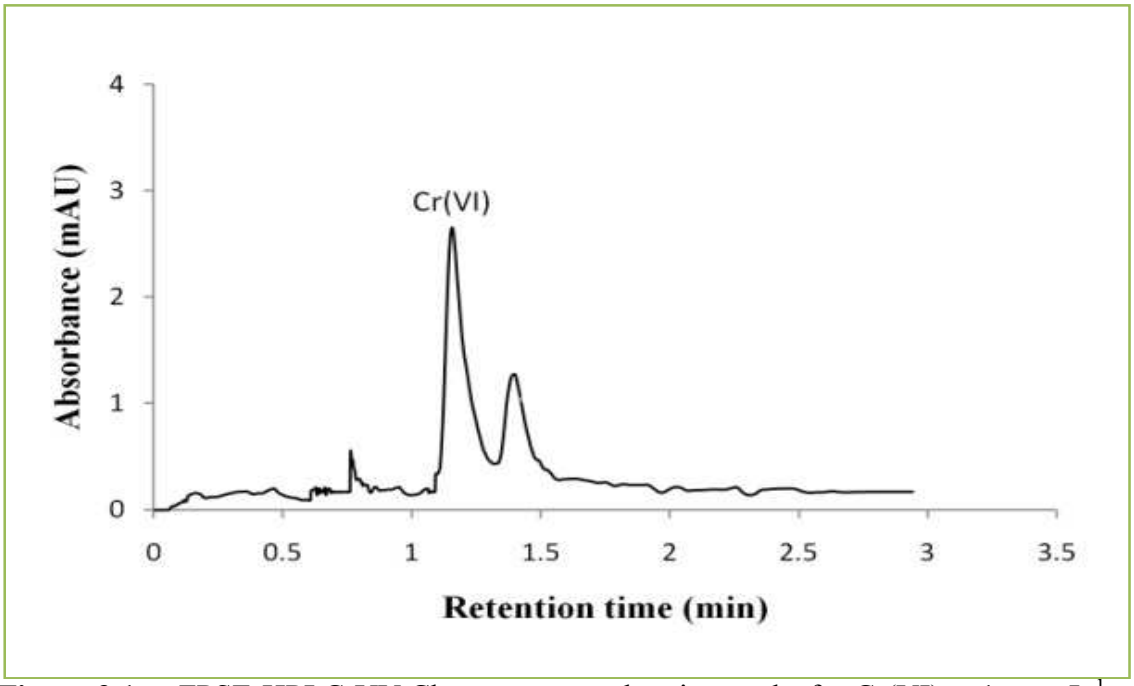

Figure 3.1 a: FPSE-HPLC-UV Chromatogram showing peaks for Cr(VI) at 1 ng.mL^{-1} under optimized chromatographic conditions as mobile phase acetonitrile : water 65:35, flow rate: 1.0 mL.min^{-1}, λ_{max} = 320 nm

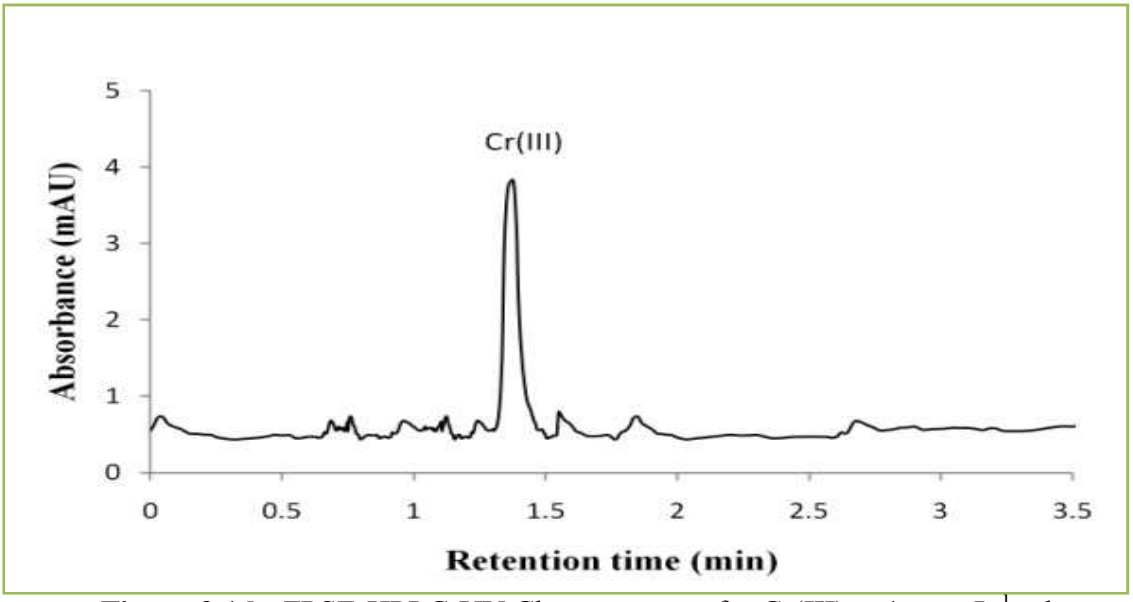

Figure 3.1 b: FPSE-HPLC-UV Chromatogram for Cr(III) at 1 ng.mL^{-1} other same as Figure 3.1. a

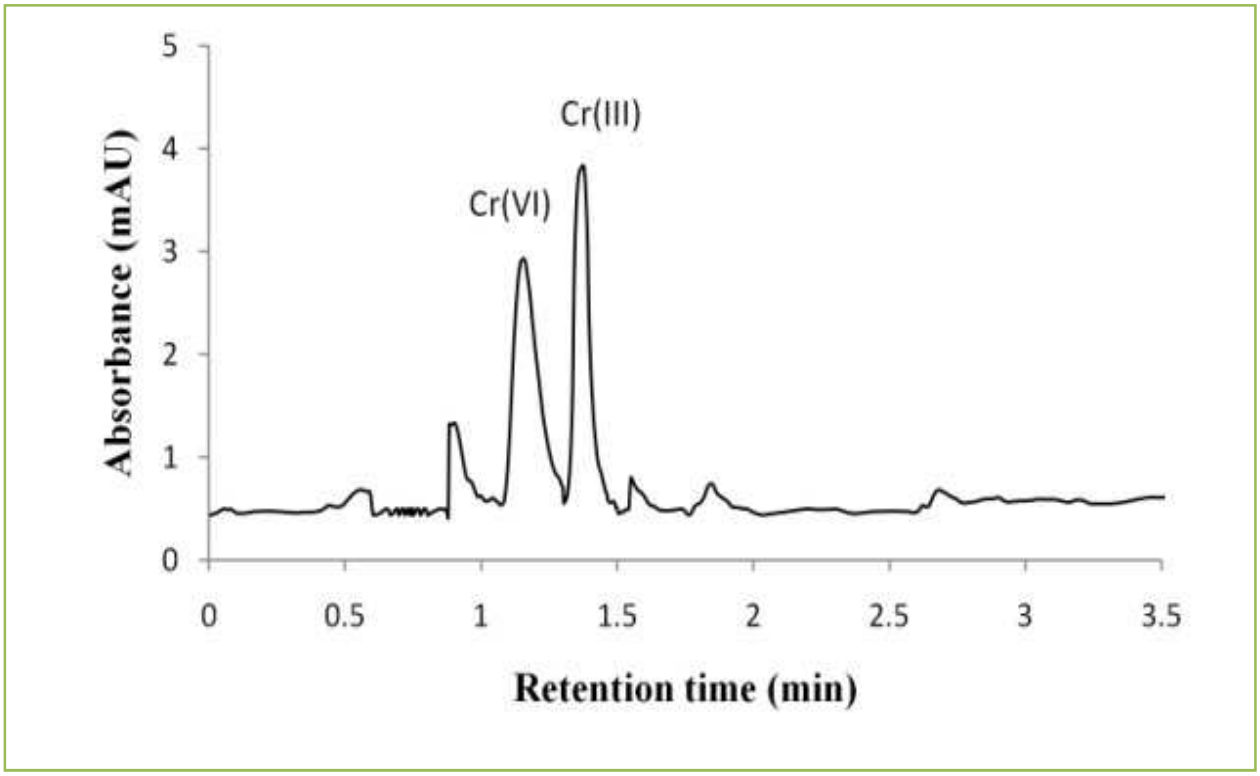

Figure 3.1 c: FPSE-HPLC-UV Chromatogram showing peaks for Cr(III) and Cr(VI) other conditions same as Figure 3.1 a

3.5 Results and Discussion

3.5.1 Spectrometric analysis of Chromium Complexes

The different kinds of interaction of both chromium ions towards dithiocarbamates make their determination easier. The IR spectra of 1:3 adduct of tris(morpholinedithiocarbamto) Cr(III) was recorded using KBr pellets. The shift of C-S band from 1083 cm^{-1} to 1050 cm^{-1} after complexation indicates that the ligand binds with metal through sulfur atoms. A band at 870 cm^{-1} may be attributed to S-O stretching vibration which confirmed the formation of oxy complex. The oxy complex formation of $Cr(MDTC)_2(OMDTC)$ was due to reduction of Cr (VI) with the reagent where Cr-O-S bond is formed in the complex [21]**.**

3.5.2 Optimization of FPSE Conditions

Efficiency of fabric phase sorptive extraction (FPSE) affected by several parameters which include type of fabric media, extraction time, eluting solvent, back extraction time and carryover effect. All experiments were performed in triplicates. During method optimization, peak areas were used to assess the effect of various parameters on extraction method. Optimized conditions were used for validation and application to the environmental samples. The following parameters were optimized.

Optimization of sample extraction time

Equilibrium extraction time is one of the most important FPSE parameters. Once the extraction equilibrium is reached, FPSE media cannot extract the target analyte any further under the given conditions. The porous sol-gel sorbent network, high primary contact surface area and the permeable cellulose substrate synergistically reduces the extraction equilibrium time. The sample containing analytes (1 $ng.mL^{-1}$) was examined to study the effect of extraction time. Extraction times from 5 to 25 min were taken for observation. As the extraction time increases, extraction of target analytes onto the FPSE fiber increases and becomes almost constant at 15 min. So the extraction time of 15 min was optimized for further experimentation shown in **Figure 3.2.**

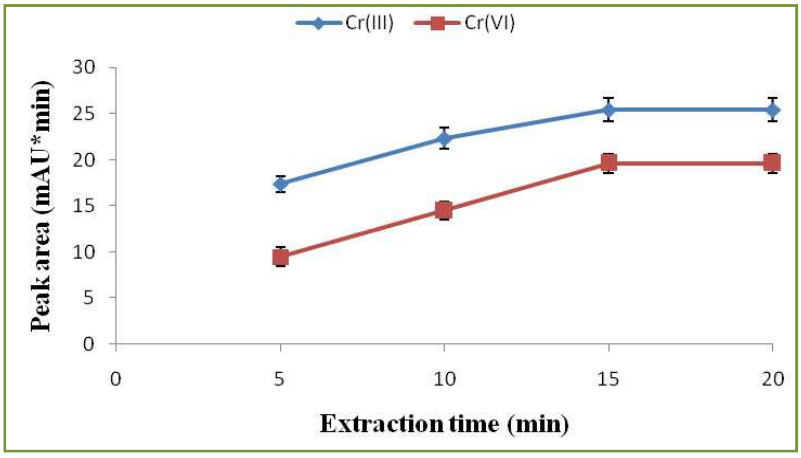

Figure 3.2: Optimization of extraction time

Effect of eluting/ back-extraction solvent

Once the target analytes are extracted into the FPSE media, a quantitative desorption into a suitable organic solvent is needed. The solvent breaks the analyte-sorbent interaction and dissolves the analyte into it. Different solvent mixtures were tried for the back-extraction of target analytes. Acetonitrile was the best elution/back-extraction solvent shown in **Figure 3.3.**

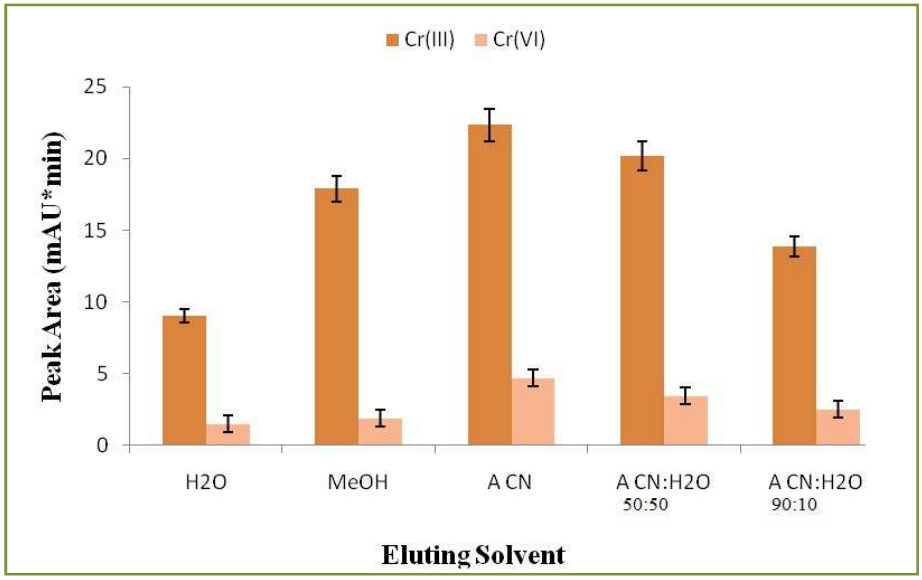

Figure 3.3: Optimization of eluting solvent

Effect of elution/back-extraction time

Elution/back-extraction time was optimized by performing the experimentation with back-extraction time ranging from 2 to 10 min. An elution/back-extraction time as 6 min was selected while keeping the other optimized parameters to their fixed values (**Figure 3.4**).

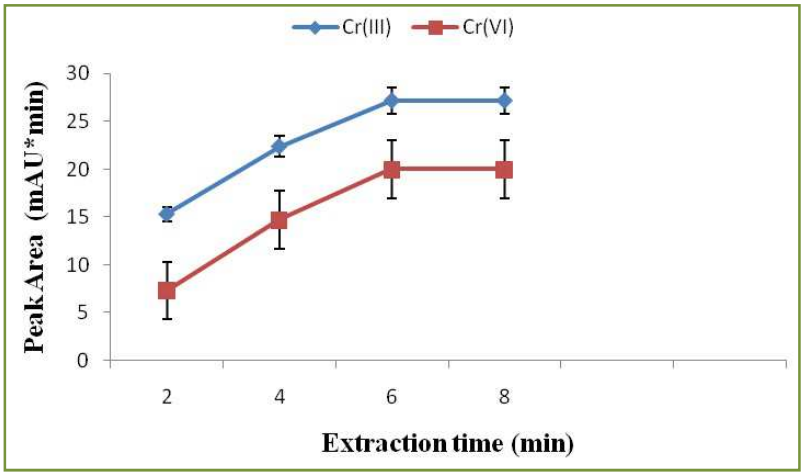

Figure 3.4: Optimization of Back extraction time

In addition to all these factors carry over effect was also studied for FPSE sorbent. A thorough clean up step was required in order to remove the matrix effect. Fiber was washed thoroughly with acetonitrile before the subsequent experimentation. The carry over effect was checked by injecting the eluent 3-4 times before analyzing the next sample. The matrix effect can also be minimized by using the new fiber for each extraction process, as it is inexpensive.

3.5.3 Method Validation

The optimized FPSE-HPLC-UV conditions were used to prepare the calibration curves in the range 1-100 $ng.mL^{-1}$ for all kinds of samples given in **Table 3.1.** Over the range, a linear response with the regression coefficient 0.997 and 0.995 was obtained for Cr(III) and Cr(VI) complexes, respectively. In the unknown samples, the concentrations of Cr(VI) can be determined by measuring the peak areas obtained due to $Cr(MDTC)_2(OMDTC)$ using the calibration curve. Proposed method is suitable for speciation of both Cr(III) and Cr(VI) in a sample using quantitative calculations based on the ratio of respective peak areas of absolute Cr(III)-MDTC complex and absolute Cr(VI)-MDTC complex. The concentration of Cr(III) can be calculated by subtracting the value corresponding to Cr(MDTC)3 obtained from Cr(VI).

Table 3.1: Analytical characteristics of developed FPSE-HPC-UV method

Parameters	Sample preparation using FPSE	
	$Cr(MDTC)_3$ in Cr(III) in Cr(VI)	$Cr(MDTC)_2OMDTC$
Wavelength (nm)	320	320
Correlation coefficient (R^2)	0.997	0.995
Retention time (min)	1.4	1.2
Regression equation	0.50x+4.8	0.275x+1.85
LOD ($ng.mL^{-1}$)	0.001	0.003
LOQ ($ng.mL^{-1}$)	0.003	0.009
RSD (%)	2.1-2.4	1.9-2.1

3.6 Analytical Applications

The effectiveness of the developed method was verified by the determination of Cr (III) and Cr (VI) complex in various kinds of aqueous samples. The environmental aqueous samples were analysed under optimized extraction conditions to demonstrate the performance and practical applicability of the developed method. A good agreement was obtained between the added and recovered analytes concentration by the developed FPSE-HPLC-UV method. The results obtained were satisfactory with recovery more than 95% for a broad concentration range. This proves that the developed procedure is suitable for genuine environmental and consuming water applications. During their determination no major interfering peaks were present at the retention time of these analytes. The well defined peaks of the analytes demonstrated that FPSE-HPLC-UV was an adequate extraction and clean up procedure for the analysis of chromium ions in aqueous environmental samples. The results of the determination are shown in (**Table 3.2**).

