การสกัดปรอทจากน้ำทะเลด้วยเฟสของแข็งที่ใช้ผงซัลเฟอร์ดัดแปรทางเคมีด้วยเอ็น-(2-คลอโรเบนโซอิล)-เอ็น’-ฟีนิลไทโอคาร์บามีดและการวิเคราะห์ด้วยอะตอมมิกฟลูออเรสเซนซ์สเปกโทรเมตรี

นางสาวธณิฎา เกตุทองสง

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาวิทยาศาสตร์ทางทะเล ภาควิชาวิทยาศาสตร์ทางทะเล
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2558

SOLID PHASE EXTRACTION OF MERCURY FROM SEAWATER USING SULFUR POWDER CHEMICALLY MODIFIED WITH N-(2-CHLOROBENZOYL)-N’-PHENYL THIOCARBAMIDE AND ANALYSIS BY ATOMIC FLUORESCENCE SPECTROMETRY

Miss Tanita Gettongsong

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Marine Science
Department of Marine Science
Faculty of Science
Chulalongkorn University
Academic Year 2015

Thesis Title	SOLID PHASE EXTRACTION OF MERCURY FROM SEAWATER USING SULFUR POWDER CHEMICALLY MODIFIED WITH N-(2-CHLOROBENZOYL)-N’-PHENYLTHIOCARBAMIDE AND ANALYSIS BY ATOMIC FLUORESCENCE SPECTROMETRY
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ธณิฎา เกตุทองสง : การสกัดปรอทจากน้ำทะเลด้วยเฟสของแข็งที่ใช้ผงซัลเฟอร์ดัดแปรทางเคมีด้วยเอ็น-(2-คลอโรเบนโซอิล)-เอ็น’-ฟีนิลไทโอคาร์บามีดและการวิเคราะห์ด้วยอะตอมมิคฟลูออเรสเซนซ์สเปกโทรเมตรี (SOLID PHASE EXTRACTION OF MERCURY FROM SEAWATER USING SULFUR POWDER CHEMICALLY MODIFIED WITH N-(2-CHLOROBENZOYL)-N’-PHENYLTHIOCARBAMIDE AND ANALYSIS BY ATOMIC FLUORESCENCE SPECTROMETRY) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ศิริชัย ธรรมวานิช, 71 หน้า.

งานวิจัยในครั้งนี้ได้ทำการสังเคราะห์ผงซัลเฟอร์ที่ถูกดัดแปรทางเคมีด้วยเอ็น-(2-คลอโรเบนโซอิล)-เอ็น’-ฟีนิลไทโอคาร์บามีดเพื่อใช้ในการสกัดปรอทจากน้ำทะเล ข้อดีของวิธีการดังกล่าว คือสามารถกำจัดผลของตัวรบกวนจากตัวอย่างน้ำทะเลทำการศึกษาสภาวะที่เหมาะสมในการสกัดปรอท เช่น pH, ปริมาณที่เหมาะสมของลิแกนด์, ชนิดของตัวชะ, อัตราการไหล, ปริมาตรของตัวอย่าง และ ความสามารถในการดูดซับของคอลัมน์ ปริมาณปรอทที่สกัดได้ทำการวิเคราะห์ด้วย เทคนิคอะตอมิคฟลูออเรสเซนซ์สเปกโทรเมตรี พบว่ากราฟความเป็นเส้นตรงอยู่ในช่วง 0-20 นาโนกรัมต่อลิตร โดยมีค่าขีดจำกัดของวิธี คือ 3.18 นาโนกรัมต่อลิตร. ร้อยละความเบี่ยงเบนมาตรฐานเป็น 1.024 ได้ทำการตรวจสอบความถูกต้องของวิธีด้วย น้ำกลั่นและน้ำทะเลเทียม

ภาควิชา วิทยาศาสตร์ทางทะเล ลายมือชื่อนิสิต ....

สาขาวิชา วิทยาศาสตร์ทางทะเล ลายมือชื่อ อ.ที่ปรึกษาหลัก ....

ปีการศึกษา 2558

5672166323 : MAJOR MARINE SCIENCE
KEYWORDS: MERCURY / SEAWATER / SOLID PHASE EXTRACTION

TANITA GETTONGSONG: SOLID PHASE EXTRACTION OF MERCURY FROM SEAWATER USING SULFUR POWDER CHEMICALLY MODIFIED WITH N-(2-CHLOROBENZOYL)-N’-PHENYLTHIOCARBAMIDE AND ANALYSIS BY ATOMIC FLUORESCENCE SPECTROMETRY. ADVISOR: ASST. PROF. SIRICHAI DHARMVANIJ, 71 pp.

A simple method has been tested and validated for the preconcentration of mercury from seawater base on sulfur chemically modified with N-(2-chloro benzoyl)-N'-phenylthiocarbamide. This technique offers an advantage of avoiding matrix effect from the saline content in water sample during analysis. Various conditions such as pH solution, ligand quantity, eluent type, flow rate, sample volume and sorption capacity have been studied in detail. The calibration graph was linear in the range 0-20 ng L^{-1} of mercury with the detection limit of 3.18 ng L^{-1}. The relative standard deviation was found to be 1.024%. The procedure was validated by analysis of certified reference material, Double deionized water and synthetic seawater.

Department:	Marine Science	Student's Signature	....
Field of Study:	Marine Science	Advisor's Signature	....
Academic Year:	2015		

ACKNOWLEDGEMENTS

I would like to express my deep and sincere gratitude to my advisor, Assistant Professor Dr. Sirichai Dharmvanij. His wide knowledge and his logic way of thinking have been a great value for me. His understanding, encouraging and personal guidance have provided a great basis for the research. Also, I am grateful to Assistant Professor Dr.Penjai somgpongchaiyakul, Assistant Professor Dr.Natdhera Sanmanee, and Associate Professor Dr. Thaithaworn lirdwitayaprasit. For their suggestion as my thesis committee and examiners.

Especially, I am greatly thankful to the Department of Marine Science, Faculty of Science, Chulalongkorn University for providing facilities, equipment, instrument and financial support throughout the course of my study. And also, I would like to thank all the staffs in the Department of Marine Science for their helpfulness and kindness.

Furthermore, I would like to express my deepest gratitude thank to my parents for their understanding and encouragement.

Finally, I would like to thank those whose names are not listed here and those who have one way or another contributed to the success of this work.

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CHAPTER I

INTRODUCTION

1.1 Statement of purpose

Mercury is an element that has been used in various applications for industries. The main sources of mercury in marine environment come from both human activities and natural source. In natural water there are three main forms of mercury, namely, element mercury (Hg^0), inorganic mercury (II) (Hg^{2+} and its complexes) and organic mercury (MMeHg and its complexes, DmeHg) (Leopold et al., 2010). Mercury can be converted into more toxic forms of DMeHg in natural water by biogeochemical transformation processes (Mason et al., 2012).

Generally, average concentration of mercury in open seawater is in the range of 10–54 ng L^{-1} as cited in (Thongra-ara and Parkpian, 2002). There are several analytical techniques for determination of mercury in seawater such as inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Chai et al., 2010), inductively coupled plasma mass spectroscopy (ICP-MS) (Gaulier et al., 2015), cold vapor atomic absorption spectrometry (CV-AAS) and cold vapor atomic fluorescence spectrometry (CV-AFS) (Mao et al., 2008), etc., but all these techniques do have some inherent limitation. However, atomic fluorescence spectrometry (AFS) have most been suggested as the most promising technique for determination of mercury in water sample.