Table 3.2: Recoveries of Cr(III) and Cr(VI) using FPSE-HPLC-UV

Analyte	Spiked Conc. (ppb)	Industrial Water		Bore well Water		Drinking Water	
		Obtained Conc. (RSD)	Recovery (%)	Obtained Conc. (RSD)	Recovery (%)	Obtained Conc. (RSD)	Recovery (%)
Cr(III) complex	1	0.89 (2.7)	89.60	0.98 (2.1)	98.70	0.97 (2.3)	97.60
	5	4.50 (2.5)	90.00	4.88 (2.0)	97.60	4.78 (2.3)	95.60
	10	9.16 (2.0)	91.60	9.75 (1.9)	97.59	9.65 (2.2)	96.70
Cr(VI) complex	1	0.87 (2.9)	87.00	0.97 (2.3)	97.50	0.98 (2.1)	98.50
	5	4.56 (2.6)	91.20	4.82 (2.2)	96.50	4.90 (2.0)	98.00
	10	9.13 (2.5)	91.30	9.86 (2.1)	98.60	9.68 (1.9)	96.80

3.7 Conclusion

Compared to other sorbent-based sorptive microextraction techniques, FPSE possessed many advantages and has been proved to be a simple, economic, high extraction efficiency, faster extraction equilibrium and efficient sample preparation technique for the detection of chromium species using HPLC-UV. Trace level determination of Cr species is accomplished by using FPSE. Real samples were analyzed after spiking with chromium complexes in order to confirm the validity of the developed method. This method shows potential as a rapid screening method to determine chromium species quantitatively in three kinds of water samples.

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Determination of Cobalt (II), Nickel (II) and Palladium (II) Ions via Fabric Phase Sorptive Extraction in Combination with High Performance Liquid Chromatography-UV Detection

4.1 Introduction

Cobalt nickel, and palladium are three typical metal ions in environmental samples causing various health problems to humans, animals and plants [1, 2]. Moreover, high exposure of these metal ions leads to lung, nasal and throat cancers [3]. Although nickel is an essential trace element but its toxic effects are well known and it is one of the most common causes of allergic contact dermatitis and respiratory diseases [4-6]. Cobalt can produce toxicological effects like vasodilation and cardiomyopathy [7]. Palladium is also the most cytotoxic element causes considerable damage to cells and degradation of DNA [8]. Therefore, interest and demand is continuously rising for simple, sensitive, economic and rapid metal determination method in biological and environmental samples.

For the analysis of cobalt, nickel and palladium ions, various techniques are used frequently but high performance liquid chromatography (HPLC) meets most of the analytical requirements of metal determination. HPLC provides several advantages over other methods for separation and quantitation of these metal ions down to the trace level concentration [9]. A number of HPLC methods have been evaluated [10,11] and a selective determination of both species can be achieved successfully by using HPLC-integrated coupled plasma mass spectrometry (HPLC-ICP-MS) [12], inductively coupled plasma-optical emission spectrometry (ICP-OES) [13], micellar electrokinetic chromatography [14] and flame atomic absorption spectrophotometry (FAAS) [15]. In the present work FPSE-HPLC-UV method is reported for the separation of cobalt, nickel and palladium complexes and their determination from aqueous

environmental samples. FPSE is relatively a new technique which permits us to detect these complexes at very low concentrations.

4.2 Experimental

4.2.1 Instrumentation

The Dionex HPLC unit consists of a P680 solvent delivery pump, a UVD 170 detector capable of detecting four wavelengths was interfaced to a computer loaded with chromeleon software (version 6.70). An Agilent Eclipse XDB reversed phase column of size 4.6 × 150 mm filled with C_{18} material (5 μm) was used for separation. The IR spectra were recorded on FTIR (PerkinElmer). A digital pH meter-101 (Delux, India) was used to adjust the pH of solutions. Gaussian 03 software was used for the optimization of the structure of metal complexes. Elico SL-164 double beam UV-visible spectrophotometer loaded with Spectra Treatz software was used to record the spectra with quartz cuvettes. A digital vortex mixer (Fisher Scientific, USA) was employed for thoroughly mixing of sol solutions. An ultrasonic cleaner-2510 (Branson Inc., USA) was used to make sol solution free of trapped gas or bubbles. Centrifugation of sol solution, to obtain particle free solution, was carried out in an Eppendorf centrifuge model 5415 R. A Barnstead Nano Pure Diamond (Model D11911) deionized water system was used to obtain ultra-pure deionized water (18.2 MΩ cm) for sol-gel synthesis.

4.2.2 Materials, chemicals and reagents

All the solvents used were of HPLC-grade and purchased from J.T. Baker Chemicals (Phillipsburg, NJ), and filtered by using Nylon 6, 6-membrane filters (Rankem, India) in a filtration assembly (from Perfit, India). Co(II) (Cobaltous chloride), Ni(II) (nickel nitrate), Pd(II) (palladium chloride), acetic acid, sodium acetate and sodium hydroxide were obtained from Merk (Mumbai, India).

4.3 Procedure

It involves the selective extraction of Co(II), Ni(II) and Pd(II) ions as MDTC complexes from aqueous solution on Polytetrahydrofuran FPSE coated media. The complex formed was then, desorbed from the fiber prior to the analysis. The complex synthesized by MDTC with these metal ions was well separate from the free ligand. The extracted amount of metal ions was quantitatively determined by UV detector at 315 nm.

4.3.1 Preparation of metal complexes

For the determination of these metal complexes, 1 mL of (0.02 M) sodium acetate-acetic acid buffer solution (pH 6.5) and 1 mL 0.02 % (w/v) MDTC solution were added into a 5 mL sample vial, then different concentrations of (1-50 $\mu g.L^{-1}$) Co(II), Ni(II) and Pd(II) was added. The total volume was made to 5 mL with triply distilled de-ionized water [11]. Subsequently, the complexes were formed with constant stirring on a magnetic stirrer at room temperature. The contents obtained were sonicated, centrifuged and injected into rheodyne loop for chromatographic analysis **(Figure 4.1).**

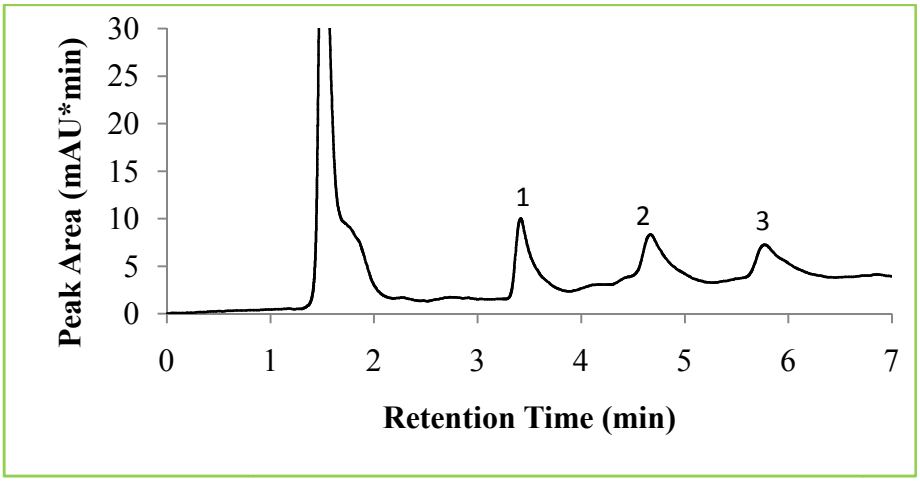

Figure 4.1: FPSE-HPLC-UV Chromatogram showing peaks for (1) Co(II) at 5 ng/mL; (2) Ni (II) at 5 ng/mL; (3) Pd (II) at 1ng/mL [MDTC = 1 mL of 0.02%, sodium acetate buffer

pH 6.5 extracted with PTHF-FPSE media under optimized chromatographic conditions (mobile phase: acetonitrile:water-60:40, flow rate: 0.7 mL/min, λ_{max} = 315 nm)

Computational Details

Quantum chemical calculations of Co(II), Ni(II) and Pd(II) complexes have been used to optimize their geometrical structures by Gaussian-03 program using Becke-3-Lee-Yang-Parr (B3LYP) exchange correlation functional with standard 3-21G basis set. Calculations were carried out for the neutral complexes [$Co(MDTC)_2$], [$Ni(MDTC)_2$] and [$Pd(MDTC)_2$] considering it as a non interacting independent units. All the structures have been optimized from initial geometries in order to guarantee the global minima energy structures shown in **Table 4.1.** The energy minimization studies of chromium complexes interpreted that both complexes are energetically stable **(Figure 4.2).**

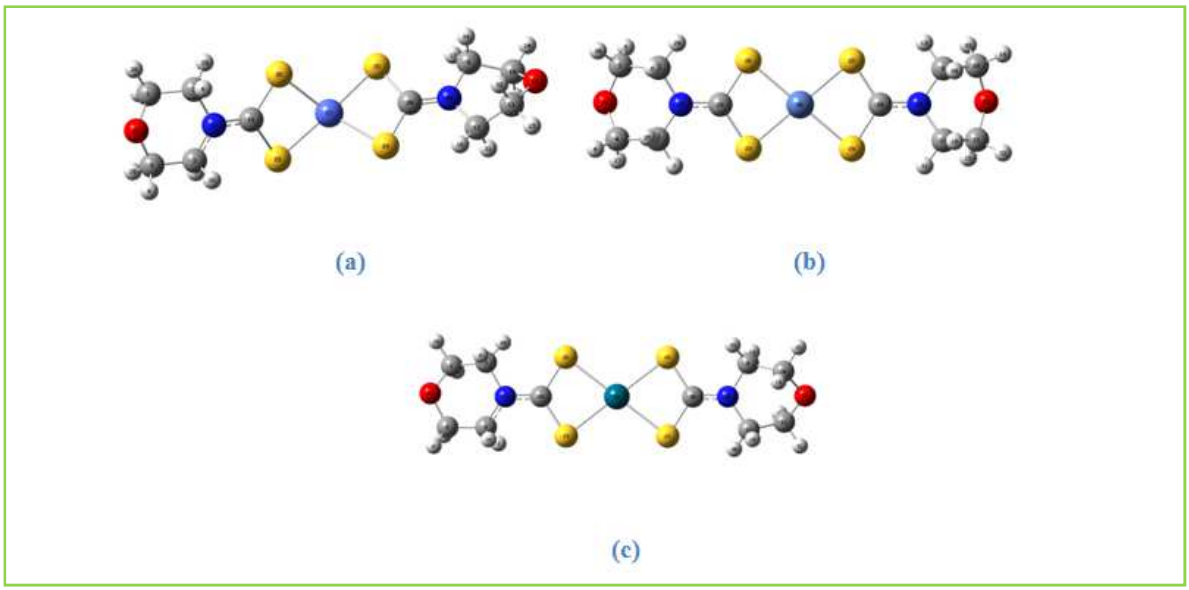

Figure 4.2: Optimized structure of (a) $Co(MDTC)_2$ (b) $Ni(MDTC)_2$ (c) $Pd(MDTC)_2$ complexes using DFT with B3LYP 3-21 G basis set.

Table 4.1: Calculated Parameters using Gaussian-03 software

S. No.	Parameters	Co(II)	Ni(II)	Pd(II)
1	Calculation method	B3LYP	B3LYP	B3LYP
2	Basis Set	3-21G	3-21G	3-21G
3	Energy (a.u)	-3608.66821	-3733.57918	-7150.22005
4	Dipole moment	1.57	2.1	1.12
5	Point Group	C1	C1	C1

4.3.2 Sample preparation

Aqueous environmental samples (Ground water and industrial waste water)

Ground water was obtained from the bore well located within Punjabi university campus, Patiala, India in pyrex borosilicate amber glass containers previously rinsed with triply distilled water. The bore well water is fed to the appliance as raw water. Industrial waste water contains high concentrations of particulate matter and suspended impurities, samples obtained from the effluent stream coming out of industrial area located in vicinity of Chandigarh city were first filtered with Whatman filter paper (grade no. 1) and then with 0.45 μm pore size Nylon-6, 6 membrane filters in a filtration assembly.

Alloys

About 100 mg of each of alloy was taken and dissolved in aqua regia. Solution was heated to near dryness and nitrate was expelled from residue by conc. HCl acid. Residue was dissolved in triple distilled water and volume was made up to 1L. An appropriate aliquot of solution was studied for Co(II), Ni(II) and Pd(II) determination by same procedure given above. All above samples were degassed with ultrasonic bath prior to the FPSE process. Spiked aqueous samples

of desired concentration of Co(II), Ni(II) and Pd(II) complexes were prepared and subjected to preconcentration with FPSE.

4.4 Results and Discussion

4.4.1 Spectrometric analysis of metal complexes

The suitable interaction of these considered metal ions towards dithiocarbamates makes their determination easier. Co(II), Ni(II) and Pd(II) metal ions react very rapidly with MDTC to form corresponding coloured and stable metal dithiocarbamates. The composition of metal chelates was investigated by changing the metal:ligand mole ratio spectrophotometrically, which was found 1:2 for these metal ions and is in accordance to literature [11]. It clearly indicates from UV visible spectra that the absorption maximum (λmax = 330nm, 336 nm and 305 nm) of the complex was found experimentally for Co(II), Ni(II) and Pd(II) whereas the morpholine-dithiocarbamate reagent show absorbance at 284 nm. The formation of complexes was also confirmed by thin layer chromatography. With solvent system ethyl acetate: hexane (10: 90), one spot for each metal complex were obtained.

4.4.2 Optimization of FPSE Conditions

Different parameters affecting the performance of FPSE procedure towards extracting the metal complexes were optimized. The optimized conditions can be used for method validation and subsequent application to analyze the real environmental samples.

Selection of fabric phase sorptive extraction sorbent

Many different FPSE coatings can be prepared, we considered only three on the basis of their polarity: sol-gel PDMDPS (a non-polar sorbent similar to PDMS), sol-gel Poly THF (of intermediate polarity) and sol-gel PEG (polar coating). When the analyses were carried out by using these three sorbents, peak area found to be maximum in case of sol-gel Poly THF. So we

have considered sol-gel Poly THF coated FPSE media as the best extracting media for the further optimization experiments. Photographic image of these three FPSE media is shown in **Figure 4.3.**

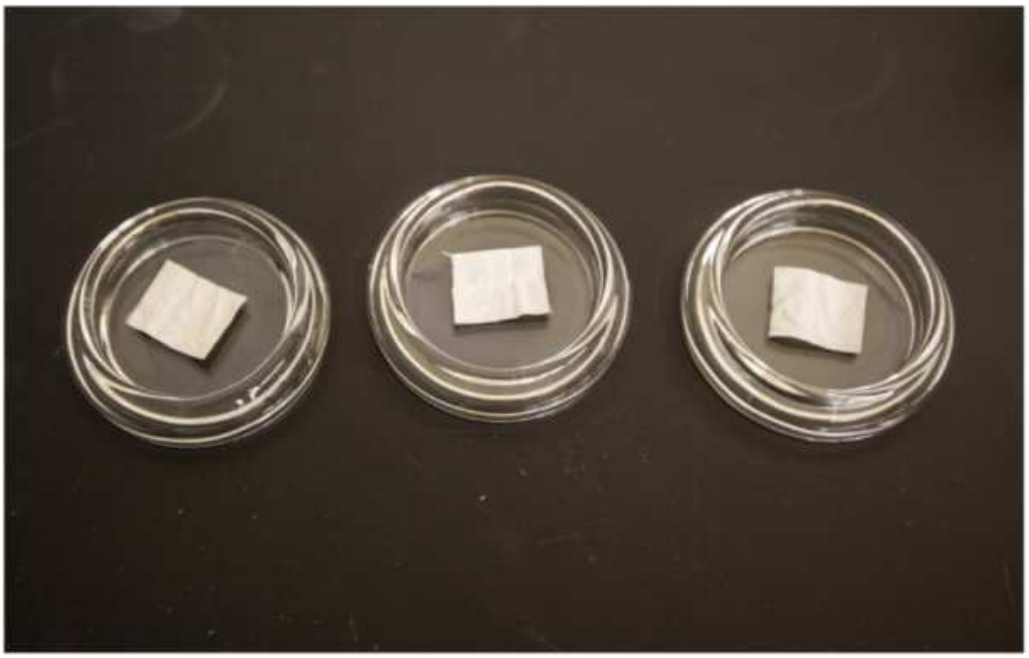

Figure 4.3: Photographic image of three FPSE media: PDMDPS; PTHF and PEG.

Effect of sample volume

Similar to other equilibrium based microextraction technique, the sample volume plays a key role in determining the extraction efficiency of FPSE and needs to be carefully optimized. Different sample volumes considered were 5, 10 and 15 containing target analytes was taken into a glass vial for optimization. When the sample volume is increased to 10 mL from 5 mL, the peak area is doubled. However, when this sample volume is further increased to 15 mL, the increase of the response is only 10-20%. Therefore, sample volume of 10 mL was fixed for subsequent experimentations.

Optimization of sorption time

Equilibrium extraction time is one of the most important FPSE parameters. The porous sol-gel sorbent network, high primary contact surface area of the FPSE media and the permeable cellulose substrate synergistically reduces the extraction equilibrium time **(Figure 4.4).** After attaining the equilibrium, FPSE media can no longer extract the analytes. The standard was examined to study the effect of extraction time. Extraction time was optimized by stirring the sample containing target analytes for different times. As the extraction time increases, extraction of target analytes onto the FPSE media increases and becomes almost constant at 20 min. So the extraction time of 20 min was optimized for further experimentation.

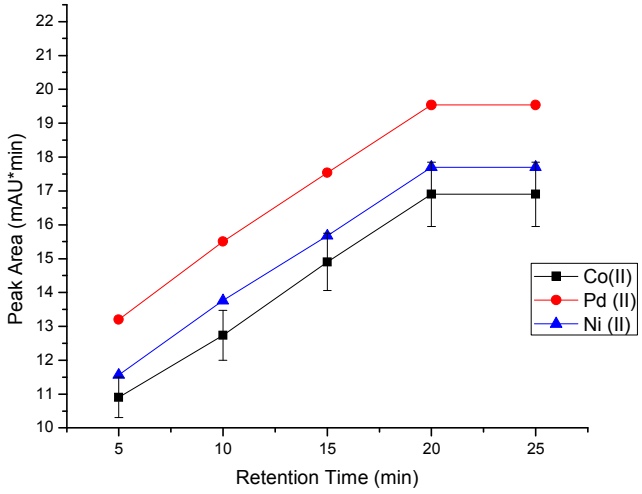

Figure 4.4: Optimization of sorption time

Effect of eluting solvent

Suitable organic solvent or solvent system is needed for desorption after extracting the target analytes into FPSE media. Different solvent/solvent mixtures were tried for the elution of target analytes. Acetonitrile was selected as the best optimum elution/back-extraction solvent by recording peak area as in **Figure 4.5.**

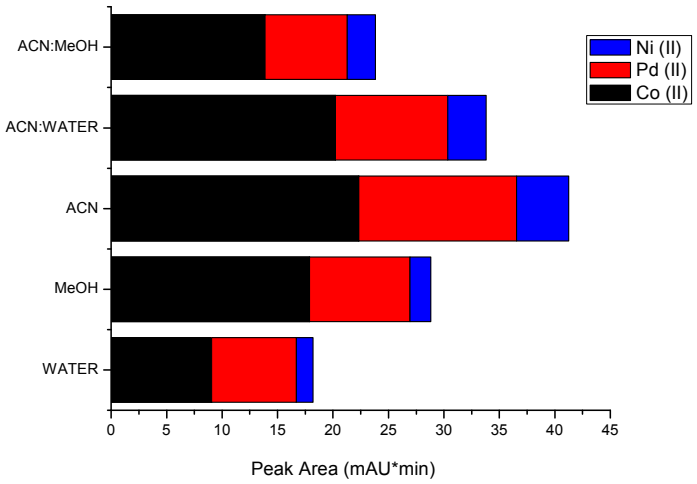

Figure 4.5: Optimization of eluting solvent

Effect of desorption time

Desorption time was optimized by performing the experimentation with time ranging from 5to 20 min. A back-extraction time of 15 min was selected as evident from **(Figure 4.6)**, while keeping the other optimized parameters to their fixed values.

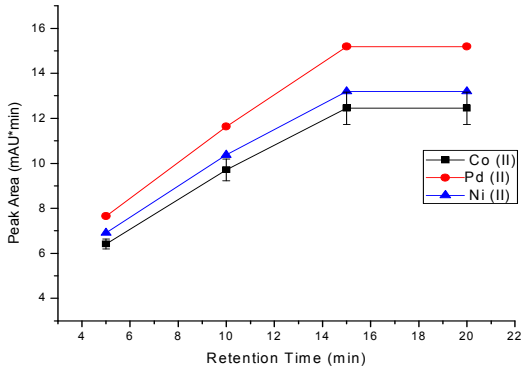

Figure 4.6: Optimization of desorption time

4.5 Precision, repeatability and detection limits

The linearity of the method for the Co(II), Ni(II) and Pd(II) complex was determined using the standard calibration curve and parameters shown in **Table 4.2.** The accuracy and precision of HPLC-UV method were evaluated for each analyte by analyzing a standard of known concentration and quantifying it using the calibration curve with FPSE. LODs were established for a signal-to-noise (S/N) equal to 3, whereas a value of 10 was used for the LOQ. Since no significant matrix effects occur with the developed method, the obtained values should not differ too much from those obtainable with real samples. Over the range of 1-100 ng/mL, the Co(II), Ni(II) and Pd(II) complex produce a linear response with the regression coefficient of 0.988, 0.994 and 0.995, respectively. The results for this detection and relative standard deviation are given in **Table 4.2.** The method provides satisfactory results in terms of recovery and relative standard deviation.

Table 4.2: Analytical characteristics of developed FPSE-HPC-UV method for determination of Co(II), Ni(II) and Pd(II) ions.

Parameters	**Sample preparation using FPSE**		
	Co(II)	Ni(II)	Pd(II)
Wavelength (nm)	315	315	315
Coefficient of determination (R^2)	0.988	0.994	0.995
Retention time (min)	3.5	4.5	5.2
Regression equation	0.060x+ 0.769	0.063x+ 0.915	0.067x+1.035
LOD (ng mL^{-1})	0.02	0.018	0.01
LOQ (ng mL^{-1})	0.066	0.059	0.03
RSD (%)	2.1-2.5	1.5-1.7	1.9-2.1
Retention Factor (k)	1.33	2.00	2.40
Selectivity (α)	1.5	1.2	1.8

Resolution	1.7	1.5	4.5

4.6 Interference of other ions

The interference of various metal ions such as Cr(III), Cr(VI), Pb(II), Zn(II), Sn(II), Ag(I), Cu(II), Mo(VI) and V(V) was studied. Different amount of these diverse metal ions were added individually to aliquots containing Co(II), Ni(II) and Pd(II). Cr(III) and Cr(VI) donot form complex at room temperature. It forms complex only on heating at a temperature of 55°C with dithiocarbamate [16]. The Zn(II), Ag(I) and Sn(II) did not interfere as no complex was observed for these metal complexes. This happens due to instability of metal complexes and is in agreement with the literature [17, 18]. Vanadium and molybdenum ions require highly acidic conditions (pH<4) for complexation, so no interference was observed. Pb(II) forms complex with dithiocarbamate but very weak in nature and even decomposed during separation [19].

4.7 Applications

The effectiveness of the developed method was verified by the determination of Co(II), Ni(II) and Pd(II) complex in various kinds of aqueous samples such as ground water and industrial waste water. To demonstrate the practical applicability and the performance of the developed method, various environmental samples were analyzed under optimum conditions. The method was also applied to alloys such as borcher and cobalt-palladium alloy. A 100 mg of alloy was taken along with HCl and HNO_3. The contents were heated on a hot plate and the volume reduced to less than half. Residue was then dissolved in triply distilled water and made volume upto 500 mL. On the basis of actual value and calculated value, the recovery of metal ions are calculated. The results obtained were satisfactory with recovery more than 90% for a broad concentration range. During their determination, no major interfering peaks were present at the

retention time of these analytes. FPSE-HPLC-UV presents an integrated approach capable of preconcentration and chromatogram of spiked ground water, industrial waste water and alloys are depicted in **Figure 4.7 (a-d).** The results of the determination are shown in **Table 4.3.**

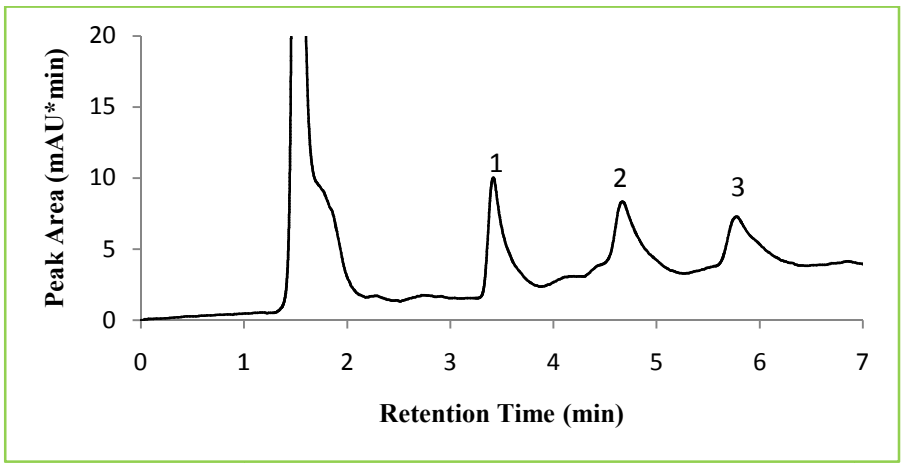

Figure 4.7 a: FPSE-HPLC-UV chromatograms in aqueous environmental samples of spiked 1-Co(II); 2-Ni(II); 3-Pd(II) in ground water sample

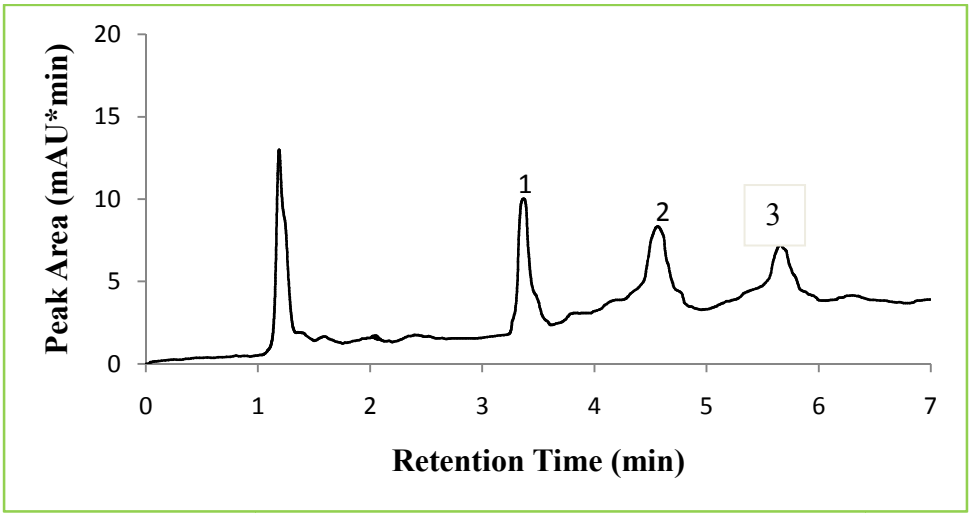

Figure 4.7 b: FPSE-HPLC-UV chromatograms in aqueous environmental samples of spiked 1-Co(II); 2-Ni(II); 3-Pd(II) in industrial water sample

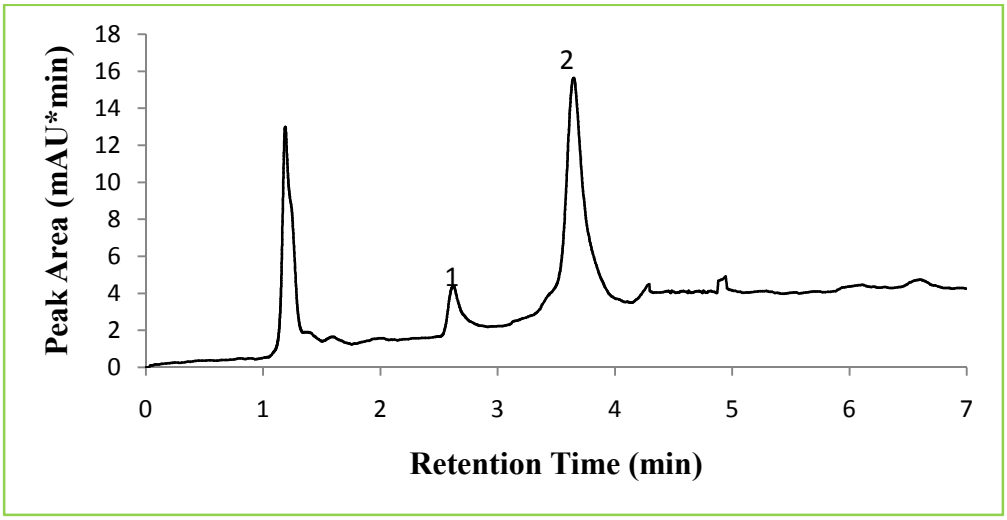

Figure 4.7 c: FPSE-HPLC-UV chromatograms in Borcher alloy

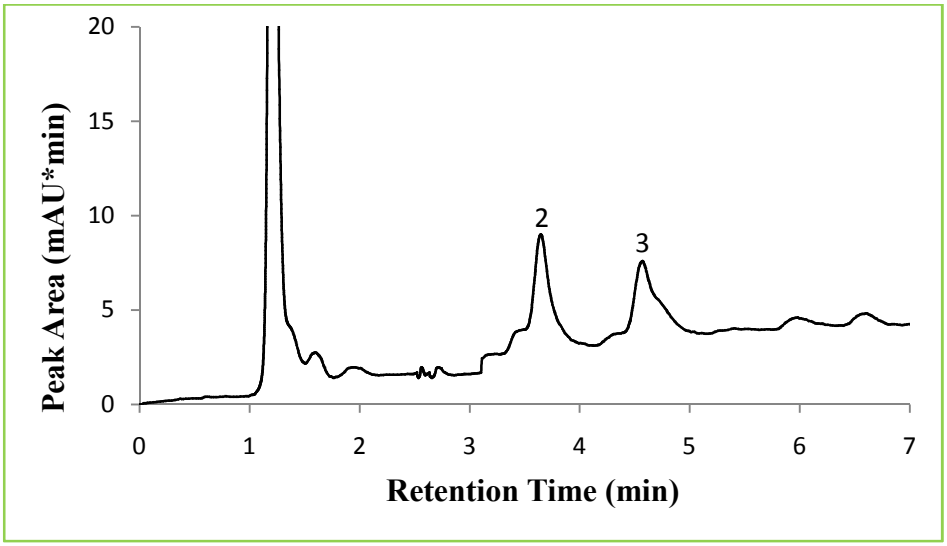

Figure 4.7 d: FPSE-HPLC-UV chromatograms in oakay alloy

Table 4.3: Recoveries of Co(II), Ni(II) and Pd(II) in aqueous environmental samples and alloys using FPSE-HPLC-UV

Analyte	Added	Industrial Water		Ground Water		Borcher Alloy		Oakay alloy	
		Found (RSD)	Recovery (%)	Found (RSD)	Recovery (%)	Found (RSD)	Recovery (%)	Found (RSD)	Recovery (%)

Co(II)	1	0.89 (2.7)	89.60	0.98 (2.1)	98.70	0.97 (2.3)	97.60	-	-
	5	4.50 (2.5)	90.00	4.88 (2.0)	97.60	4.78 (2.3)	95.60	-	-
	10	9.16 (2.0)	91.60	9.75 (1.9)	97.59	9.65 (2.2)	96.70	-	-
Ni(II)	1	0.87 (2.9)	87.00	0.97 (2.3)	97.50	0.98 (2.1)	98.50	0.97 (2.2)	97.00
	5	4.56 (2.6)	91.20	4.82 (2.2)	96.50	4.90 (2.0)	98.00	4.77 (2.1)	95.4
	10	9.13 (2.5)	91.30	9.86 (2.1)	98.60	9.68 (1.9)	96.80	9.80 (2.1)	98.0
Pd(II)	1	0.89 (2.8)	89.00	0.98 (2.7)	98.00	-	-	0.97 (2.9)	97.0
	5	4.83 (2.6)	96.66	4.91 (2.5)	98.20	-	-	4.95 (2.9)	99.0
	10	9.69 (2.5)	96.69	9.78 (2.5)	97.8	-	-	9.86 (2.7)	98.6

4.8 Conclusion

FPSE has been demonstrated to be a rapid, convenient, simple and an efficient tool for the determination of metal ions after complex formation for the first time. In this work, three different FPSE sorbent chemistries, sol–gel PDMDPS, sol–gel PTHF, and sol–gel PEG were investigated and sol-gel PTHF was optimized for the rapid and efficient analysis of Co(II), Ni(II) and Pd(II) as MDTC complex in environmental water samples and alloys. The sensitivity, precision and accuracy of FPSE are proved to be satisfactory. Compared to the other sorptive microextraction techniques FPSE obsess many advantages including low cost, simple, less use of toxic and hazardous organic solvent which is in agreement with green chemistry and many more described above. The application of this methodology to the analysis of ground water, industrial

waste water and alloys with good recoveries substantiated its future potential in analyzing environmental samples with good conviction.

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Development of High Pressure Liquid Chromatographic method for the analysis of aluminium metal ion and its application to environmental samples

5.1 Introduction

Aluminium is the third most abundant element and major constituent of rocks in the earth crust [1]. It is ubiquitous in the environment. The main route of aluminium input to humans is through the food, medicine, drinking water, cosmetics and use of cooking utensils. Aluminium is a well known flocculating and coagulating agent in purification of water which increases the level of its found at parts per billion in most of portable drinking water. Although aluminium compounds are not as toxic as heavy metals, but an excessive intake of aluminium in human body can cause serious health problems such as damage of nervous system, loss of memory alzheimer's disease, anemia, osteomalacia, neurological syndrome and it is also responsible for intoxication in hemodialysis [2-4]. It is highly used in many industries to make millions of products and highly important for the world economy. People who work in aluminium production industries may suffer lung problems due to breathing in aluminium dust. As per WHO guidelines, the permissible level of aluminium in drinking water is only 0.2 mg/L [5].