However, a suitable analytical technique is still necessary to develop it. Because of in natural water mercury is at extremely low concentration with matrix interference. The preconcentration technique such as liquid-liquid extraction (Li et al., 2007) ion-exchange (Chen et al., 2013) and solid phase extraction (Hashempur et al., 2008, Pourreza and Ghanemi, 2009, Pourreza et al., 2009, Panichev et al., 2014) are generally used before determination of mercury as a mean to increase sensitivity and reduce matrix interference.

Solid phase extraction, which is the technique that use an absorbent to separate an analyte from the sample, have become popular technique for preconcentration of trace element because of its advantages in removing interference from matrix, easy to operate and inexpensive. Thus, to improve sensitivity of solid phase extraction this can be done by developing a suitable absorbent.

Since, mercury is classified as a soft acid and has good affinity toward sulfur-functionalized with ligand. Such as xanthate (Cui et al., 2015), thiol group (Zhang, Wang et al. 2009, Hadavifar, Bahramifar et al. 2014, Wang, Chen et al. 2014, Pavase et al., 2015), pyridine based on thiol (Sonmez and Bicak, 2002) and dithiozone (Shah and Devi, 1996) which lead to the choice of N-(2-chloro benzoyl)-N’-phenyl thiocarbamide chemically with sulfur powder use as an absorbent for mercury preconcentration from natural water.

In this research, a simple and inexpensive method for the solid phase extraction of mercury from seawater based on the adsorption of itself on sulfur powder chemically modified with N-(2-chloro benzoyl)-N'-phenyl thiocarbamide was tested for its optimum conditions. Various conditions such as pH solution, ligand quantity, elute type, flow rate, sample volume and sorption capacity have been studied in detail. Consequently, the procedure was validated by analysis of double deionized water and synthetic seawater.

1.2 Purpose of this research

1) To optimized parameters for the mercury extraction from seawater by using developed sulfur absorbent.

2) To study relationship between saline water and effective of column.

1.3 Scopes of this research

In this research was focus on the optimum and validate method for preconcentration and determination of mercury from saline water. Modified sulfur absorbent was prepared. Then, the optimized parameters such as pH, ligand quantity, amount of an absorbent, the elute type, flow rate of absorption and elution, sample volume and salinity content were investigated. In addition, the optimized parameters were validated and applied for real saline water sample.

1.4 The benefit of this research

A simple and high precision and inexpensive method for preconcentration and determination mercury from saline water sample at nanogram level using AFS was obtained.

CHAPTER II

THEORY AND LITERATURE REVIEW

2.1 Mercury measurement

There are several methods for determination of trace elements in environmental sample. The spectrometry is one of the most popular method for determination of trace elements in seawater because of it is high accuracy and precision. Since mercury is one of the trace elements in natural water. The technique employed for mercury determination are usually classified in the same category as those employed for trace elements. However, mercury is relatively more volatile than other elements. Specific consideration has to be taken into account.

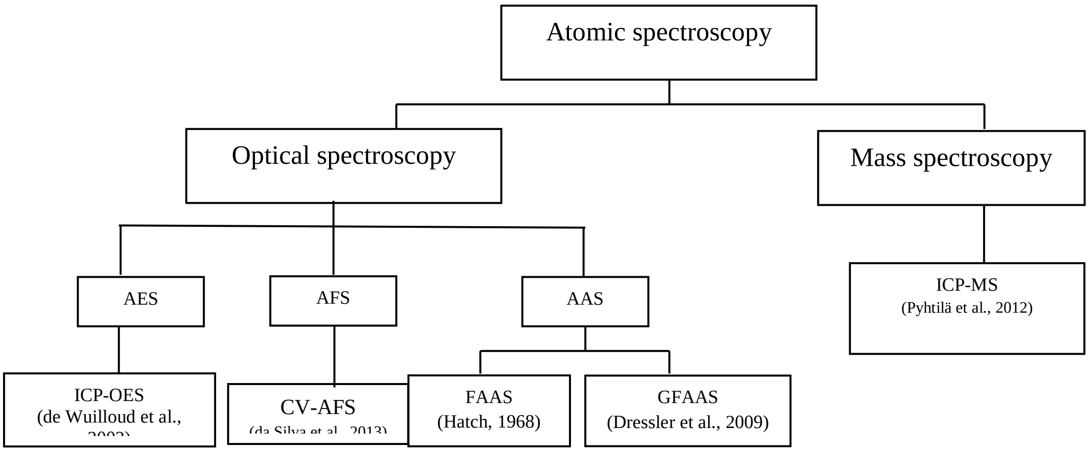

Figure 1 Reviews the spectroscopy techniques for determination mercury in water sample.

Historically, atomic spectroscopy has been a first method for determination of trace elements in water sample. Monteiro et al., 2002 designed the sequential reduction of mercury (II) and methyl mercury based on the on-line selective reduction of the species using two gas–liquid separators and Cold vapor-atomic absorption spectrometry (CV-AAS) detection with the detection limits of 400 and 600 ng L^{-1} for mercury (II) and methyl mercury, respectively.

Flame atomic absorption spectroscopy (FAAS) had also been largely employed because of its ease of use, cheap to operate and relatively high precision (Hatch and Ott 1968). However, its detection limit is not low enough to determination the concentration of mercury in environmental sample. To overcome these problem, Graphite furnace atomic absorption spectroscopy technique was later introduced to perform measurement at lower detection limit than FAAS (Orani et al., 2015). Though GFAAS has a lower detection limit but chemical interferences and limitation in dynamic range are still unavoidable. Dressler et al., 2009 tried to improve the sensitivity of AAS by combining ion exchange and amalgamation with AAS for determining mercury speciation in urban landfill leachate by cold vapor - atomic spectrometry (CV-AAS) with detection limits of mercury (II) and methyl mercury were 9 and 12 ng L^{-1}, respectively. Again, this technique is still not capable to achieve detection limit low enough for determination of mercury in seawater.

The cold vapor atomic emission spectrometry (CV-AES) was later studied by Yuan et al., 2014. This technique offers several advantages such as ease of use, low gas consumption and small-size sample, but its disadvantage is the spectral interferences.

Another improvement came with Inductively coupled plasma mass spectrometry (ICP-MS) in EPA method 200.8 for determination of trace elements in water and waste (De Smaele et al., 1999, Deshommes et al., 2010). The main advantage of this technique is its detection limit. Chen et al., 2013 studied the speciation of mercury compounds by cation exchange chromatographic separation and inductively coupled plasma mass spectrometry (ICP-MS). The detection limits for mercury (II), methyl mercury, ethyl mercury and phenolic mercury were 19, 27, 31 and 22 ng L^{-1}, respectively. Since then, cold vapor generation couples with spectrometry was become very popular. A cold vapor - inductively coupled plasma mass spectrometry (CV-ICP-MS) technique determining of mercury in humic-rich natural water samples performed with a lowest detection limit of 0.7 ng L^{-1} (Pyhtilä et al., 2012). However, the disadvantages of inductively coupled plasma mass spectrometry are still spectral interferences, time consumption and expensive operational cost.

The latestest attempt came when EPA (Environmental protection agency) published “Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry” (EPA Method 1631) which was specifically developed to provide reliable technique of mercury determination in water sample (Leopold et al., 2008). Major disadvantages of AFS are the lifetime of gold traps and contamination can quench the fluorescence signal (Wu et al., 2012). A comparison was then made between ICP-MS and Gas chromatography-atomic fluorescence spectrometry in marine samples by (Armstrong et al., 1999). The limits of detection were $9x10^{-4}$ and $2.5\ x10^{-4}$ ng for ICP-MS and AFS, respectively.

Recently, AFS has become the most suitable choice for determination of mercury in natural water. In addition, EPA recommenced that AFS has been useful method for determination of mercury because of many benefits such as high selectivity, sensitive and time consumption. Nevertheless, direct injection of mercury from real sample water is still difficult. Since concentration of mercury is extremely low. Another reason is the high concentration of matrix elements in the samples, which can affect the signal of mercury and clogging in instrument and cause memory effect in the detection system (Dwinna, 2009). So once again preconcentration had a chance to show its usefulness in reducing these problems.

2.2 Preconcentration

Mercury has extremely low concentration in seawater. Thus, preconcentration step is very necessary to improve sensitivity and removal of matrix from sample. The methods for determination of mercury are reviewed below.

Preconcentration techniques are usually required for a reliable measurement of mercury in seawater. The advantages of preconcentration techniques are not only to eliminate the matrix but also to enrich the trace element concentration (Dalman et al., 2007, Ashkenani et al., 2009). Preconcentration techniques for pre-treatment and separation trace elements from natural water can be classified in to three main types: coprecipitation, solvent extraction and solid phase extraction.

Coprecipitation can separate mercury from natural water sample by transferring trace elements into precipitate form. Solvent extraction is another method that can separate an analyte by using liquid reagent used as a solvent. Disadvantages of these two techniques are the large amount of reagents and effect of emulsion that cause of loss of an analyte, and time consuming. On the other hand, solid phase extraction technique has been more widely used since it can be applied in small size column, easy for operation and less time consumption.

2.3 Solid phase extraction

The advantage of this techniques are elimination of a large amount of matrix elements, requiring small sample volume, low cost, no needs for special apparatus, and its capability in removal interference from matrix (Dwinna, 2009). According to the hard soft (Lewis) acid base principle (Table 1) mercury is classified as a soft acid and has an extremely high affinity in binding with sulfur atom (soft base) through covalent attachment and can be immobilized strongly in the adsorbent (Wajima and Sugawara, 2011).

Table 1 Peason Hard Soft[Lewis] Acid Base Species (Vas and Vekey, 2004).

	Hard	Boderline	Soft
Acids	Na^+, K^+, Mg^{2+}, Ca^{2+}, Cr^{3+}, Al^{3+}, Ga^{3+}, Co^{3+}, Fe^{3+}	Cu^{2+}, Zn^{2+}, Pb^{2+}, Bi^{3+} Ni^{2+}, Co^{2+}, Fe^{2+}	Cu^+, Au^+, Ag^+, Hg^{2+}, Hg^+, Cd^{2+}
Base	H_2O, NH_3, OH^- Carbonate, Sulphate Carboxylates, Ethers Nitrate Alcohol	Aniline Imidazole Pyridine Nitrite Azid	Phenyl groups Thiols Ethylene Thioethers Cyanide

Nevertheless, the mercury – thiols interaction is not specific (Yetimoğlu et al., 2009). There are many articles about developed of absorbent containing supported ligands for improve selectivity and efficiently for removal of mercury from aqueous solutions such as xanthate (Cui et al., 2015), thiol group (Zhang et al., 2009, Hadavifar et al., 2014, Wang et al., 2014, Pavase et al., 2015), pyridine based on thiol (Sonmez and Bicak, 2002) and dithiozone (Shah and Devi, 1996). As mentioned thiol group show a good performance ligand for improve selectivity for an absorbent for preconcentration mercury from natural water (Table 2).

Table 2 The different types of absorbent and ligand had been used for preconcentration of trace elements for water sample (Leopold et al., 2010).

Ligand	Absorbent	Analytical method	LOD (ng L^{-1})	Real samples
5,5'-dithiobis(2-nitrobenzoic acid)	Octadecyl silica membrane disks	AAS	2	water samples
1-acylthio semicarbazide	Activated carbon	ICP-OES	120	natural water
Gold	Silica	AFS	0.18	natural water and waste waters
2-mercapto benzimidazole	Agar powder	AAS	20	drinking waters, waste waters
2-(2-oxoethyl) hydrazine carbo thioamide	Silica gel	ICP-OES	100	river water and tap water
Sodium dodecyle sulfate	Magnetite nanoparticles	ICP-OES	40	Tap water, well water and mineral waters
1,5 - diphylcarbazide	Magnetic nanoparticles	CV-AAS	160	
Dithiozone	Silica gel	CV-AAS	20	

2.4 Sorption capacity

The principle of solid phase extraction is a method for separation of an analyte from aqueous solution onto solid support (absorbent). The distribution coefficient (K_d) shows ratio between concentration of an analyte in solid phase and concentration of analyze element in aqueous phase. The efficiency of absorbent can be expressed by sorption capacity.

The Langmuir isotherm model is a general model for adsorption on a completely homogenous surface. The maximum adsorption depend on saturation of the monolayer (Bai et al., 2011, Murugesan et al., 2012). Moreover, mercury can be adsorbed monolayer on the solid support so that can related with Langmuir isotherm model.

The mathematical description of this model is

$$\frac{C}{N_f} = \frac{1}{bN_{fs}} + \frac{C}{N_{fs}}$$

Where C is the equilibrium concentration of the metal ions in the solution (mol L^{-1})

N_f is the adsorbed value of the metal ion at equilibrium concentration (mol g^{-1})

N_{fs} is the maximum adsorption capacity (mol g^{-1})

b is the Langmuir binding constant

2.5 Quality control

Calibration is a common acceptable part in analytical procedures, which is external standard and internal standard addition method. External standard calibration curve is a procedure in analytical method that having no matrix effect. Internal standard addition calibration curve is a procedure in analytical method that can minimize error from matrix effect (Chan et al., 2004).

2.5.1 Internal standard addition method

Internal Standard addition or spike method is a common procedure suitable for analyzing aqueous sample, in the presence of matrix. In order to determine relative respond of an analyte, the known amount of analyte were added to the sample. The relative respond is used in estimation of either operative interference or analyte concentration. There are several step for spike method procedure. First, unknown concentration (V_x) of sample are transferred to a series of volumetric flask (V_i as a volume). Second, Added a series of a small volume of standard (V_s) with known concentration (C_s). Third, added the reagent and adjusted the volume each of the solutions. Finally, measure each of the solutions to receive a signal value (S) (Chandran and Singh, 2007).

$$S = \frac{V_s C_s}{V_t} + \frac{V_x C_x}{V_t}$$

The graph plot between S and V_s as shown in figure 2, the slope m = $\frac{C_s}{V_t}$ and intercept b = $\frac{V_x C_x}{V_t}$ and unknown concentration (C_x) can be calculated form equation

$$\frac{b}{m} = \frac{V_x C_x}{V_t} \text{ x } \frac{V_t}{C_s}$$

$$C_x = \frac{bC_s}{mV_x}$$

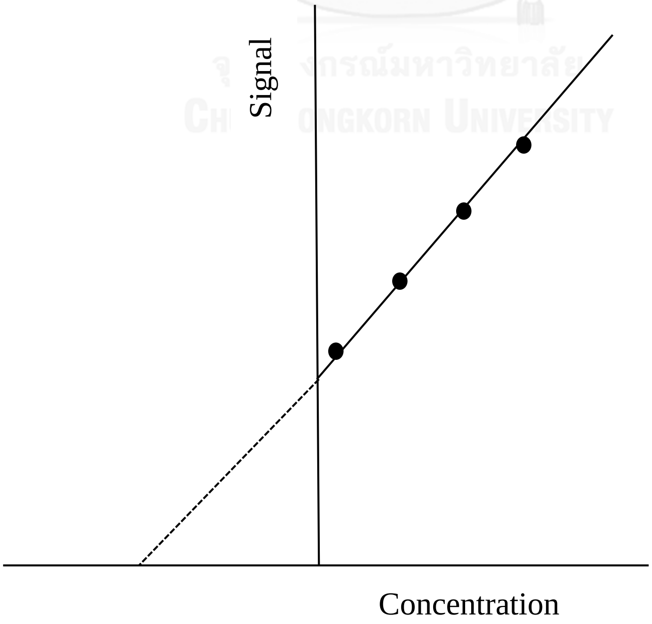

Figure 2 Linear calibration curve for spike method.