The biological effects of aluminium have received much focus in recent years. It is found that abnormally high levels are linked to pathologies [6-8]. The presence of this element in the drinking water is of more concern as it causes potential threats to the health of humans. In the present work 8-hydroxyquinoline-5-sulfonic acid (8-HQSA) as a chelating agent has been selected due to its ability to rapidly form the stable complexes with a large range of metals, and the fact that a number of metal-ligand complexes of this type are fluorescent. 8-HQSA does not cause any interference in detection of metal in an un complexed form. The ligands can form the complex with metal by the three ways as a post column derivatization as in post column reaction

detection system for aluminium species developed by Jones and coworkers [9-10]. Secondly, form metal complexes "pre column" and thirdly as addition to the mobile phase, where by a combination of "pre column" and "on column" complexation may be utilized [11]. In this work 8-HQSA complex with metal ion used pre column. Meaney and coworkers [12, 13] used a reverse phase system for the separation of iron and aluminium complexes of 8-HQSA metal complexes were formed pre column and separated on octadecyl silica (ODS) column using acetonitrile mobile phase containing 8-HQ and KNO_3 but this method results in broad peaks and the detection method was UV-Vis at 400 nm with the detection limits of 10 and 50 μg L^{-1}. This is also recently illustrated by Feng et al [14] that the retention behavior of Zn(II), Cd(II), Al(III), Ga(III) as 8-HQSA complexes on ODS column with mobile phase containing buffer, tetrabutylammonium bromide and 8-HQSA. This part of a chapter reports on the development of a simple and sensitive HPLC reverse phase method suitable for the determination of trace levels of aluminium in environmental samples with fluorescence detection. The following method is based on pre column formation of metal 8-HQSA complexes. The fluorescence of negatively charged 8-HQSA complex is enhanced by cationic surfactants Cetyltrimethylammonium bromide (CTAB) due to their micellar action. Such surfactants have lots of advantages including the solublization of Al-HQSA complex, enhancement of fluorescence intensity and elimination of the need for reagent in the mobile phase. Enhancement is the result of strong interaction between the positively charged surfactants and the negatively charged complex.

5.2 Experimental

5.2.1 Apparatus

The Dionex HPLC Unit consisted of following components; a P680 solvent delivery pump, FLD detector detecting at two wavelengths only excitation and emission wavelength and interfaced to a computer loaded with Chromeleon software, in conjunction with HP Laser 1010 printer. A

supelco C_{18} stainlees steel reversed phase column of Ascentis express with C_{18} material (10 cm × 4.6, 5 µm) hard core shell was used for the separation.

5.2.2 Materials and Reagents

All the used solvents were of HPLC grade purchased from J.T Baker Chemicals, USA and filtered by using 0.45-µm Nylon 66- membrane filters (Rankem, India) in filtration assembly (from Perfit India). Water was deionized (Riviera, SCHOTT DURAN, Mainz, Germany) and filtered. Al(III) (Aluminium sulfate), 8-Hydroxyquinoline-5-sulfonic acid were obtained from Merck, India. 0.01 g of 8-hydroxyquinoline-5-sulfonic acid was dissolved in 10 mL of triply distilled water. 10 mL of stock solution of Al (1000 ppm) was prepared by dissolving 10 mg of $Al_2(SO_4)_3$ (Merck) in deionized water. 0.5M of Cetyltrimethylammonium bromide (CTAB) was prepared by dissolving required amount of CTAB in deionized water. Working standard solutions were prepared after suitable dilutions of the stock solution and sonicated every time before use. All HPLC grade solvents were degassed using an ultrasonic bath prior to use for about 15 minutes. The pH was adjusted on digital pH meter using 0.1M NaOH and acetic acid. Canned coke was purchased from local market.

5.3 General Procedure

An aliquot amount of 50 μgL^{-1} of aluminum $Al_2(SO_4)_3$ solution was transferred to a 5 mL flask and 0.1% (200 mL) of the ligand 8-HQSA solution (in water) was added followed by the addition of a 0.05 M (500 mL) surfactant CTAB (to solubilize the complex). The solution was mixed well and allowed to stand for 1 min, and the pH of solution was adjusted to 8.5 by adding 20 mL of 0.1 M sodium hydroxide. Total volume of solution was made up to 2 mL with triply distilled water.

The separation of Al-HQSA complex was performed by using the C_{18} column. The composition of water and methanol with acetonitrile was varied to optimize the conditions. The metal complex was detected under isocratic flow of acetonitrile-methanol-water (55: 30: 15) at a flow rate of 0.5 mL min^{-1}. This provides a fast elution of complex at a retention time of 2.5 min.

5.4 Sample preparation

Tap water samples

The tap water sample was taken from our lab in a Pyrex borosilicate amber glass bottle, which was rinsed with triply distilled water. The water sample was then filtered through Nylon 6, 6 membrane filters in a filtration assembly and sonicated to degas. The sample was prepared in a 10 mL beaker and 200 mL of ligand (0.1%) 8-HQSA was spiked with 100 mL of aluminum metal of appropriate concentration. A 500 mL volume of 0.05 M surfactant was added and the pH was adjusted to 8.5 with 20 mL of 0.1 M NaOH. The total volume of the formed complex was made 2 mL with tap water. The solution obtained was sonicated and filtered with the syringe filters before analysis.

Canned coke samples

One milliliter of canned coke (Moon Beverages Limited Coca-Cola Company originated in USA since 1886) sample was prepared by diluting 10 times the coke sample with deionized water and filtered using 0.45 mm Nylon 6,6 membranes. The sample was spiked as described in the "Tap water samples" section.

5.5 Results and Discussion

The aim of this work was to develop a sensitive and a selective method for the determination of Al(III) in water samples. The course of investigation was accomplished with HPLC-FLD method as it requires an inexpensive detection system as compared with other chromatographic

techniques having detection systems such as ICP-MS. As Al(III) forms a strongly fluorescent complex with 8-HQSA, this approach was chosen for further method development and applied to real samples. The structure of the complex of aluminum, Al–HQSA, is shown in **Figure 5.1** (15).

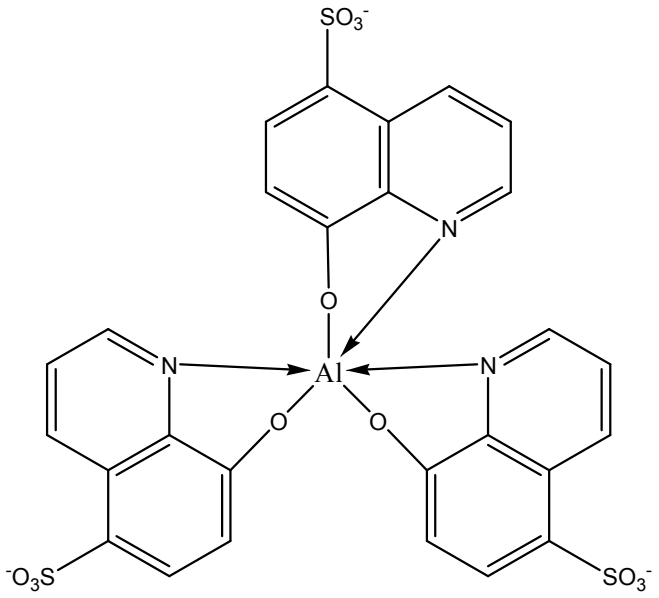

Figure 5.1: The proposed structure of Al-HQS complex

5.5.1 Optimization of different Parameters

In this chromatographic work, a number of parameters exist which can be changed to control the selectivity and analyte resolution. Therefore, in this study it was necessary to understand and develop suitable conditions that provided the desired selectivity, efficiency and sensitivity.

Effect of Surfactant Concentration

This effect was studied by varying the concentration of surfactant in the solution. **Figure 5.2** shows the effect of surfactant CTAB on the peak area of the aluminum complex. The appropriate concentration found for the relevant studies was in between 0.02 and 0.09 M. It was found that the concentration up to 0.05 M was enough for the solublization of Al-8-HQSA complex. The

concentration found above 0.1 M could not be used because column back pressure exceeded normal operating conditions.

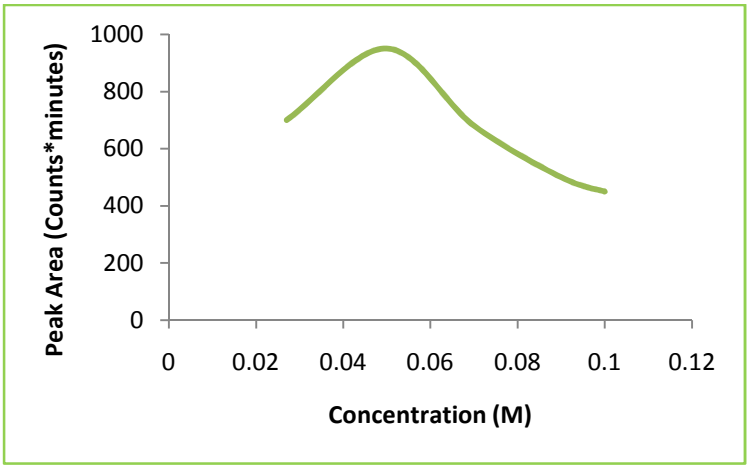

Figure 5.2: Effect of CTAB concentration on chromatographic peak of Aluminium

Effect of mobile phase

It is important to optimize mobile phase for the separation of the aluminum species. Mobile phase selection depends upon resolution of peaks as well as signal response and separation time. Feng et al. has taken the mobile phase containing the concentration of 8-HQSA of 0.5 mM by adding the different volumes of 0.1% 8-HQSA for the Al(III) –8-HQSA complex (29). However, in our study, 8-HQSA as a mobile phase has not been taken. The varying concentration of water and methanol in the various ratio have been as shown in **Figure 5.3** taken and the final conditions were optimized as acetonitrile-methanol-water (55: 30: 15). With varying the amount, it was observed that with the increase in the amount of water, a single sharp peak was obtained with no tailing. However, with the use of methanol in higher ratios the shoulder appears in the peak and it was not separated.

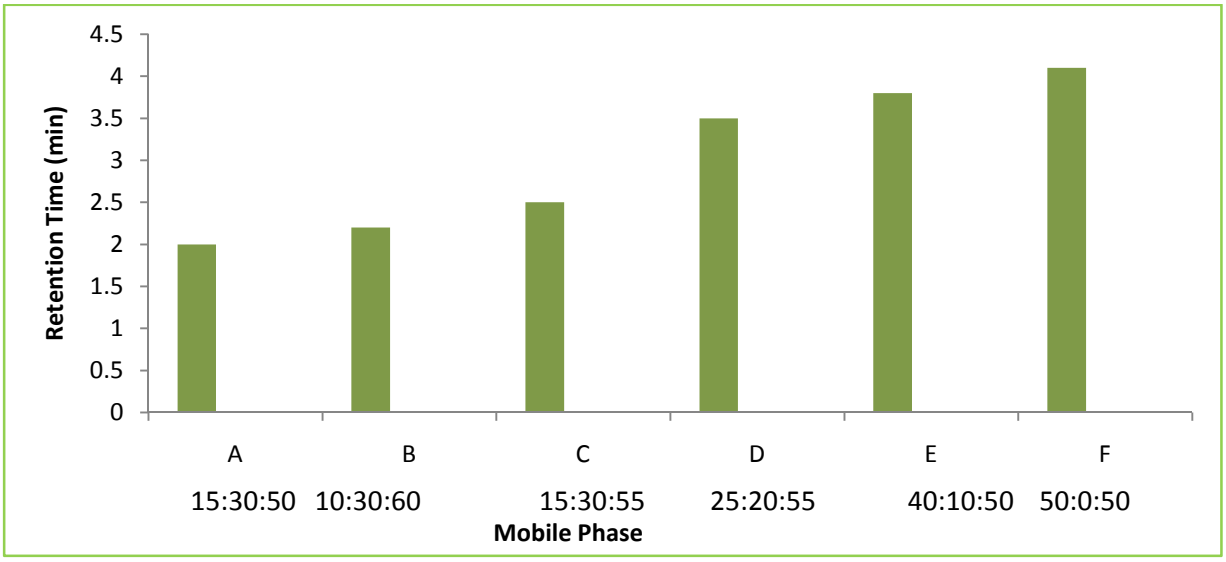

Figure 5.3: Effect of mobile phase

Effect of pH on pre-column chelation

The sample analyzed must be within the pH range of 6.0 –9.0 because below this pH interference of many other ions was found. These metal ions react with the ligand, and no complexation occurs with aluminum. As the pH also affects the fluorescence phenomenon, so complexation was performed with in this range. The pH was adjusted to 8.5 by adding 0.1 M NaOH. Using all these optimized parameters, the chromatograms of ligand and metal complex are shown in **Figures 5.4 (a, b)**

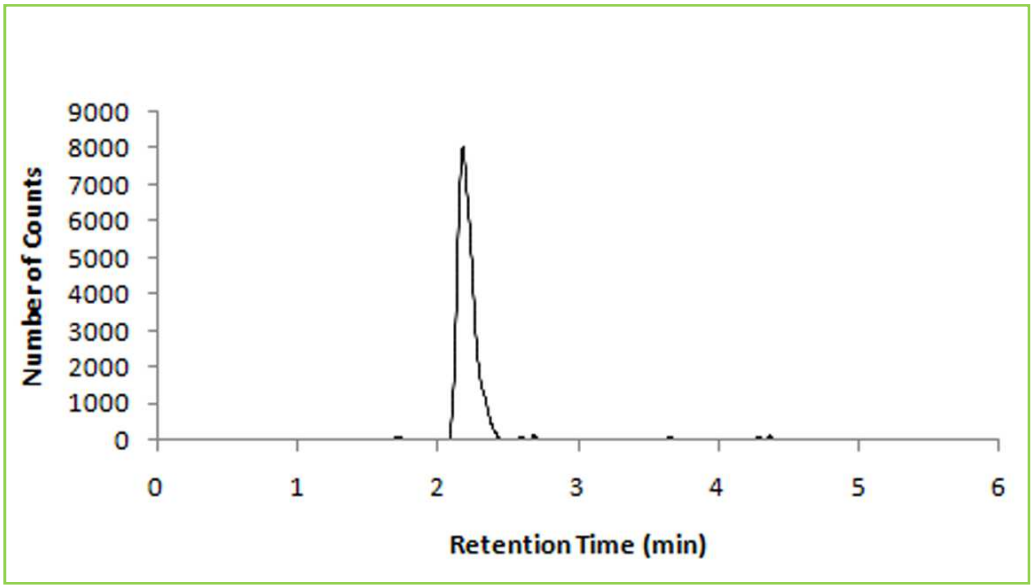

Figure 5.4 a: Chromatogram of a ligand HQS

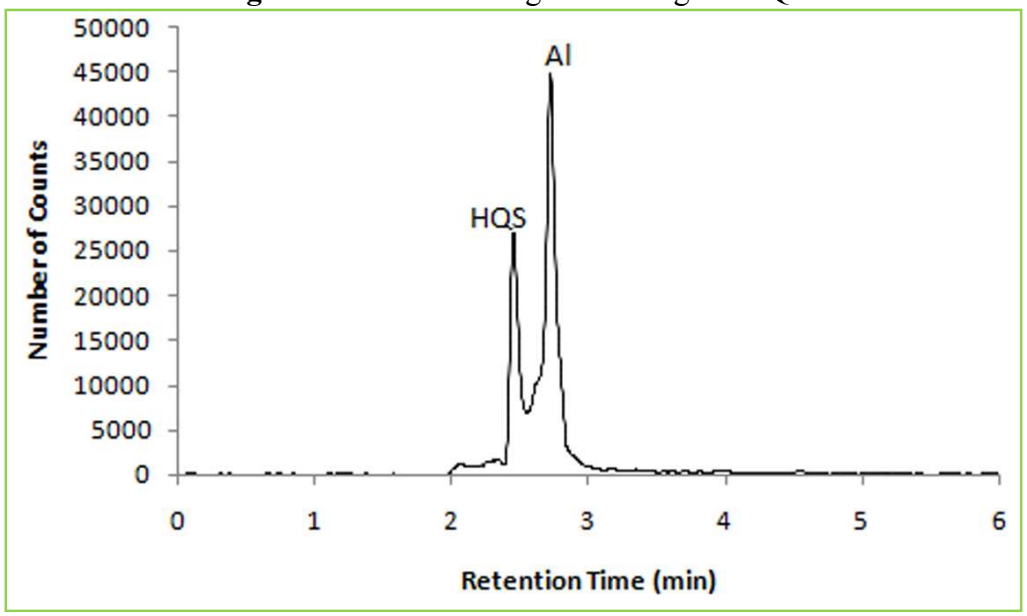

Figure 5.4 b: Chromatogram of a metal ligand complex (Al-HQS complex)

5.6 Method Validation

The linearity of the method for the Al(III) was determined using the standard calibration curve and the parameters are summarized in Table I. Calibration curve for the Al(III) as Al-HQSA complex was prepared in the range 1–200 μgL^{-1} ,on HPLC-FLD with five concentration levels separately. The calibration curve was described by the linear regression equation:

$$y = mx + c$$

where y is the peak area, x is the concentration, m is the slope and c is the intercept. The limit of detection (LOD) was set at the concentration when the signal-to-noise ratio was equal to 3: 1. Over the range of 1-200 μgL^{-1}, the Al(III) standards produced a linear response with the regression coefficient of 0.997. The detection limits were calculated for the metal complexes as per accordance to the published guidelines, as three times the standard deviation. Similarly the method of quantification was also evaluated as 10 times the standard deviation. The accuracy and precision of the HPLC-FLD method was evaluated for each analyte by analyzing a standard of known concentration and quantifying it using the calibration curve. The results for this detection and relative standard deviation (RSD) are shown in **Table 5.1**. The method gives the satisfactory results in terms of recoveries and RSD. These detection limits are better when compared with methods reported in the literature shown in **Table 5.2.**

Table 5.1: Analysis of calibration Curve of Al (III) HQS by HPLC-FLD.

PARAMETERS	***Al(III) added as $Al_2(SO_4)_3$***
Wavelength	410 and 510 nm
Linear Range	1-200 μgL^{-1}
Average Peak Area	25468.9
Average Peak Height	226784.79
Retention Time (min)	2.5
Correlation Cofficient	0.997
LOD (S/N=3) (μgL^{-1})	0.05
LOQ (S/N=10) (μgL^{-1})	0.15
Relative Standard Deviation (RSD)	2.5

Table 5.2: Comparison of the Analytical Methods used for the determination of aluminium in various water samples with reported literature.

Method Type	***Preconcentration***	***Sample Matrix***	***LOD(ngL^{-1})***	***Reference***

ICP MS	SingleDrop Microextraction (SDME)	Lake Water	3.7	[30]
UV –Vis	HPLC (Kromacil C_{18})	River	51.9	[18]
Spectrophotometric	Al as Morin complex	River	6	[21]
8-HQS	Solvent Extraction (chloroform)	River(Drinking and waste water)	7.4	[22]
8-HQS	Rp-HPLC(Spherisorb ODS)	Natural Water	1.8	[17]
8-HQS	Rp-HPLC (Ascentis Express)	Tap Water, Coke	0.05	DM

5.7 Analytical Applications

This method developed was applied to tap water and canned coke. The concentration of aluminum in these samples of tap water and coke was examined by using the described procedure in "Sample preparation." There was a good agreement between the results obtained from the proposed method and the reported method (12). The procedure was adopted to analyze the separation in the coke sample at a flow rate of 0.5 mL min^{-1} in between the elution time of 2-4 min. The chromatograms for the unspiked and spiked sample of coke are shown in **Figure 5.5 a and b,** respectively. Similarly, **Figure 5.5 c and d** show the chromatograms obtained from an injection of a tap water sample and similar spiked tap water sample with the concentration of 20 ng mL^{-1}. However, it is clear from the chromatogram that in between the elution time of 3-4 min with a flow rate of 0.5 mL min^{-1}, there is no interfering peaks arising from the tap water and retention time migrated from 2-4 to 3-4 min. The results obtained were satisfactory with recovery more than 85%.

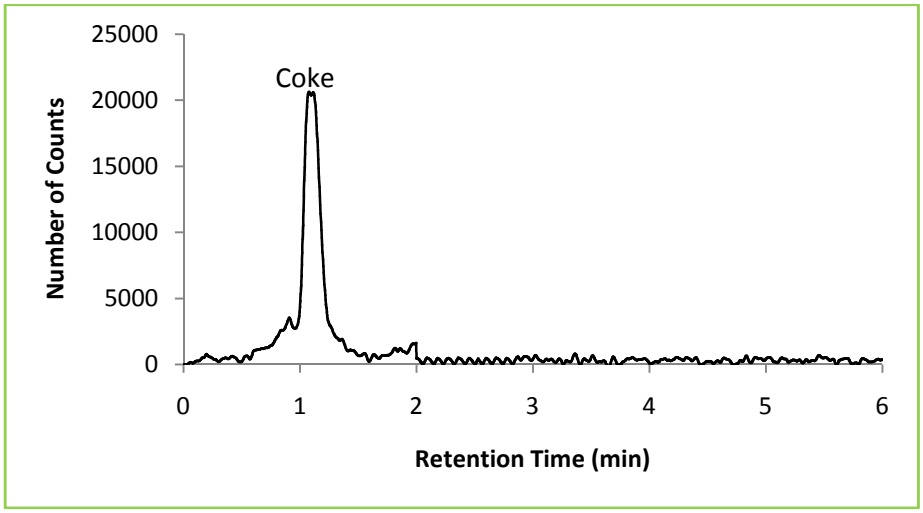

Figure 5.5 a: Chromatogram of standard solution of Canned Coke

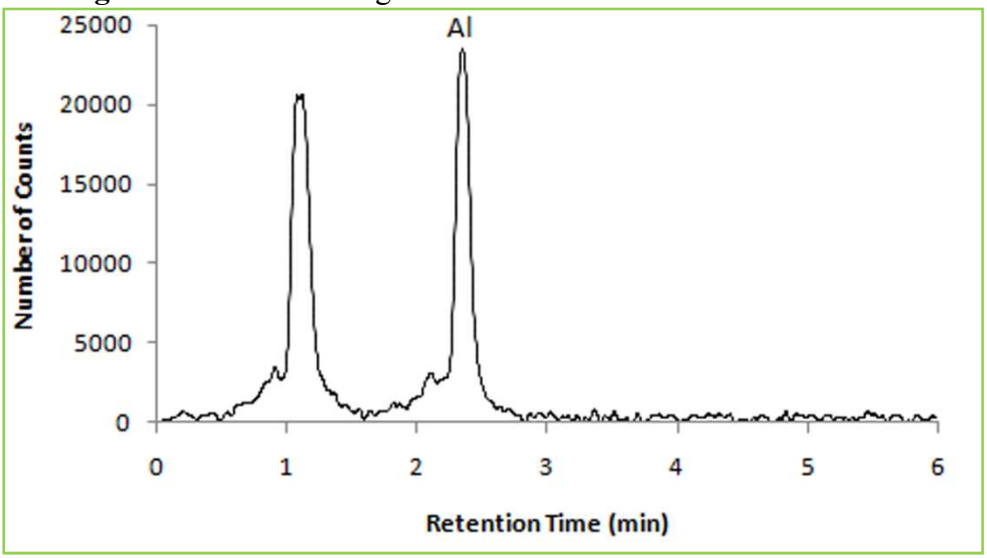

Figure 5.5 b: Chromatogram of spiked sample of aluminium in coke standard solution

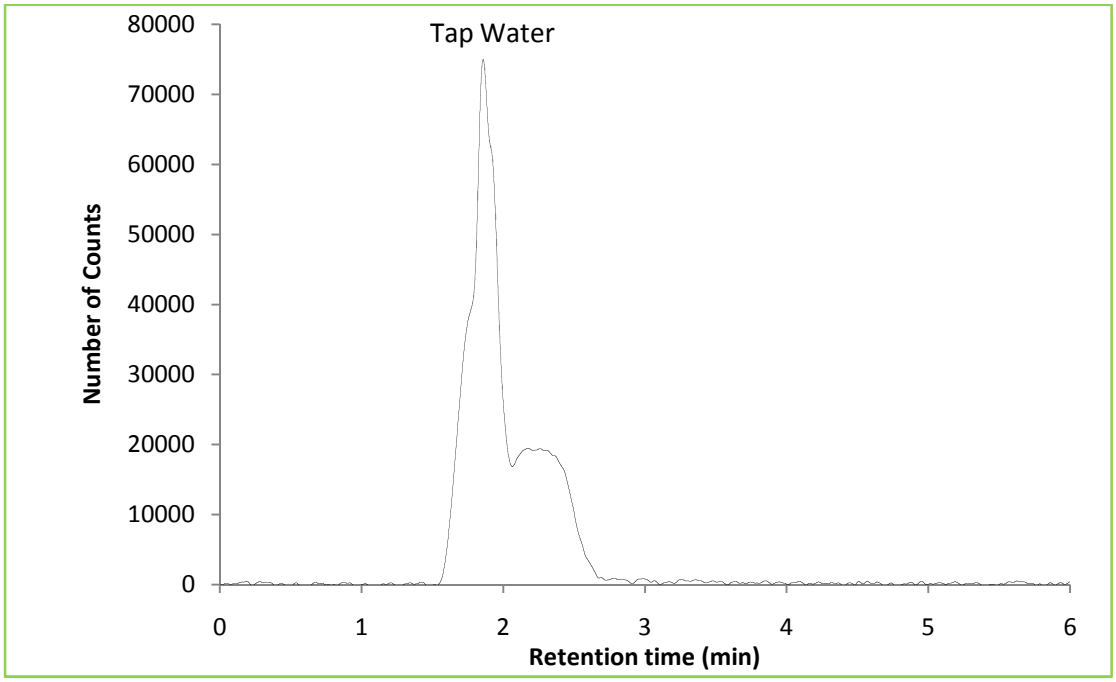

Figure 5.5 c: Chromatogram of tap water

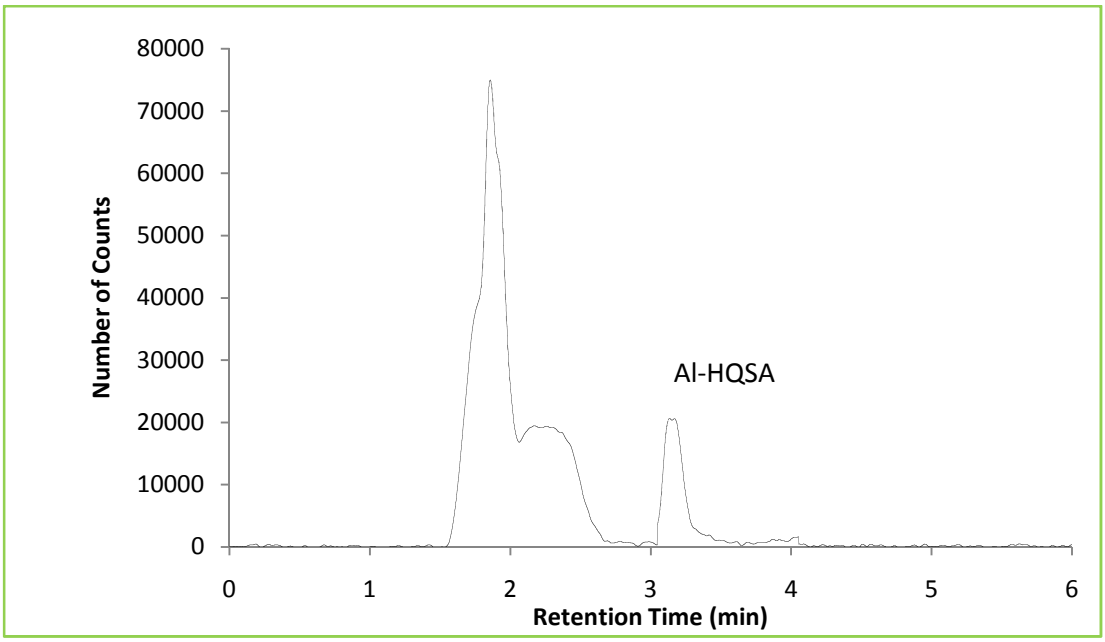

Figure 5.5 d: Chromatogram of spiked sample of aluminium in tap water

5.8 Conclusion

A simple and selective method has been developed for the determination of aluminum in environmental water samples and coke based on the RP-HPLC combined with fluorescence detection. The developed method does not require any post-column reaction and time-consuming sample preparation. The response is linear and the selectivity of the method is such that Al can be detected even at very low concentration. The developed method does not require any use of a reagent in the mobile phase. The use of cationic surfactant CTAB enhances both selectivity and sensitivity of the determination. The ligand alone is fluorescent but in the presence of a surfactant, it mobilized with it and comes at higher retention time. It also eliminates the need of purification, which is time consuming. The method showed improved detection limits of 0.05 mgL^{-1} for Al(III) over reported methods (Table II). Thus, the method can be considered as a choice for the determination of aluminum in various aqueous environmental samples.

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Development of FPSE-HPLC-UV Method for analysis of phenyltin compounds in environmental and canned food samples

Abstract

This paper narrates a novel fabric phase sorptive extraction-high performance liquid chromatography-ultra visible detection (FPSE-HPLC-UV) method for the simultaneous extraction and analysis of four phenyltin derivatives that include triphenyltin hydroxide, triphenyltin acetate, triphenyltin chloride and tetraphenyltin in environmental (agricultural waste water and municipal waste water) and canned food sample. The selected analytes were well resolved by waters nova pack C18 column (3.9 x 150 mm, 4μm) in isocratic elution mode within 15 minutes. The new microextraction media has been analytically evaluated using phenyltin derivatives as model compounds. The factors affecting the extraction efficiency of FPSE have been probed and the optimized extraction conditions have been determined. Under these optimum conditions, the limits of detection (LODs) for sol-gel C18 coated FPSE media in combination with HPLC-UV for the analysis of the phenyltin derivatives were in the range of 10-100 ng/mL with precision (relative standard deviation) at 10 ng/mL concentration with good absolute recoveries and less relative standard deviation. To the best of our knowledge, this is the pioneer FPSE extraction procedure applied on environmental and canned food sample for the simultaneous determination of phenyltin derivatives and could be mimic as a rapid and robust green analytical tool.

Keywords: Phenyltin derivatives, Tetraphenyltin, Triphenyltin chloride, triphenyltin acetate, triphenyltin hydroxide, Fabric phase sorptive extraction (FPSE), High performance liquid chromatography (HPLC)

6.1. Introduction

The environment is flooded with huge burden of toxic compounds originated from both natural and anthropogenic activities. The presence of these compounds even in minute quantities is considered critical with disastrous consequences. Organotins (OTs) are the organometallic pollutants, where aryl or alkyl group are directly bounded to tin (Sn). The number and structure of the organic moieties bounded to Sn can significantly alter its physicochemical properties [1-2]. The industrial and agricultural utility of phenyltins compounds has led to presence of it in food products and environment, resulting into wide spread risk of human exposure. According to the Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO), the maximum permissible limit for tin in canned foods is 250 mg kg^{-1} [3]. Phenytins can elicit a wide range of endocrine and nervous-system effects, particularly tri-substituted species [4-5]. In general the elemental tin and its inorganic salts are considered to be harmless while the organotins like Tributyltin and Triphenyltin are very toxic and can promote harmful effects on non-target aquatic organisms, even at a low ng/L level [6].

In this study Tetraphenyltin [$(C_6H_5)_4Sn$; TPT], triphenyl tin acetate [$(C_6H_5)_3SnOOCH_3$; TPTA] triphenyl tin hydroxide [$(C_6H_5)_3SnOH$; TPTH] and triphenyltin chloride [$(C_6H_5)_3SnCl$; TPTC] were studied as target analytes because of its wide use in insecticides, fungicides, bactericides, acaricides, preservatives, catalysts, stabilizers for PVC, antifouling paints, water repellents, fire retardants for woolen & cotton fabrics, moth repellants of textiles, disinfectors of hospitals and clothing and UV-oxidation resistance stabilizers, therefore found in numerous compartments such as water, plastic, textile and agriculture products etc [7-13]. As a result there is a variety of pathways for their entry into the environment and have long term persistence in the environment. Their extremely toxicity and brutal effects on both mammals [14] and marine organisms [15] including humans and also their high bioaccumulation prospective have led to the

control of pollution levels of phenyltin species, especially at low concentration levels. In addition, long-term exposure to phenyltin derivatives can lead to anomalies in DNA mutation and human red blood cells [16]. Therefore, the development of rapid, precise, novel and sensitive analytical methods for determination of these analytes is of special importance. Till now numerous protocols including gas chromatography (GC) [17-18], capillary electrophoresis [19-20], liquid chromatography (LC) or supercritical fluid chromatography (SFC) with detection method like atomic absorption spectrometry (AAS) [21-22], atomic emission spectrometry (AES) [23], inductively coupled-plasma mass spectrometry (ICP-MS) [24-25], electron spray ionization mass spectrometry (ESI-MS) [26-27], ultraviolet (UV) [28-29] flame photometric detection (FPD) [30-31] or dispersive liquid liquid microextraction (DLLME) [32] methods has been successfully developed for their determination. Although these techniques are very useful but possess some serious drawbacks including laborious, time consuming, high consumption of organic solvents and sample solutions, and often lead towards significant loss of analytes and poor reproducibility (DLLME, SPE), high cost, fragility of the fiber, due to bubble formation reduction in rate of extraction, and non-equilibrium in most cases. Therefore novel, versatile and high-performance adsorbents with a simple sample preparation are still highly desirable.

Recently Kabir et al [33-35] has developed fabric phase sorptive extraction technique which involves the successful integration of the advantages of rich surface chemistry of cellulose fabric and advanced material properties of sol-gel derived hybrid organic-inorganic extraction sorbents leading to the formation of a highly sensitive, solvent minimized and an efficient microextraction technique. The synergistic combination of sol-gel derived hybrid organic-inorganic sorbent and intrinsically porous network of cellulose surface as the substrate has allowed the utilization of high amount of sol-gel extraction sorbent onto the cellulose substrate

matrix, which leads to a phenomenal increase in analyte retention capacity with fast extraction equilibrium. As phenyltin compounds are inherently hydrophobic compounds. Thus the selective extraction of these analytes from aqueous sample matrix would be highly facilitated with a hydrophobic sorbent as the extraction phase material and consequently sol-gel C18 coated FPSE media containing long hydrophobic C_{18} chains has been evaluated and successfully applied in the present study. FPSE technique using sol-gel C_{18} coated FPSE media is thoroughly investigated as an advanced analyte enrichment tool to develop a green sample preparation technique.