2.5.2 Quality control

The validation of analytical method was performed to ensure the performance of the developed method, in terms of the reliability and accuracy.

2.5.2.1 Accuracy

The accuracy of an analytical procedure is the closeness of mean test results of the developed method to a true value or an acceptance reference value. The true value of accuracy can be demonstrated by several ways (Chandran and Singh, 2007, Ermer and Miller, 2014). The result of the method is either compared with certified reference material or reference method. Or, accuracy assessment can be calculated by using of recovery from spiked samples. Spiking recovery can be calculated through percentage of recovery, which is the amount of metal eluted from column per the amount of metal in initial sample.

2.5.2.2 Precision

The precision of an analytical procedure is described by the closeness of individual measurement which is in agreement between series of the results obtained from multiple sampling of the same homogenous sample under optimum conditions.

There are three types of precision that can be demonstrated for an analytical method. First, repeatability precision (intra-run precision), which is the precision under condition that the result obtained with the same method in the same laboratory using the same operator and equipment within short time. Second, intermediate precision (inter-run-precision), which is the precision under condition that the result obtained with the same method in the same laboratory using different operator and equipment within extended of time. Third, reproducibility precision (inter-laboratory precision), which is the precision under condition that the result obtained with the same method in the different laboratories using different operator and equipment. The precision can be expressed by using percent relative standard deviation (RSD) or standard deviation (SD) (Taverniers et al., 2004).

2.5.2.3 Sensitivity

The sensitivity of an analytical procedure is defined as the increase in signal with unit increase in concentration or the ability of the analytical method to identify small differences in concentration of the analyte (Chandran and Singh, 2007). The sensitivity can be obtained by plotting the signal against the analyte concentration, which is the slope of calibration graph.

2.5.2.4 Limit of detection and Limit of quantitation

The limit of detection (LOD) of an analytical procedure is the lowest of concentration of an analyte in the sample that can be detected but not necessarily be quantitated. The limit of quantitation (LOQ) of an analytical procedure is the lowest of concentration of an analyte in the sample that can be detected with acceptable precision (Chandran and Singh 2007). The limit of detection (LOD) and the limit of quantitation (LOQ) can be obtained by measuring three and ten times of standard deviation of blank signal, respectively.

2.5.2.5 Linearity

The linearity of an analytical procedure is defined as the ability (within range) to receive test results, which are directly proportional to the amount of an analyte concentration in the sample over the range of interest. The linearity can be expressed by measuring the signal of standard solutions in the range of interest. The linearity is evaluated as a relationship between signal and known concentration of an analyte as can be seen in figure 3. The range of an analytical procedure refer to interval between the upper and lower amounts of an analyte in the sample, which is suitable amount of accuracy, precision, and linearity has been demonstrated. (Chandran and Singh, 2007)

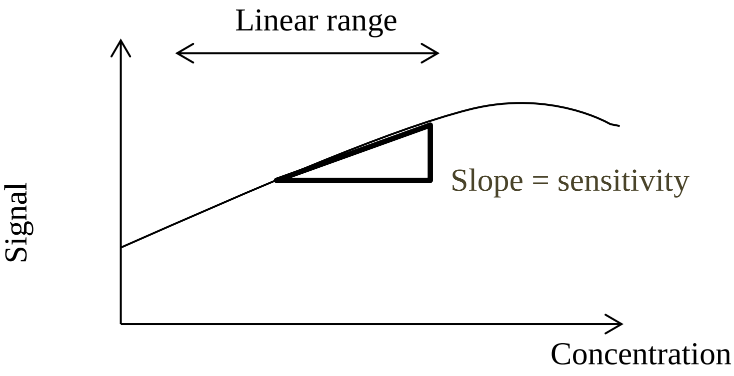

Figure 3 The linear calibration curve for standard addition (Spiked) method.

2.6 Literature reviews

Recently, many solid phase extraction for a preconcentration techniques for a preconcentration step to separate mercury ions from real sample water were published. Agar functionalized with 2 - mercaptobenzimidazole column was studied for determination of mercury in water samples. After preconcentration step, the cold vapor atomic absorption spectrometry (CV-AAS) was employed. The limit of detection obtained under optimum conditions was 20 ng L^{-1}. The relative standard deviation (RSD.) for the determination were 400 and 2000 ng L^{-1} of mercury (II) ions and methyl mercury were 2.6 and 1.9%, respectively (Pourreza and Ghanemi, 2009).

Silica is one of the widely used as a support material. Segada and Tyson. (2007) preconcentated methylmercury and inorganic mercury in water samples by extraction with silica C18 modified with ammonium pyrrolidine dithiocarbamate (APDC), followed by slurry sampling cold vapor atomic absorption spectrometry (SS-CVAAS) in a flow injection (FI) system. The detection limits were 0.96 and 0.25 ng L^{-1} for methyl mercury and inorganic mercury, respectively. Bai et al.(2011) determined traces of lead, cadmium, copper, and mercury from aqueous solution by extracting with silica-supported dithiocarbamate adsorbent. The adsorption process was studied by using Langmuir, Freundlich and Dubinin–Radushkevich (D–R) adsorption isotherms. The adsorption capacities were found to be 0.34, 0.36, 0.32 and 0.40 mmol g^{-1} for lead, cadmium, copper, and mercury, respectively.

Cellulose can also be used as a support material. A cellulose column modified with diphenylthiocarbazone ligand use for the preconcentration of inorganic mercury in water samples involving complexation with modified cellulose column. Desorption of mercury from column by using polyethylene glycol. The concentration of mercury was determined using visible spectrometry. The calibration curve was linear in the range of 0 - $2x10^6$ ng L^{-1} of mercury with a detection limit of $2x10^3$ ng L^{-1}. The relative standard deviation (RSD.) of the method was found to be 3.5% (Rajesh and Gurulakshmanan, 2008).

Resin chelating with N,N'-di (carboxymethyl-dithiocarbamate) for extracted heavy metals such as copper, lead, and nickel from aqueous solutions. The kinetic and isotherm date were evaluated with Laguerre-second-order equation and Langmuir model, respectively. The maximum adsorption capacities were in order of lead > copper > nickel. The electrostatic attraction found in the range of 0.1–1 mmol L^{-1} and the chelating adsorption was in the range of 1–5 mmol L^{-1}. Enthalpy change values were 16.80, 17.49 and 12.54 kJ mol^{-1} for copper, lead and nickel, respectively (Jing et al., 2009).

Sulfur absorbent is another absorbent have been used for preconcentration of trace elements in real water sample. Pourreza et al. (2009) modified sulfur powder column with N-(2-chloro benzoyl)-N'-phenylthiourea and determination mercury in water samples by cold vapor atomic absorption spectrometry. The limit of detection (LOD) with 250 and 1000 mL sample volume were 12 and 3 ng L^{-1}, respectively. Pourreza et al. (2010) modified sulfur powder column with 2 – mercaptobenzo Thiazole (2-MBT) in the 1-butyl-3-methylimidazolium hexafluorophosphate ionic liquid solution for preconcentrated of cadmium in water samples with limit of detection was 4.6 ng L^{-1} and the relative standard deviation (R.S.D.) of 25 and 150 ng

L^{-1} of cadmium were 4.1 and 2.2%, respectively. The method was validated by the analysis of a certified reference material DORM-3, water and fish sample.