Though gas chromatography (GC) is the most common approach in phenyltin analysis, but the low volatility and pre column derivatization complicates the analysis of phenyltin compounds by GC-MS [36,37]. The derivatization process makes the sample preparation more laborious and time consuming, which increases the probability of contamination and errors, and usually accompanied by sample loss. Because of inherent simplicity, prevention of sample loss and suitability for condition of volatility, high performance liquid chromatography (HPLC) is used preferentially over gas chromatography. The concern over the toxicity of phenyltins has led to the development of precise and reliable analytical method for their determination in canned food and environmental samples.

In this paper we describe the preparation, characterization of sol-gel coated FPSE media and the development of novel analytical method for the efficient extraction of trace amounts of four phenyltin species of high environmental interest from aqueous solution. The hydrophilic cellulose fabric substrate incorporated in the core of the adsorbent aids in the extraction kinetics by attracting water molecules containing phenyltin species towards its surface for a successful sorbent-analyte interaction, resulting in trapping of the analyte on FPSE media. To the best of my knowledge this kind of work serves the novel, better and superior pathway for effective

extraction and determination of phenyltin species. Therefore, the objective of this investigation was to develop a rapid, selective and sensitive FPSE-HPLC-UV for the determination of some important phenyltin derivatives/compounds in environmental aqueous samples and canned food.

6.2 Experimental

6.2.1 Apparatus

The HPLC system consist of pump (Dionex P680, Dionex Softron GmbH, Germering, Germany) with four solvent chambers, a Waters Nova pack C18 4µm reversed phase analytical column (3.9 x 150 mm), a Dionex UVD170U detector operated at 254 nm connected to a computer loaded with Chromeleon software for data acquisition. Separations were carried out at room temperature at 20-25 °C. Sample was directly injected into a rheodyne 20 µL loop on the valve for analysis. A digital vortex mixer (Fisher Scientific, USA) was employed for thoroughly mixing of sol solutions. An ultrasonic cleaner-2510 (Branson Inc., USA) was used to make sol solution free of trapped gas or bubbles. Centrifugation of sol solution, to obtain particle free solution, was carried out in an Eppendorf centrifuge model 5415 R. A Barnstead Nano Pure Diamond (Model D11911) deionized water system was used.

6.2.2 Materials, chemicals and Reagents

Acetonitrile (HPLC grade; UV cut off 190 nm), was purchased from J.T. Baker chemicals (USA). Triple distilled water was used as one of the elution solvent for chromatographic separation. Aqueous and non aqueous solvents were filtered with 0.45 µm Nylon-6, 6 membrane filters and 0.40 µm syringe filter (Rankem, India) in a filtration assembly (Perfit, India). Tetraphenyltin (> 97 %), triphenyl tin acetate (> 98 %), and triphenyl tin hydroxide (**> 96 %)** and triphenyltin chloride were purchased from Sigma aldrich (USA). C_{18} fiber, acetone, dichloromethane, methyltrimethoxysilane (MTMS), and trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich (USA). Sodium hydroxide and hydrochloric acid were purchased

from Thermo Fisher Scientific (USA). Standard stock solutions of each phenyltin compound (1000 ppm) were prepared in acetonitrile (HPLC grade). These solutions were stored at -10°C in dark bottles. Ultrasonic degassing was performed with ultrasonic bath (Sarthak Scientific Services, Panchkula, India). The required working solutions were prepared from stock solutions using acetonitrile solvent.

6.3. Spiking procedure

Stock solutions were prepared by dissolving each of Ph_3SnCl, Ph_3SnOH and $Ph_3SnOCOCH_3$ in acetonitrile (1mg/1mL). Working standard solutions were prepared from stock solutions. The stock and working standard solutions were covered with aluminum foil and stored at -18°C. The spiked samples of desired concentrations of agricultural waste, municipal waste etc were prepared from the stock solutions. The spiked samples were stirred at room temperature overnight on a magnetic stirrer (800-900 rpm/min) to obtain a homogeneous clear solution. The contents obtained were sonicated and injected into rheodyne loop for chromatographic analysis (Figure 6.1).

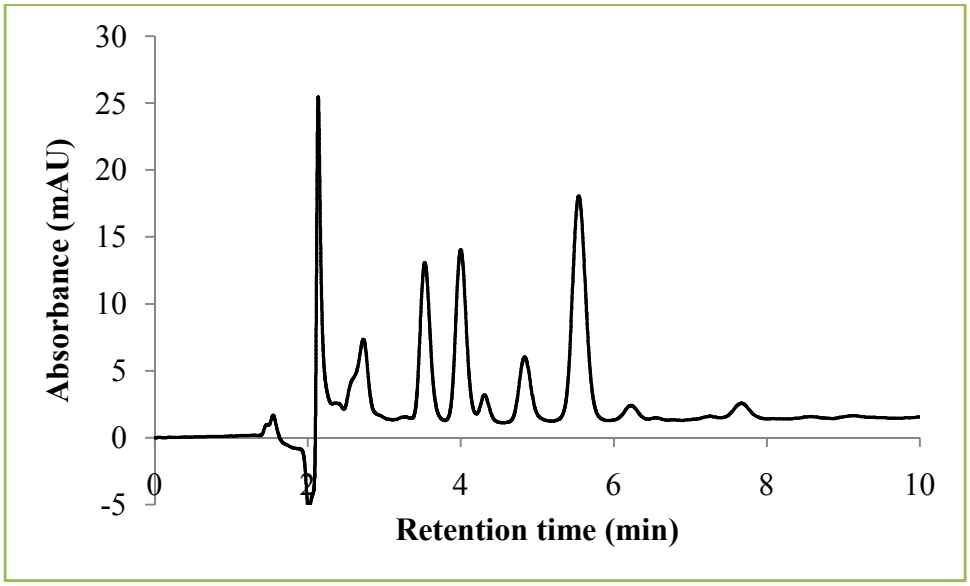


Figure 6.1: Chromatogram of spiked aqueous samples at 0.1µg/mL (a) triphenyltin acetate; (b) triphenyltin hydroxide; (c) tetraphenyltin; (d) triphenyltin chloride. Column: Waters Nova pack; mobile phase: 90:10 (v/v) acetonitrile: water; flow rate 1 mL/min.

6.3.1 Sample Preparation

6.3.1.2 Preparation of agricultural waste water and municipal water samples

Agricultural waste water was collected from the agriculture field of village Sadhubela, Patiala, Punjab, India. Municipal water was collected from the area near to TN Plastic industries, Panipat, Haryana, India. Water samples were collected and transported to the lab in Pyrex borosilicate amber glass bottles. Samples were immediately stored in the freezer to avoid any microbial decomposition. Since both kind of water samples contain a lot of impurities and suspended material. Prior to analysis a fraction of stored aqueous samples were sonicated for about 10 minutes to expel dissolved gases and then filtered using 0.22 µm nylon filters to remove the impurities. It was analyzed with the optimized FPSE-HPLC-UV conditions. Fractions of each filtered water sample were spiked with selected target analytes of phenyltin derivatives to make samples bearing 100 ng/mL respectively. No traces of tin were detected in the samples. The

agricultural and municipal waste water were spiked with phenyltin components (100 ppb) and were analyzed with FPSE-HPLC-UV at optimized conditions.

6.3.2 Canned food sample

Canned food sample containing 0.25 g was accurately weighed on a Teflon digestion vessel on a micro analytical balance and then treated with a mixture of 6 mL nitric acid and 1mL hydrogen peroxide in the microwave digestion system [38]. The extracts thus obtained were re-dissolved in 25 mL of triply distilled water, filtered using 0.22 μm nylon filters and subsequently analyzed by HPLC-UV.

6.3.3 Urine sample

Urine samples were acquired from the person who has consumed tin canned food. The supernatant sample collected after the process of centrifugation was stored at - 4°C in glass tube and kept in a refrigerator until the time of sample pretreatment. The urine was diluted to about ten times with triply distilled water, filtered with Nylon-6, 6 membrane filters, and was then degassed on ultrasonic bath for half an hour. Spiked Sample was prepared and pre-concentrated with FPSE media before the analysis. The FPSE eluted sample was injected into rheodyne loop and analyzed chromatographically.

6.4 Results and Discussion

Malik and coworkers [39] has developed a new era of microextraction sol-gel technology for synthesizing different variety of sorbents. Preparation of fabric phase sorptive extraction media coated with any sol-gel sorbent involves a number of steps including (a) pretreatment of sorbent for sol-gel coating (b) preparation of sol solution (c) sol-gel coating on the target substrate.

6.4.1 Pretreatment of sorbent for sol-gel coating

Foremost the cellulose fabric was soaked in deionized water for 15 minutes under constant sonication. Then the fabric was cleaned with ample amount of triply distilled water followed by 1M sodium hydroxide solution for 1 hour. The base treated fabric was again need to wash with profuse amount of distilled water followed by treating with 0.1 M hydrochloric acid solution for 1 hour. Finally the treated fabric was washed with deionized water and dried overnight in an inert atmosphere. The dried fabric was stored in a clean airtight glass container for the future use as a coated sorbent.

6.4.2 Preparation of sorbent for sol-gel coating

Taking into the consideration of non polar hydrophobic nature of selected phenyltin derivatives, it is quite apparent that a non polar sorbent would provide the best affinity towards the target selected analytes to selectively isolate them from complex environmental water sample matrix. The substrate used in fabric phase sorptive extraction plays dynamic role in determining the overall polarity and selectivity of FPSE media. Support selected for FPSE media was 100% cellulose containing various finishing chemicals, starch softeners etc. To remove the hindrance of such chemicals fabric requires surface treatment that would remove the chemicals and regenerate surface hydroxyl functional groups to proficiently anchor the sol–gel polymeric network. The sol solution required for creating the sol-gel coated C18 media was prepared by using the modified form of previously described formulation [35, 40]. The sol solution was prepared by mixing sol-gel precursor octadecyltrimethoxysilane, sol-gel catalyst trifluoroacetic acid (TFA), dichloromethane, acetone and water. All the ingredients were mixed well in order to get homogeneous sol solution. The molar ratio between methyl trimethoxysilane and octadecyltrimethoxysilane were maintained at 1: 0.38. The mixture was then vortexed for 5

minutes and centrifuged or sonicated. The cellulose fabric substrate was then gently immersed into the homogeneous sol solution for about 2 hours.

6.4.3 Characterization of sol-gel C18 coated FPSE media

Mechanism of extraction of an analyte by fabric phase sorptive extraction media largely depends on the porous nature of sol gel sorbent. As can be seen from the SEM images, each cellulose fiber is constituted of various macro pores which make an easy access of sol solution into the cellulose fabric matrix formed uniformly on the surface of microfibrils of cellulose. Thus it accomplishes the faster extraction equilibrium in a very short period of time. Figure 6.2 represents the scanning electron micrographs (SEM) of (a) uncoated cellulose substrate; (b) sol-gel C18 coated fabric phase sorptive extraction media

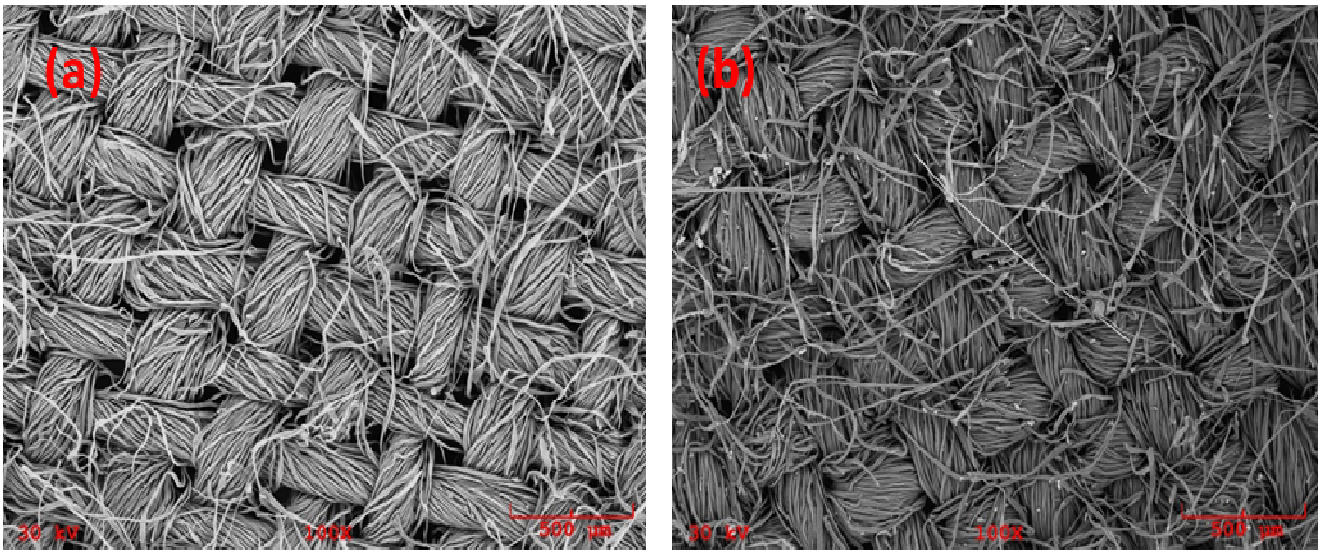
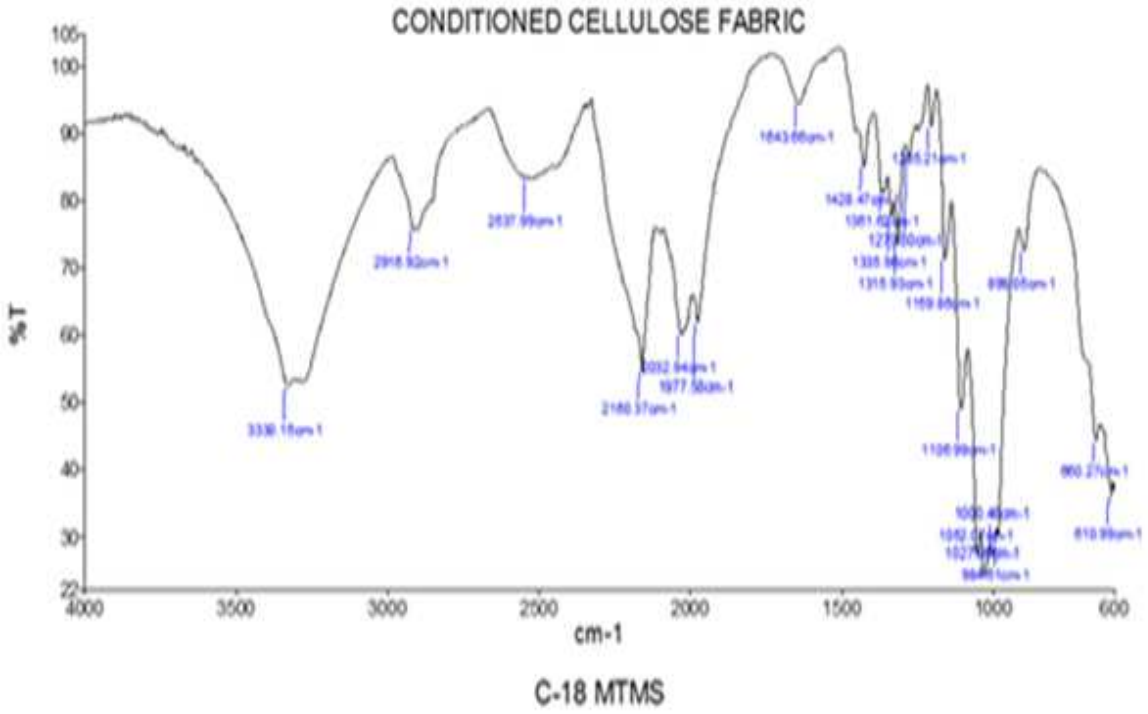


Figure 6.2: illustrates FT-IR spectra representing (a) uncoated cellulose fabric; (b) octadecyl trimethoxysilane; (c) sol-gel C18 coated fabric phase sorptive extraction media.

Figure 6.3 interprets two FT-IR spectra of uncoated cellulose substrate (top) and sol-gel C18 coated FPSE media (bottom). The FT-IR spectra of uncoated cellulose fabric have characteristic absorption between 2894 cm^{-1} and 1308 cm^{-1}; 3000 cm^{-1} and 3300 cm^{-1} ; and at 1024 cm^{-1} correspond to C–H, O–H and C–O vibration or C–H bending vibration, respectively.