According to previous study, sulfur chemically with N-(2-chloro benzoyl)-N’-phenylthiocarbamide showed a good performance for preconcentration of mercury. However, the effective in seawater and sorption capacity still need to studies to fulfill the understanding.

CHAPTER III

Experiment

3.1 Instrument

3.1.1 Cold vapor atomic fluorescence spectrometer

Determination of mercury (II) ions concentration was performed by an atomic fluorescence spectrometer (mercurDUO plus, AnalytikJenaR, Germany). For Hg^0 vapor generation, 2% stannous (II) chloride in 4% hydrochloric acid, 2% hydrochloric acid and argon gas were used as a reducing agent and carrier solution, respectively. The Mercur parameter used enrichment without reloading mode which is enriched mercury vapor by using gold collector. During measurement mercury is thermally desorbed from gold collector and swept to the cell by an argon stream. Then, the tubes and components have to be rinsed by acids after each sample measurement. The condition for cold vapor atomic fluorescence spectrometry are shown in Table 3.

Table 3 The conditions for cold vapor atomic fluorescence spectrometry.

Parameter	Operation time
Sample load time	10 s
Gas flow	10 (NL/h)
Reaction time	6 s
Purge time 1	30 s
Waiting time AZ	5 s
Purge time 2	15 s
Gas flow	5 (NL/h)
Purge time 3	25 s
Gas flow	10(NL/h)
Heating time collector 1	20 s
Cooling time collector 1	50 s
Sample pump speed	Level 3
Wash time acid	10 s

3.1.2 pH meter

The pH of sample solution was measured by pH meter.

3.1.3 Hand refractometer

The salinity of solution was measured by hand refractometer.

3.1.4 Peristaltic pump

The flow rate was controlled by peristaltic pump.

3.2 Chemicals

All chemicals used in this research are listed in Table 4.

Table 4 List of chemicals

Chemicals	Grade
Acetone	LAB
Ammonium thiocyanate	AR-Grade
Anhydrous magnesium sulfate	LAB
Aniline	AR-Grade
2 – chloro benzoyl chloride	AR-Grade
Citric acid	LAB
Dimethyl formamide	LAB
Ethanol	LAB
Hydrochloric acid	LAB
Hydroxylamine	LAB
Mercury standard solution (1,000 mg L^{-1})	LAB
Methylene chloride	LAB
Nitric acid	LAB
Polyethylene glycol-400	AR-Grade
Potassium bromide	LAB
Potassium bromate	LAB
Potassium persulfide	LAB
Permanganate	LAB
Sodium hydroxide	LAB
Sulfuric acid	LAB
Sulfur powder	AR-Grade
Stannous (II) chloride	LAB

3.3 Preparation of Labware

Contamination is the most important factor for trace metal determination. All Labware were washed with detergent. Rinsed three times with deionized water (17.8 Ω). then soaked in 10% super pure hydrochloric acid and 10% nitric acid for a week. Bottles are cleaned by heating to 80^{o}C in 0.1% permanganate, 0.1% potassium persulfide and 2.5% nitric acid in water bath for 2 hours. The bottles are cooled, rinsed with 2 mL of hydroxyl aminohydrochloride and rinsed three times with deionized water. Place in a clean bench until outside surface are dried. Keep in zip-lock bags and stored in plastic boxes until use.

3.4 Preparation of chemicals

3.4.1 Citrate solution

Citrate solution was prepared by dissolving 2.1 g of citric acid in 50 mL volumetric flask.

3.4.2 Sodium hydroxide solution

Sodium hydroxide solution was prepared by dissolving 0.4 g of sodium hydroxide in 50 mL volumetric flask.

3.4.3 Citrate buffer

Citrate buffer was prepared by using sodium hydroxide solution to adjust the pH of citrate solution.

3.4.5 Hydrochloric acid solution (2% v/v)

Hydrochloric acid solution was purified before used by sub-boiling technique. Next, 2% hydrochloric acid solution was prepared by dilution of super-pure hydrochloric acid with deionized water.

3.4.6 Stannous (II) chloride solution

Stannous (II) chloride solution was prepared by dissolving 20 g of stannous chloride in 2% of super pure hydrochloric acid. The volume was adjusted to 1,000 mL with deionized water.

3.4.7 Bromate/Bromide solution

Bromate/Bromide solution was prepared by dissolving 1.1 g of Bromate and 1.5 g of bromide in 100 mL volumetric flask. Make up to 100 mL with deionized water.

3.4.8 Synthetic seawater

Synthetic seawater was prepared by using 12.5 g of sodium chloride and 4 g of magnesium sulfate for 14 psu and 12.5 g of sodium chloride and 8 g of magnesium sulfate for 28 psu. A salinity of synthetic seawater was measured by hand refractometer.

3.4.9 Secondary mercury standard solution

Secondary mercury standard solution was prepared by added 20 mL of deionized water in a 100 mL volumetric flask, added 0.1 mL of stock mercury standard solution. Make up 100 mL with deionized water. This solution containing 1 mg L^{-1} of mercury (II) ions and should be replaced monthly.

3.4.10 Working mercury standard solution

Working mercury standard solution was prepared by added 20 mL of deionized water in a 100 mL volumetric flask. Then, added 1 mL of secondary mercury standard solution. Bring to 100 mL with deionized water. This solution containing 10 μg L^{-1} of mercury (II) ions and should be replaced daily.

3.4.11 Synthesis N-(2-chloro benzoyl)-N'-phenyl thiocarbamide

The following step for synthesis ligand shown in Figure 4. N-(2-chloro benzoyl)-N'-phenyl thiocarbamide was synthesized by mixing 0.317 mL 2 - chloro benzoyl chloride, 0.215 g ammonium thiocyanate and 0.94 g poly ethylene glycol-400 with 20 mL of chloroform in a round bottom flask. Added, 2 drops of dimethyl formamide into the solution, then the white color of solution turned to yellow color immediately. The mixture was stirred continuously at the room temperature for 1 hour. Next, added mixture of 0.23 mL aniline and 5 mL chloroform into the solution. The mixture was stirred for another 2 hours. After that, the 15 mL of deionized water was added into the mixture. The organic layer was separated by using filtration and dried with anhydrous magnesium sulfate. Then, the solvent was removed in a rotary evaporate and N-(2-chloro benzoyl)-N'-phenyl thiocarbamide was separated by crystallization. The product was characterized by NMR-spectra (Apendix).

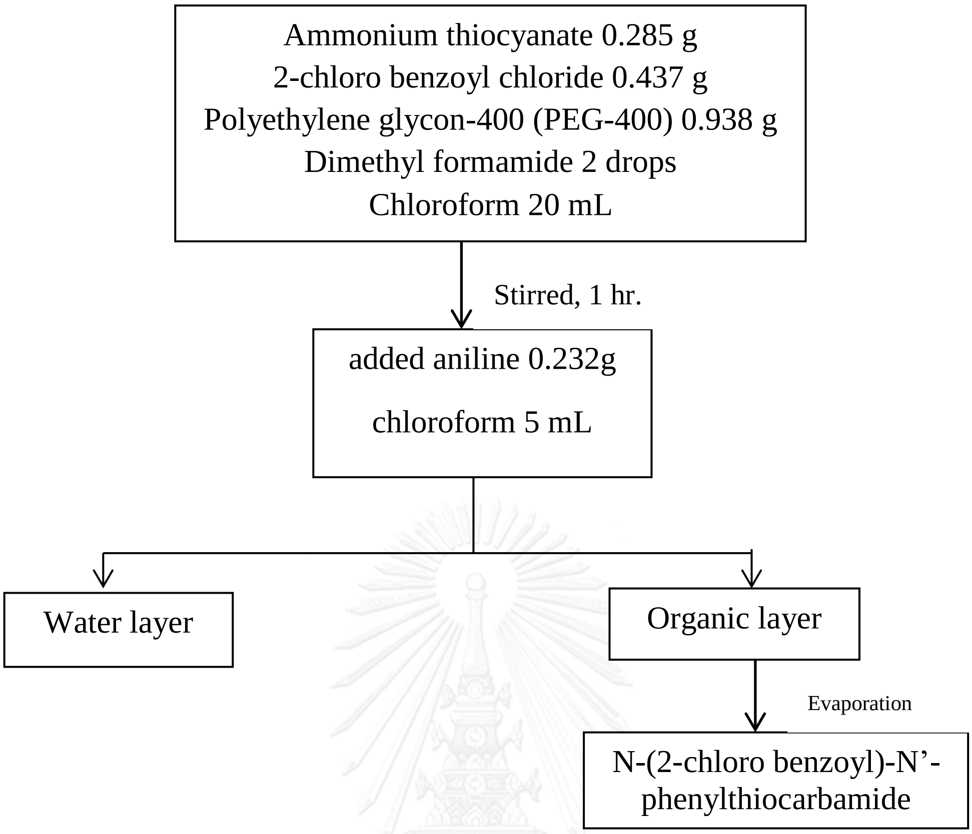

Figure 4 The following step for synthesis ligand (Pourreza et al., 2009).

3.4.12 Preparation of modified sulfur compound

The 40 mg of N-(2-chloro benzoyl)-N'-phenyl thiocarbamide was mixed with 20 mL of acetone in a 50 mL beaker. The mixture was heated until the temperature reached 45 ^{o}C for 30 minutes. Then, the solvent was removed by a rotary vacuum evaporator. The following step for preparation of modified absorbent show in Figure 5.

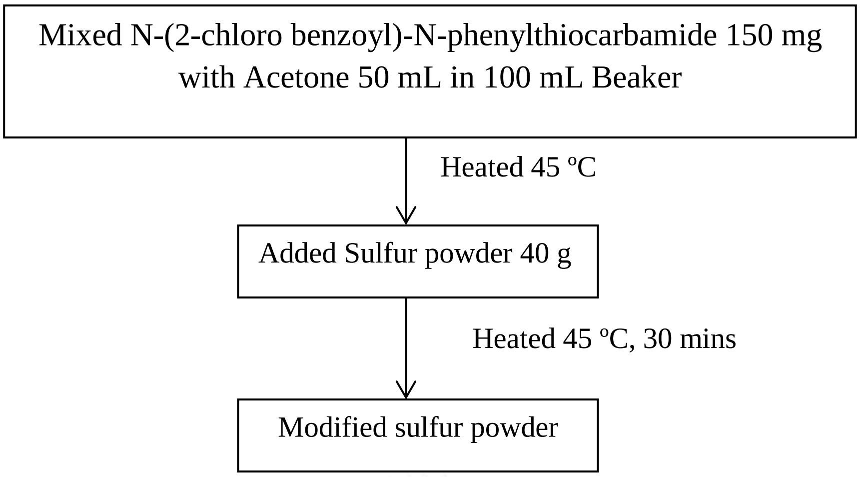

Figure 5 The following step for preparation of modified absorbent.

3.4.13 Preparation of column

The glass column 0.7 cm in diameter and 9.0 cm in a length was used for preconcentration of mercury. The 2 g of modified sulfur compound was placed in the column. Cotton was placed at the bottom for allowing modified sulfur compound to settle properly. Figure 6 show the modified column.

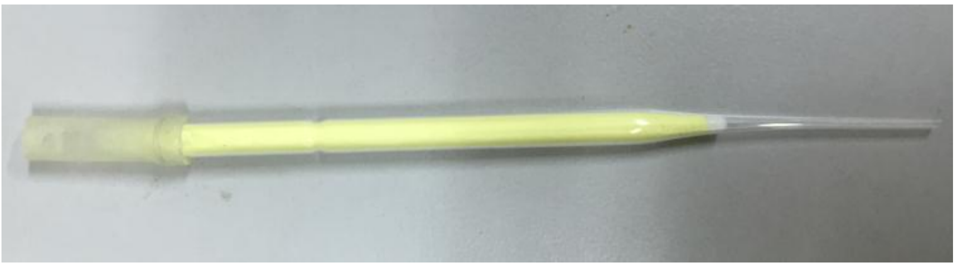

Figure 6 The modified column.

3.5 Optimization

3.5.1 Optimization absorbent conditions

3.5.1.1 Procedure of mercury determination

For preconcentration of 250 mL mercury solution containing mercury (II) ions in the range 5-20 ng L^{-1}. The 5 mL of citrate buffer was loaded to the column, maintaining a flow rate by peristaltic pump. Next, the 250 mL of sample solution was passed through a column. General procedure steps are shown in Figure 7. After retention of mercury (II) ions, the adsorbed complex was measured by using cold vapor atomic fluorescence spectrometry at 253.7 nm. For total mercury determination, added a strong oxidant such as bromine monochloride. After pre-reduction of excess oxidant by 12% hydroxyl aminohydrochloride. Mercury (II) ions was reduced to elemental mercury (Hg^0) by stannous (II) chloride.

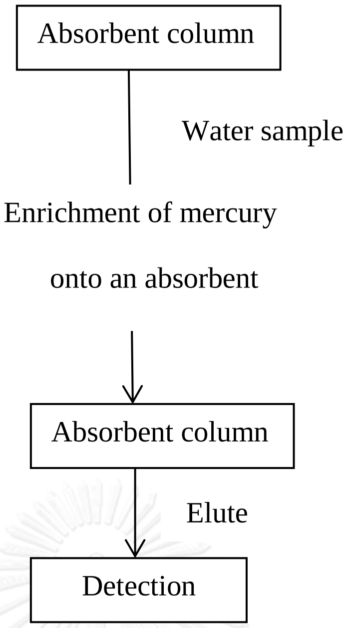

Figure 7 General procedure steps for solid phase preconcentration method.

The concentration of mercury at 10 ng L^{-1} mercury (II) ions was used as a sample to study the optimization condition because of limitation of linearity range cover in the range of 0-20 ng L^{-1}. All sample was studies with three measurements and each sample was studied three times.

3.5.1.2 Effect of pH on stabilization of absorbent

The stabilization of the absorbent use as a conditioning step for improve the promoting area contraction and remove any contaminant. The effect of pH solution was studied by varying the pH of citrate buffer in the range 2 – 4. The pH of citrate buffer was adjusted by adding different concentration of 2M sodium hydroxide solution.

3.5.1.3 Effect of N-(2-chloro benzoyl)-N’-phenyl thiolcarbamide quantity

Because of the limitation of reactive site of sulfur powder so in this section was to study the suitable amount of functional ligand with an absorbent. The effect of N-(2-chloro benzoyl)-N’-phenyl thiolcarbamide was study by varying the amount of N-(2-chloro benzoyl)-N’-phenyl thiolcarbamide in a range of 2 – 8 mg functionalized with 1 g sulfur powder.

3.5.1.4 Effect of amount of absorbent

The maximum capacity of an absorbent were control the reactive site and the maximum amount of metal adsorbed per gram of the absorbent. The amount of absorbent was studied in range of 1 – 3 g.

3.5.2 Optimization of parameters

3.5.2.1 Effect of sample volume

The maximum capacity of an absorbent was the maximum amount of metal adsorbed per gram of the absorbent. The sample volume was studied in the range of 50 – 250 mL.

3.5.2.2 Effect of the elute type

Normally, the acid reagents were used as an eluent for elute trace element from an absorbent (Abrankó et al., 2007, Issaro et al., 2009). A variety of acid reagents were tested in order to elute the adsorbed complex from the column. Hydrochloric acid, sulfuric acid and nitric acid were studied as eluents.