The distinctive peaks of sol-gel C18 coated FPSE media (bottom) appeared at 2890 cm^{-1} and 2851 cm^{-1} which represent symmetric vibration of $–CH_2$ and asymmetric vibration of $–CH_3$, respectively. These peaks are also seen in uncoated cellulose fabric media. In addition, the presence of approximately 1467 cm^{-1}, 1268 cm^{-1} and 1977 cm^{-1} in the sol-gel C18 FPSE media strongly suggests the successful integration of octadecyl moieties into the sol-gel network. The considerable reduction of the O-H stretching vibrations (at 3325 cm^{-1}) in sol-gel C18 coated FPSE media compared to uncoated cellulose indicates the chemical assimilation of the sol-gel C18 network to the cellulose structure via the process of condensation. FPSE offers remarkably superior thermal and stable due to the chemical integration of sol-gel sorbent to the substrate surface. This technique has innovatively incorporated both the solid phase microextraction (SPME) and solid phase extraction (SPE) into a single technology podium.



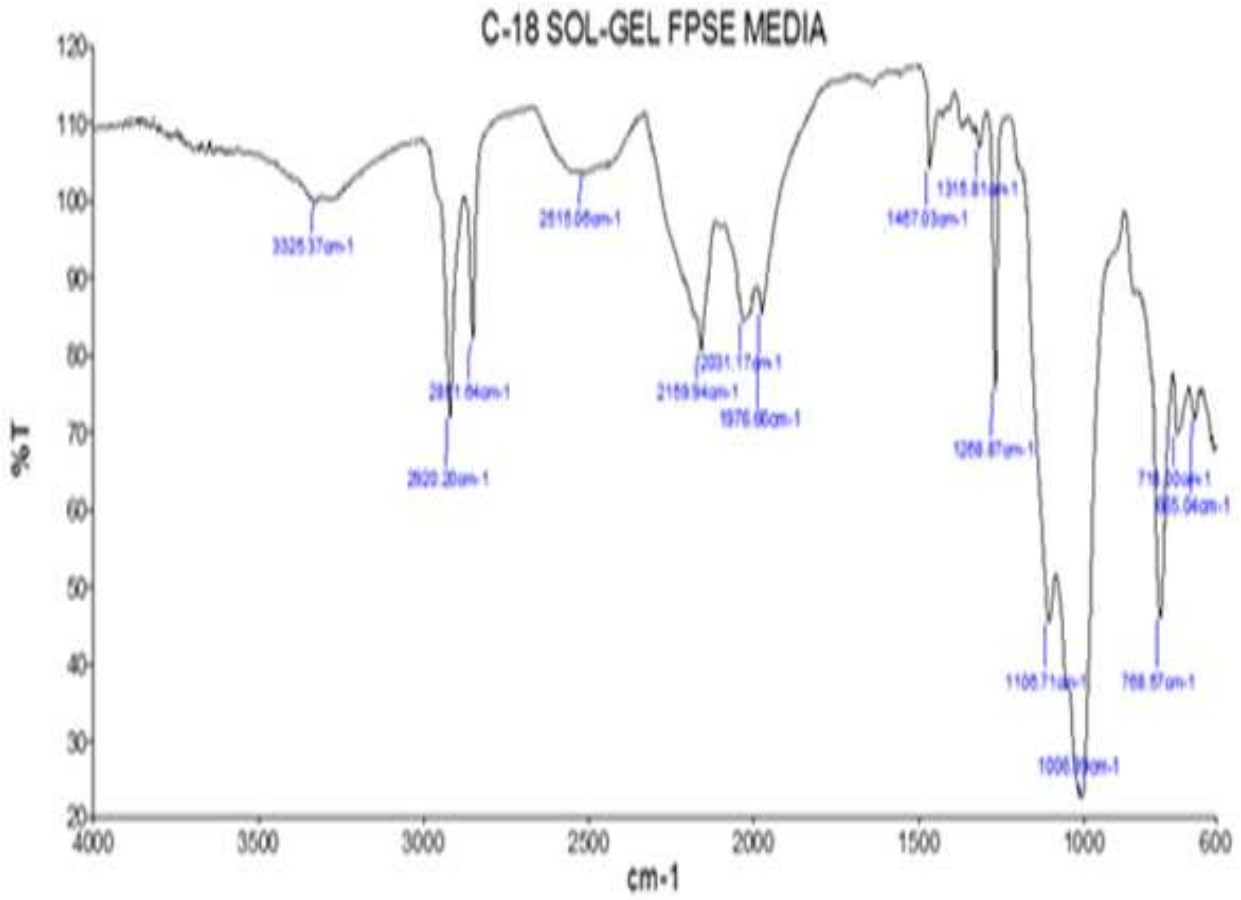


Figure 6.3: FT-IR spectra of (top) uncoated cellulose substrate; (bottom) sol-gel C18 coated FPSE medium.

6.4.1 FPSE Method

The method using fabric phase sorption extraction was used with silica C_{18} as a sorbent material. To optimize FPSE procedure, factors affecting the recovery such as the extraction time, back extraction time, effect of ionic strength and the composition and volume of the elution solution and carryover effects were studied.

6.4.1. Optimization of eluting solvent

Effect of different eluting solvents on elution recoveries of organotins was studied. Once the target analytes were extracted onto the FPSE media, a quantitative desorption into a suitable

organic solvent/solvent-system was required. Methanol, ethanol, acetonitrile, toluene and hexane (ranging from high polarity to low polarity) were tried for quantitative desorption of target analytes from sol-gel coated FPSE media (figure 6.4). Ethanol and methanol (100%) were not found to be suitable for the extraction. Toluene has a definitive advantage for the extraction of phenyltin compounds with well resolved and fine peaks. Therefore, toluene was selected as the back extracting solvent for further experimentation. Recoveries with toluene were further increased when the aqueous phase was treated with salts like sodium chloride.

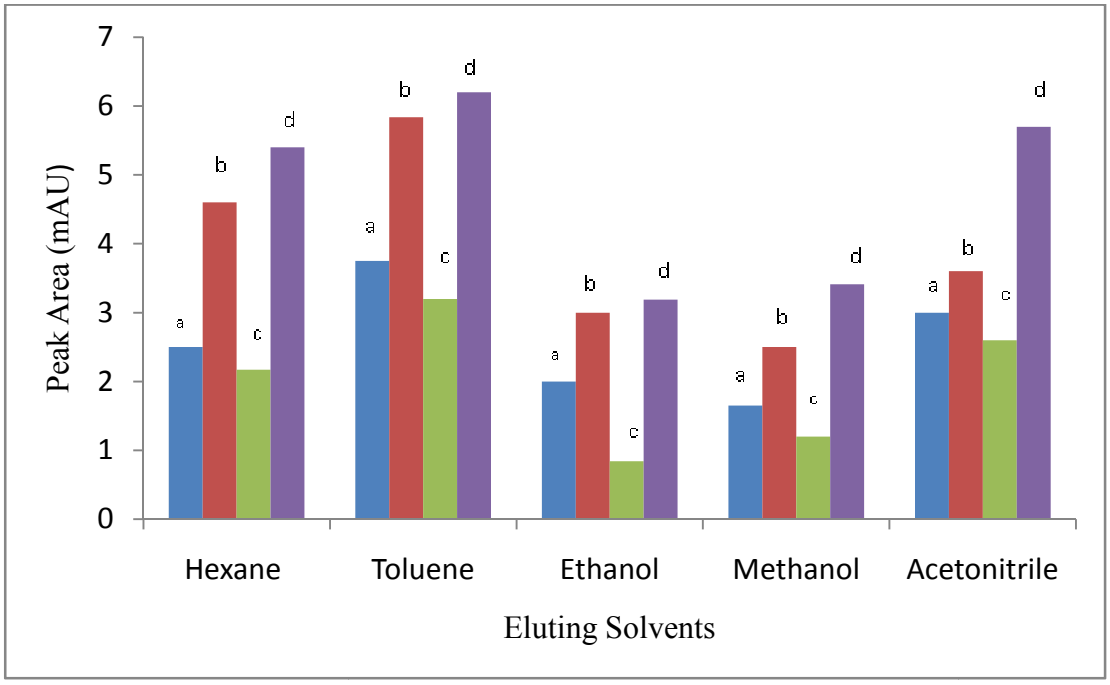


Figure 6.4: Effect of eluting solvent on extraction recoveries of phenyltins

6.4.2. Optimization of extraction time

Extraction time is duration required to reach the state of extraction equilibrium between the extraction media and the matrix. The extraction time was studied by exposing the samples to PSE media from time 5 to 30 min. Analytes recovery increases with increase in extraction time

upto 20 min and it becomes constant later on (figure 6.5). So the extraction time of 20 min was selected for the subsequent analysis.

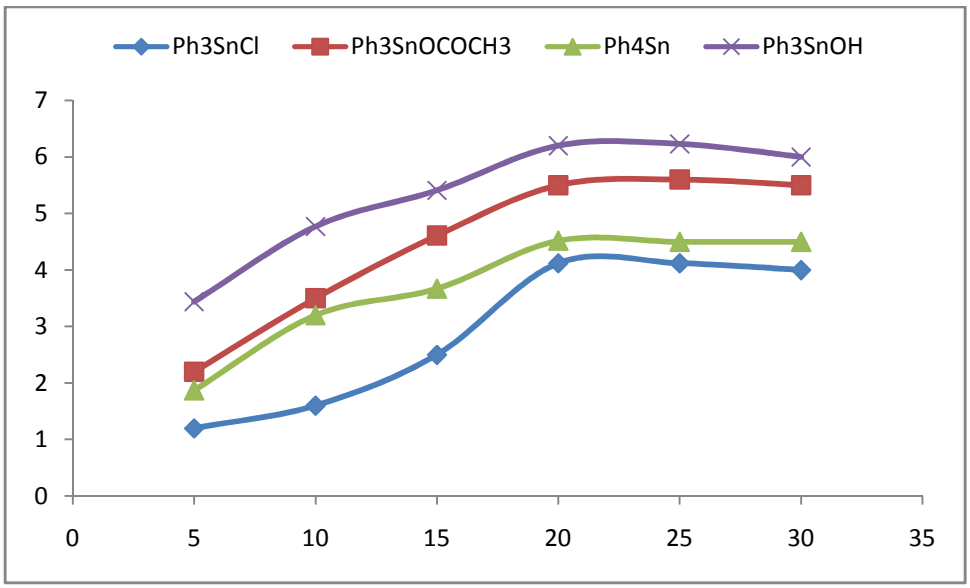


Figure 6.5: Effect of extraction time on recoveries of phenyltins

6.4.3. Optimization of back extraction time

The influence of back extraction time on analytes recovery was tested by varying time from 5 to 25 min. Maximum recovery was obtained at 15 min, while slightly reduced recoveries were obtained at 20 and 25 min, which probably may be due to re-adsorption process on fabric (fig. 6.6). So, back extraction time of 15 min was optimized for further studies.

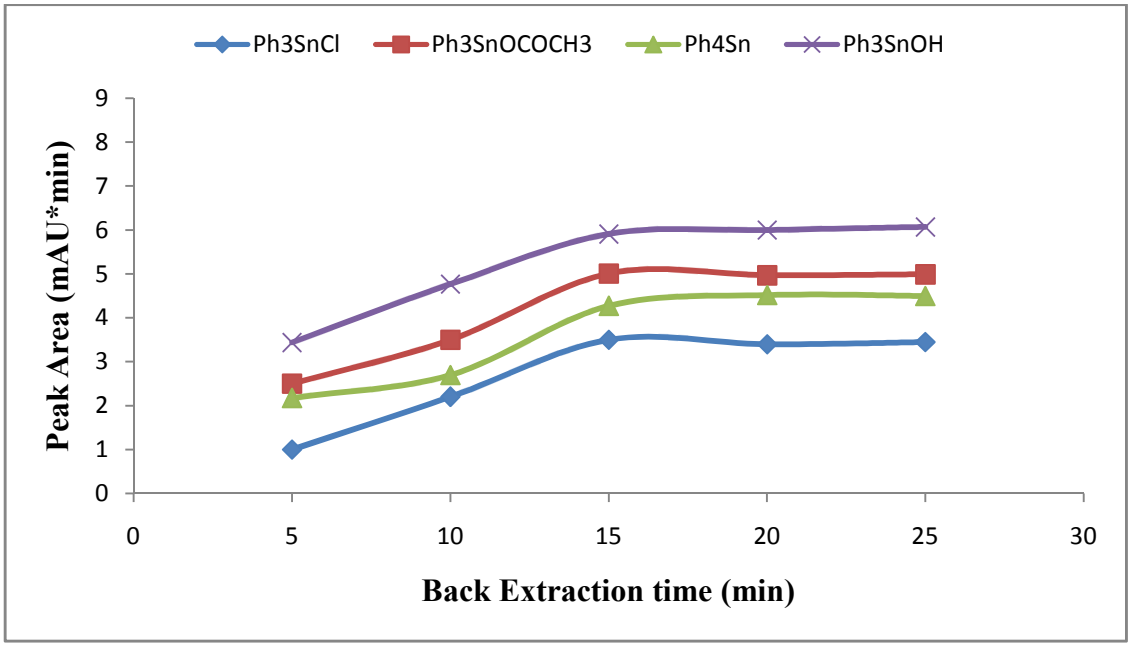


Figure 6.6: Effect of back extraction time on recoveries of phenyltins

6.4.4. Effect of pH

The effect of pH of the sample matrix was examined on the extraction of phenyltin derivatives for a pH range 3 to 9. It was observed that the maximum extraction sensitivity was at pH 7.0.

The pH of 7.0 was observed for the aqueous solution after the addition of analytes to the water sample without adding any buffer solution. The extraction was decreased both in acidic and alkaline condition shown in figure 6.7.

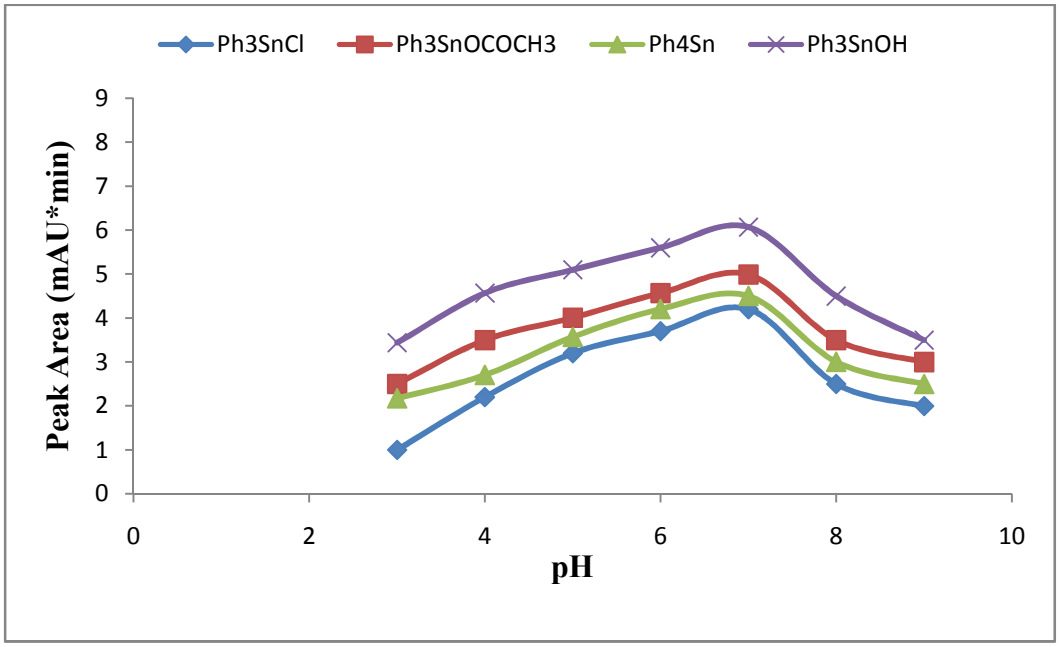


Figure 6.7: Effect of pH

6.4.5. Carryover effect

To avoid the carryover effect the fabric was washed with eluting solvent 2-3 times and then with water before subsequent experiment. The carryover was checked by injecting elution solvent to the chromatographic instrument. To eliminate the memory effect, 2 mL x 3 of toluene was incorporated for washing followed by 2 mL acetonitrile to clean the fabric, before analyzing the next sample. However, due to their easy availability and economic nature, FPSE media can be used as a single use microextraction device to lessen the solvent consumption in washing.

6.4.6. Effect of salt addition

Addition of salt is a common practice to increase the solubility of target analytes to the sorption media. The effect of addition of sodium chloride on adsorption characteristics of phenyltin compounds was studied. Salt addition might effects the FPSE by competitive interaction between the salting in and salting out effects. The salting in effect increases the solubility of target analytes in aqueous media where as salting out has opposite effect. Different concentrations of

sodium chloride (1-10%) were added to check the response. Probably due to non polar nature of sorptive media there was no appreciable change in the adsorption characteristics and extraction efficiency. This indicated that the sol gel C18 fabric phase sorptive extraction media is stable in various conditions and extraction efficiency is independent of salinity.

6.5 Method Performance

6.5.1 Preparation of Calibration graph

The optimized FPSE-HPLC-UV conditions (Table 6.1) were used to prepare calibration curves for the spiked samples (10-100 ng/mL). The calibration curves of phenyltin compounds were obtained by performing linear regression analysis on spiked agricultural, municipal and canned food samples ranging from 10-100 ng/mL and were found linear over this range. The calibration curves were described by the equation; $y = mx + c$, where y is peak area ratio, x is the concentration, m is the slope, and c is the intercept. The developed method was validated to deliver optimal analytical performance for the overall target analyte suite. Reproducibility was studied by evaluating the relative standard deviations for three replicate injections for each concentration of target analytes. The repeatability of the method was evaluated intra and interday using a triplicate analysis of each sample. Detection limits were calculated from a signal that was three times the noise. The LOD was defined as the lowest concentration that gave a signal to noise ratio that was equal to 3. The LOQ was defined as the lowest concentration that gave a signal to noise ratio that was equal to 10.