3.5.2.3 Effect of flow rate

The flow rate of sorption and elution would affect to effectiveness of solid phase extraction in the column. The effect of flow rate on sorption was studied in the range 5 – 15 mL min^{-1}. On the other hand, the effect of flow rate on elution was studied in the range 2 – 8 mL min^{-1}. The flow rate was maintained by peristaltic pump.

3.5.2.4 Effect of salinity

Salinity in Thai river covers in the range of 0-28 psu (Thongra-ara and Parkpian, 2002). the effect of salinity was studied in the range of 0 – 28 psu of synthetic seawater.

The sorption capacity was studied in order to know a maximum capacity of the absorbent.

3.6 Sorption capacity

Sorption capacity represented to the maximum amount of metal adsorbed per gram of the absorbent. The sorption capacity was studied by varying the concentration of mercury (II) ions in total volume of 250 mL.

3.7 Method validation

Method performance (Table 5) was validated by using standard mercury (II) ions solution. The validation was studied under optimum conditions previously tested. The calibration curve was obtained at the concentration of 0, 5, 10 and 20 ng L^{-1}. The accuracy and precision were presented as percentage of recovery and percentage of relative standard deviation (RSD), respectively. The limit of detection was calculated from standard deviation of three times measurement of a reagent blank.

Table 5 Brief definition of various validation characteristics

Characteristics	Definition
Accuracy	Closeness agreement between determined value and true value
Precision	Closeness agreement of a series of determined
Linearity	Ability (within range) to receive test results
Detection limit	Lowest amount of an analyte in a sample that can be detected
Quantitation limit	Lowest amount of an analyte in a sample that can be detected and acceptable precision

3.8 Application of natural water sample

To demonstrate the performance of validated preconcentration method of mercury (II) ions based on modified sulfur powder, natural water samples were filtered by online membrane filter (as show in Appendix). After that, the samples divide for studied of mercury (II) ions and for total mercury (Figure 8). The water sample total mercury was digested by BrCl for 5 mins in 80 ºC the excess of BrCl was removing by hydroxylamine solution. Both of water samples were spiked mercury in the range 5 – 20 ng L^{-1}. Next, real water sample and spike samples were investigated under optimum conditions. The determination of mercury (II) ions concentration was done by cold vapor atomic fluorescence spectrometer (CV-AFS).

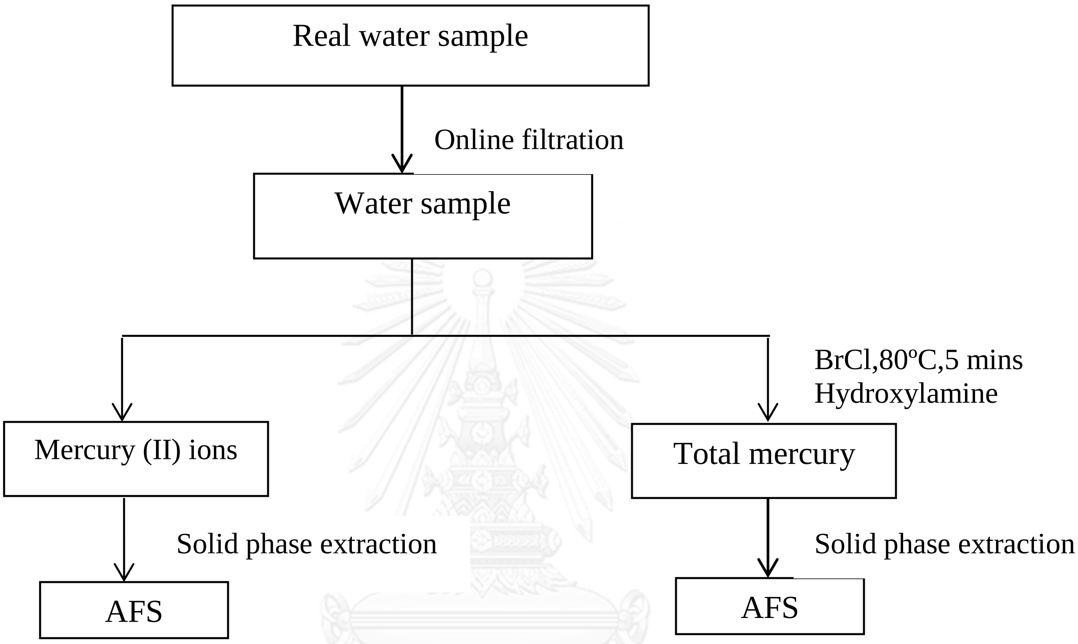

Figure 8 The following step for determination mercury in natural water sample.

CHAPTER IV

Results and Discussion

4.1 Synthesis of N-(2-chloro benzoyl)-N'-phenyl thiocarbamide

The synthesis of N-(2-chloro benzoyl)-N'-phenyl thiocarbamide was shown in Figure. The ^{1}H-NMR spectrum of N-(2-chloro benzoyl)-N'-phenyl thiocarbamide was recorded in $CDCl_3$ (Appendix). As can be seen that N-(2-chloro benzoyl)-N'-phenyl thiocarbamide was successfully synthesized (Figure 9).

Figure 9 The synthesized N-(2-chloro benzoyl)-N'-phenyl thiocarbamide.

4.2 Optimization absorbent conditions

The solution containing 10 ng L^{-1} of mercury (II) ions was used as a sample. For raw data are shown in Appendix.

4.2.1 Effect of ligand N-(2-chloro benzoyl)-N’-phenyl thiocarbamide quantity

Solid phase extraction is a well-known technique for trace element preconcentration in natural water. Mercury (II) ion is a soft acid and has a good affinity toward with sulfur group. Therefore, sulfur powder can be used as a solid support. The efficiency of solid support could be improved by making it functional with suitable ligand such as xanthate, thiol group, pyridine based on thiols and dithiozone. The N-(2-chloro benzoyl)-N’-phenyl thiocarbamide is one of the thiol group. Thus, N-(2-chloro benzoyl)-N’-phenyl thiocarbamide was selected for investigated.

The effect of N-(2-chloro benzoyl)-N’-phenyl thiocarbamide quantity was studied by varying quantity of N-(2-chloro benzoyl)-N’-phenyl thiocarbamide in the range of 2-8 mg of N-(2-chloro benzoyl)-N’-phenyl thiocarbamide combined with 1 gram of sulfur powder. The percentage recovery of various amount of N-(2-chloro benzoyl)-N’-phenyl thiocarbamide loaded on sulfur powder are shown in Figure 10. It was found that the percentage recovery increased from 75.4% at 2 mg g^{-1} of N-(2-chloro benzoyl)-N’-phenyl thiocarbamide chemically with sulfur powder to reach the peak at 98.86% of 4 mg g^{-1}. After that, the percentage recovery decreased slightly. It is likely over capacity of solid support happened after 4 mg g^{-1}. Thus, the 4 mg g^{-1} of N-(2-chloro benzoyl)-N’-phenyl thiocarbamide loaded with sulfur powder was selected for this studied.