Table 6.1: Optimized FPSE-HPLC-UV conditions for phenyltin derivatives

FPSE	**Fabric sol-gel coating**	**C18 fiber**
	Eluting solvent	Toluene
	Extraction time	20 min
	Back extraction time	15 min
	pH	7.0
	Salting effect	Nil
HPLC	Pump	Dionex P680
	Column	Waters spherisorb C18
	Mobile Phase	90:10 (acetonitrile:water)
	Injection volume	20µL
	Flow Rate	1.00 mL/min
	Wavelength	254 nm

6.5.2 Linearity, Accuracy and Precision

The method was validated to deliver the optimal analytical performance for target analytes. The linearity of the method for the phenyltin derivatives was determined using the standard calibration curve and other analytical parameters (**Table 6.2).** The accuracy and precision of FPSE-HPLC-UV method were evaluated for each analyte by analyzing a standard of known concentration and quantifying it using the calibration curve. The precision expressed as relative standard deviation, RSD was evaluated under intraday and interday conditions at 10 ng/mL

concentration. As it can be seen in Table 6.2, the repeatability (n = 3) ranged from 2% to 3%. The reproducibility ranged from 2.7 to 4.2%. The accuracy of phenyltin compounds attributed to the degradation during the analysis of standard prepared compounds after 3-4 days. So regarding the stability of compounds it is recommended to do sample preparation and follow the procedure of analysis on the same day. The developed method is selective because of non interference of foreign substances in the concerned region. Once optimized was done, the proposed analytical method was applied for the determination of the target phenyltin derivatives in environmental water samples. A recovery study was performed in order to assess the applicability of the proposed method to determine phenyltin derivatives in agricultural and municipal waste water. Since the target analytes were not detected, the samples were spiked with target analytes at concentration 10 ng/mL.

Table 6.2: Analytical characteristics of developed FPSE-HPC-UV method for determination of Organotin compounds (10-100 nm).

Parameter	**$Ph_3SnOCOCH_3$**	**Ph_3SnOH**	**Ph_4Sn**	**Ph_3SnCl**
Regression Equation	$0.126x + 0.398$	$0.153x + 0.369$	$0.088x + 0.92$	$0.11x + 0.335$
Coefficient of determination	0.987	0.991	0.989	0.987
Retention time (min)	3.5	4.1	5.1	5.9
Intraday RSD (%)	2.5-3.0	2.0-2.5	2.2-2.6	2.0-2.4
Interday RSD (%)	3.6-4.2	2.7-3.2	3.0-3.5	3.3-3.9
Limit of detection	0.29	0.26	0.34	0.30

(LOD) (ng/mL)				
Limit of Quantification (LOQ) (ng/mL)	0.96	0.86	0.11	0.10
Retention factor	0.66	0.95	1.42	1.80
Selectivity	1.42	1.5	1.26	2.71

6.6 Applications

Research and development over the years has provided reliable and selective analytical techniques that can be used for water research analysis, monitoring and health risks assessment including sampling, testing and validation, although some challenges still survive in regard to the accessibility of efficient and economic sampling techniques. To assess the wider applicapability of developed method, it was applied to agricultural waste, municipal waste water. The performances of the new analytical method were tested in real urine samples collected from healthy volunteers after single oral administration of canned food. Samples were extracted by FPSE and then analyzed using HPLC-UV according to the method here in reported. The sample of this target canned food was protein powder and amount of tin content found was 0.152 mg/kg. However this content is very low but may also present in higher amount in some kind of acidic canned foods. Although the maximum permissible limit of tin 200 ppm was not exceeded. But when these cans remain open for certain hours then the dissolution of the tin from the can surface occurs and tin concentration more than 200 ppm were observed. So it is recommendable that after opening the can the contents should immediately be transferred to some other glass vessels.

The recoveries from real samples were determined by comparing the recovered amounts of phenyltin with original spiked concentrations. The relative recoveries obtained from the spiked waste water sample (Fig. 6.8 a-d) were around 90 to 102% The recoveries range more than 90% obtained were quite satisfactory for a broad concentration range (**Table 6.3).**

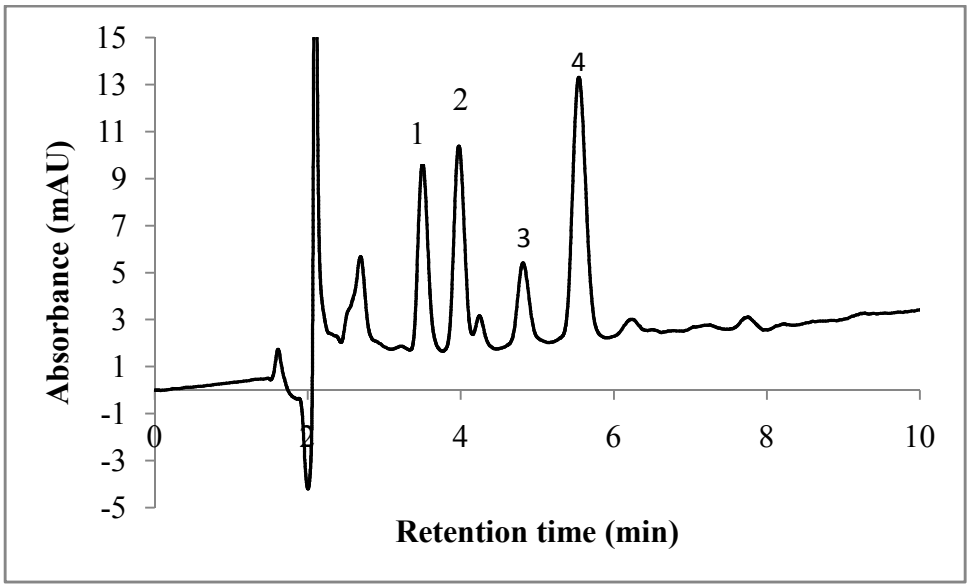


Figure 6.8 a: FPDE-HPLC-UV Chromatogram in agricultural waste water sample of spiked 1-triphenyltin acetate; 2- triphenyltin hydroxide; 3-tetraphenyltin; 4- triphenyltin chloride

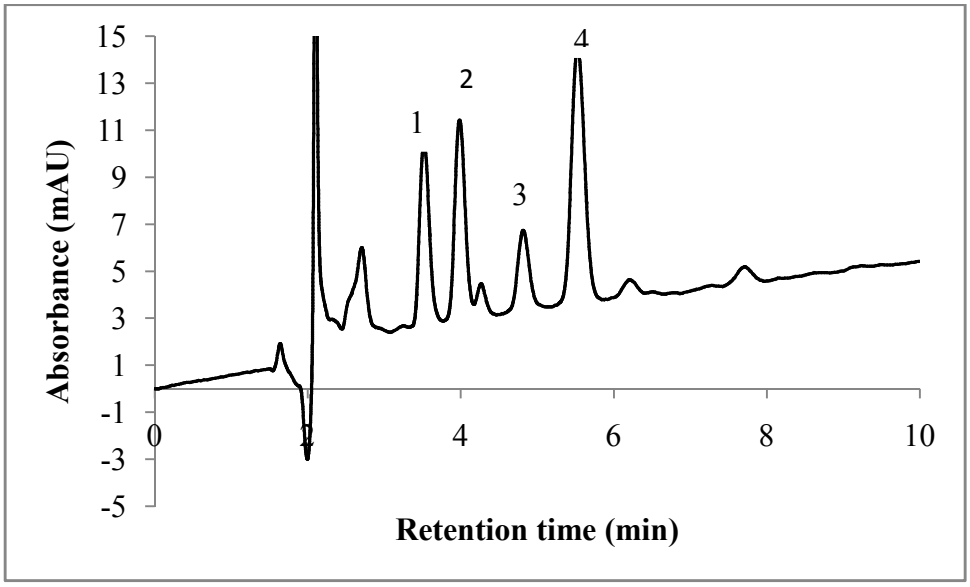


Figure 6.8 b: FPSE-HPLC-UV Chromatogram in municipal waste water sample of spiked 1-triphenyltin acetate; 2- triphenyltin hydroxide; 3-tetraphenyltin; 4- triphenyltin chloride

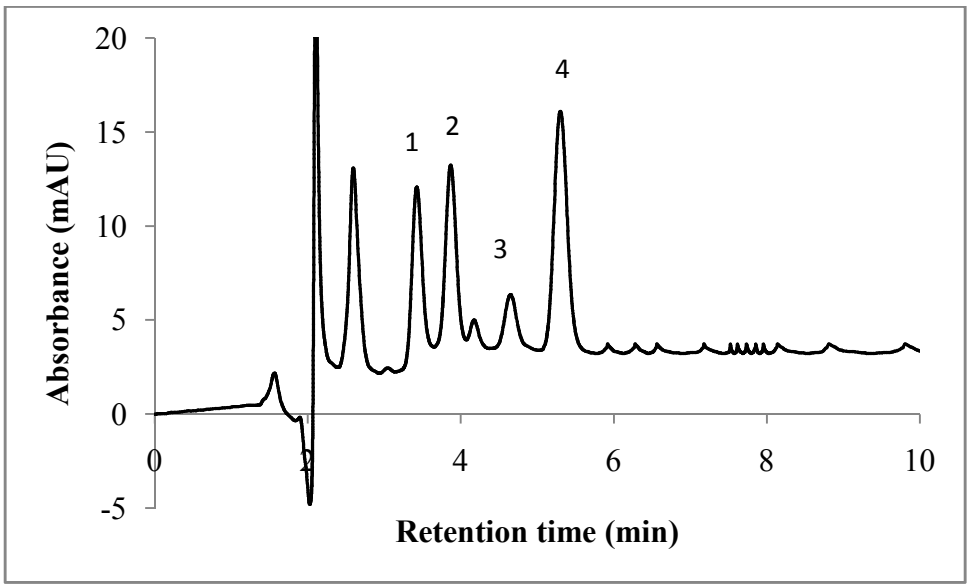


Figure 6.8 c: FPDE-HPLC-UV Chromatogram in canned food sample of spiked 1-triphenyltin acetate; 2- triphenyltin hydroxide; 3-tetraphenyltin; 4- triphenyltin chloride

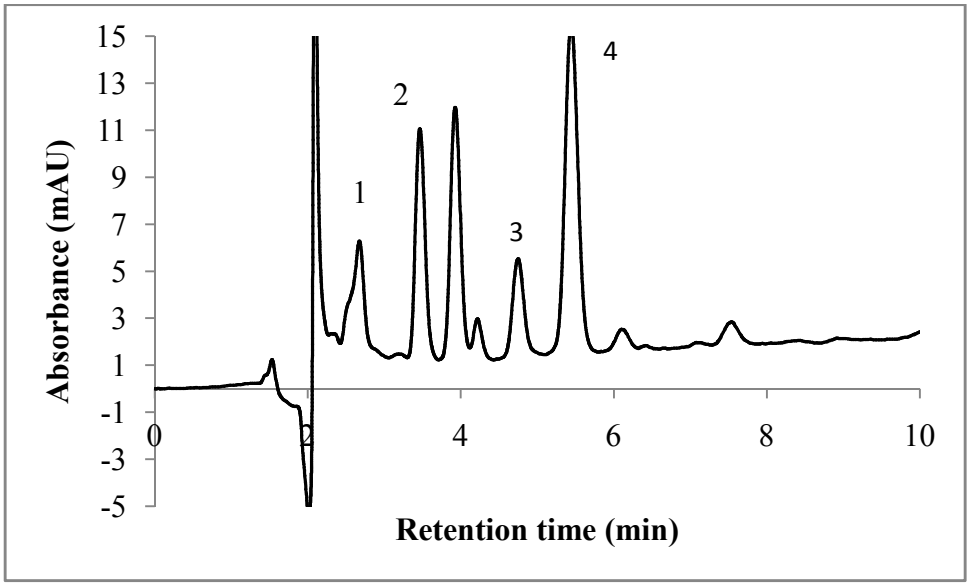


Figure 6.8 d: FPDE-HPLC-UV Chromatogram in urine sample of spiked 1-triphenyltin acetate; 2- triphenyltin hydroxide; 3-tetraphenyltin; 4- triphenyltin chloride

Table 6.3: Recoveries of phenyltins in aqueous environmental and canned food sample

Analyte	Added	Agricultural waste Water		Municipal waste Water		Canned food		Urine sample	
		Found (RSD)	Recovery (%)	Found (RSD)	Recovery (%)	Found (RSD)	Recovery (%)	Found (RSD)	Recovery (%)
Triphenyl tin acetate	0.1	0.092 (3.5)	92.00	0.094 (3.7)	94.00	0.102 (3.6)	102.00	0.092 (3.9)	92.00
	0.5	0.467 (3.2)	93.40	0.488 (3.5)	97.60	0.501 (3.4)	100.20	0.489 (3.7)	97.80
	10	9.274 (3.0)	92.74	9.755 (3.4)	97.59	9.967 (3.2)	99.67	9.897 (3.6)	98.97
Triphenyl tin hydroxide	0.1	0.088 (2.9)	88.00	0.097 (2.8)	97.00	0.099 (3.2)	99.00	0.097 (3.0)	97.00
	0.5	0.475 (2.7)	95.00	0.482 (2.6)	96.40	0.499 (3.0)	99.80	0.477 (2.8)	95.40
	10	9.336 (2.5)	93.36	9.864 (2.4)	98.60	9.987 (2.9)	99.87	0.980 (2.7)	98.00
Tetra phenyl tin	0.1	0.092 (3.6)	92.00	0.095 (3.7)	95.00	0.099 (3.5)	99.00	0.097 (3.9)	97.00
	0.5	0.452 (3.4)	90.40	0.491 (3.5)	98.20	0.496 (3.3)	99.20	0.495 (3.7)	99.00
	10	9.696 (3.2)	96.69	0.978 (3.3)	97.8	9.899 (3.1)	98.99	0.987 (3.5)	98.70
Triphenyl tin chloride	0.1	0.091 (3.4)	91.00	0.095 (3.1)	95.00	0.103 (3.6)	103.00	0.096 (3.2)	96.00
	0.5	0.483 (3.2)	96.60	0.491 (3.1)	98.20	0.503 (3.2)	100.60	0.493 (3.1)	98.60
	10	9.675 (3.1)	96.75	9.765 (2.8)	97.65	10.12 (3.0)	101.20	9.784 (2.9)	97.84

6.8 Conclusion

Fabric phase sorptive extraction is a promising solvent-minimized sample preparation technique for the high-efficiency extraction of different analytes from various matrices. By choosing the suitable fabric phase sorptive extraction sorbent chemistry and optimizing sorption and elution conditions, it can be used in solving many difficult and unsolved analytical problems. All the parameters related to FPSE, such as extraction and desorption times, eluting solvent, effect of salt addition, pH of the sample have been optimized in order to get the better recoveries for all compounds. The inherent porosity of sol-gel sorbent and characteristic permeability of flexible cellulose fabric substrate results in rapid extraction of phenyltin derivatives and accomplishes extraction equilibrium in a short period of time. Since it is a rapid, solvent-minimized, user-friendly and direct method that simplifies the overall sample preparation protocol, it can be used in routine analysis with high sample throughput at low cost. The flourished FPSE-HPLC-UV method shows a good sensitivity and selectivity and it offers low detection limits that ranged from 2.4-3.6 ng/mL and lower relative standard deviation which are appropriate in the analysis of phenyltin derivatives.

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- **Heena**, Rajesh Kumar, Susheela Rani and Ashok Kumar Malik*, Development of a Rapid and Sensitive Method for the Determination of Aluminum by Reverse-Phase High-Performance Liquid Chromatography Using a Fluorescence Detector.

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- **Heena**, Rajesh Kumar and Ashok Kumar Malik Novel Fabric Phase Sorptive Extraction Tehnique. Analytical Proceedings published in chemistry for a better tomorrow: current trends and challenges 2014, 13-22.
- **Heena**, Gaurav, Susheela Rani and Ashok Kumar Malik Fabric phase sorptive extraction using a cellulose fabric modified with sol-gel polytetrahydrofuran nanocomposite and their quantification via HPLC with UV detection. Journal of chromatography and separation techniques, 2016, 7(3), 1-6.
- **Heena,** Ramandeep Kaur, Susheela Rani, Ashok Kumar Malik, Abuzar Kabir and Kenneth G. Furton Determination of Co(II), Ni(II) and Pd(II) ions via Fabric phase sorptive extraction using a sol-gel polytetrahydrofuran nanocomposite and their analysis via HPLC with UV detection, Separation science and technology, 2016,
- **Heena,** Irshad Mohiuddin and Ashok Kumar Malik Importance and various challenges of trace metal speciation in environment system. International journal of engineering, technology management and applied science. International journal of engineering, technology, management and applied sciences. 2016, 4(6), 58-62.
- **Heena,** Ashok Kumar Malik Review on recent applications of metals on high performance liquid chromatography and a short accont on metal speciation Critical reviews in analytical chemistry (Accepted)

- **Heena,** Ramandeep Kaur and Ashok Kumar Malik Development of new methods for the determination of aluminium quercetin complex Journal of chromatographic science (Accepted)