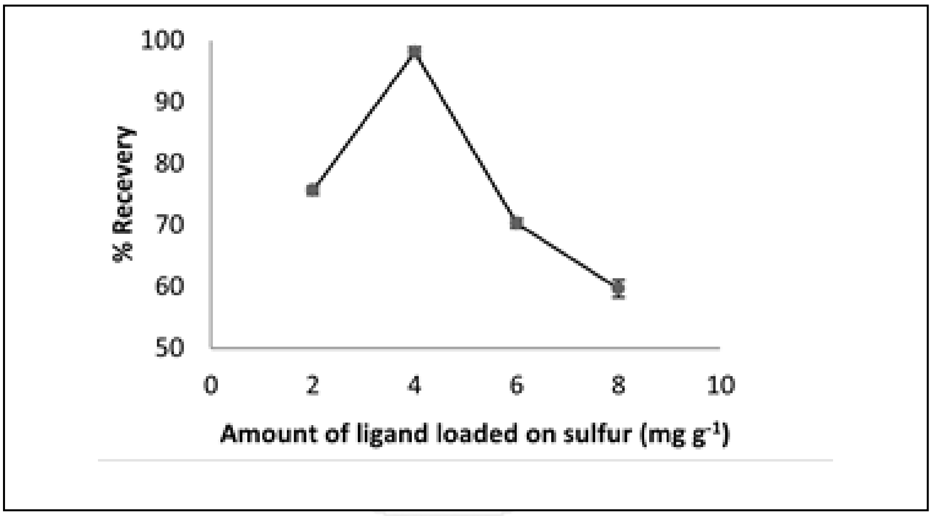

Figure 10 Effect of the amount of ligand loaded on sulfur (mg g^{-1}) on the percentage recovery of 10ng L^{-1} of mercury (II) ions solution.

4.2.2 Effect of stabilization of column

The conditioning to stabilize the absorbent was done through the buffer solution. This stabilization process will help promoting better surface area contraction, and removing of any contaminant presented. Citric-citrate buffer was found to be suitable for conditioning the modified sulfur column for determination trace elements (Pourreza and Ghanemi 2010). In order to investigated pH effect, values. The pH value was varied in the range of 2-4. The pH value of solution was adjusted using either citric acid or sodium hydroxide solution. The graph of percentage recovery of mercury (II) ions versus pH of the solution after preconcentration experiments are shown in the Figure 11. The maximum of mercury (II) ions preconcentration increased with increasing pH, from its minimum at pH 2 and reach to the maximum at pH 2.5. Then, when the pH of solution increased to pH 4 the percentage recovery decreased slightly. This implied that the donor site of N-(2-chloro benzoyl)-N'-phenyl thiocarbamide functionalized with sulfur powder absorbent could be protonated. Thus, The N-(2-chloro benzoyl)-N'-phenyl thiocarbamide functionalized with sulfur powder absorbent might be stable in the pH range of 2.5 – 3. The pH at 2.5 was selected as the optimum values for sorption of mercury (II) ions.

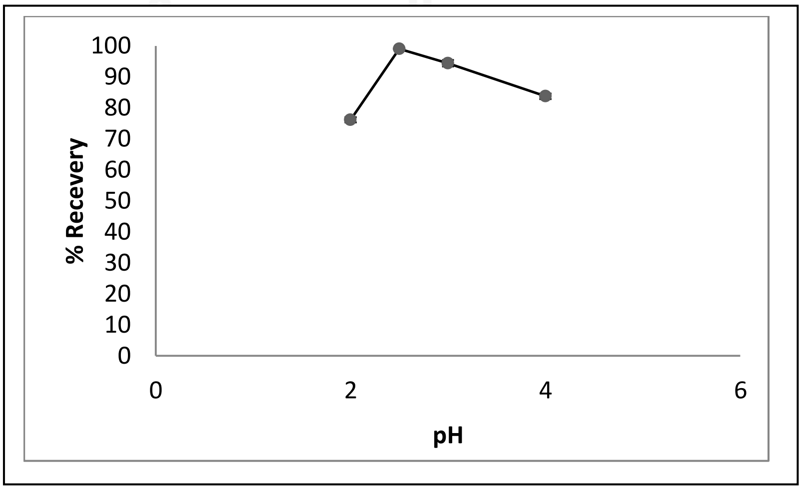

Figure 11 Effect of the pH values of the solution on the percentage recovery of 10 ng L^{-1} of mercury (II) ions solution.

4.2.3 Effect of amount of absorbent

The effect of amount of absorbent was studied by using different amount of absorbent in the range of 0.5 – 3 g (Figure 12). it was found that the amount of absorbent at 2 g is the most suitable amount.

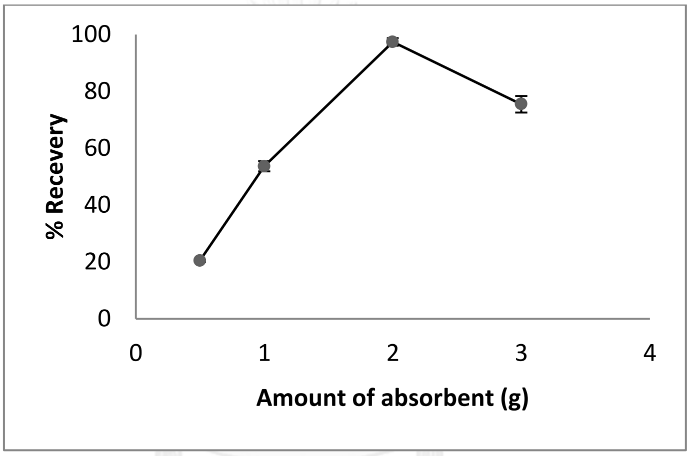

Figure 12 Effect of the amount of absorbent on the percentage recovery of 10 ng L^{-1} of mercury (II) ions solution.

4.3 Optimization parameters

4.3.1 Effect of sample volume

From previous experiments, the column was packed with 2.0 g of absorbent with the flow rate of loading and eluting at 16 and 6 mL min^{-1}, respectively were used.

In this section, the effect of sample volume on the recovery of analyzed of mercury (II) ions was investigated. The solution was prepared by dilute concentration of mercury (II) ions in the volume sample of 50 – 300 mL. The sample was passed through the column at the flow rate of 16 mL min^{-1} and elute at the flow rate of 6 mL min^{-1}.

The results are presented in Figure 13. It was indicated that the recovery of mercury (II) ions is quantitative (>95%) up to 250 mL sample volume. The sample volume of 250 mL was selected as a sample volume of sample solution.

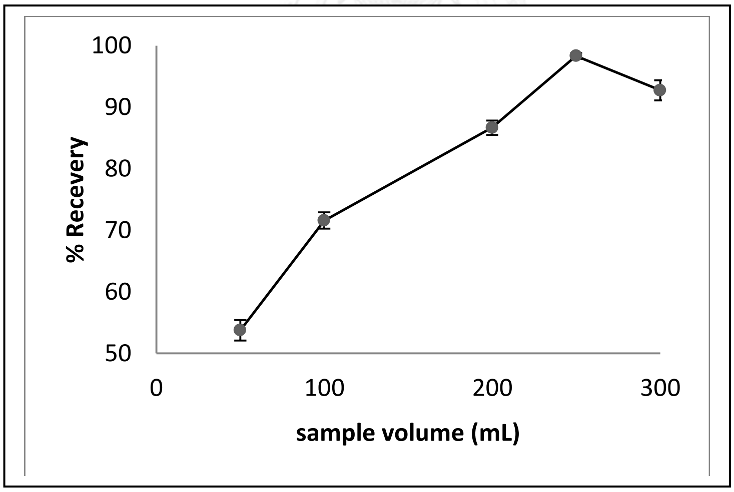

Figure 13 Effect of the sample volume on the percentage recovery of 10 ng L^{-1} of mercury (II) solution.

4.3.2 Effect of flow rate (sorption and elution)

The flow rate of sorption and elution are important factor that determine the time required for the adsorption processes to complete successfully. In this section, we divided the experiments into two sections, Firstly the studied of the effect of flow rate on sorption and secondly the studied of the effect of flow rate on elution.

In term of studied effect of the flow rate of sorption was varied at 5, 10 and 15 mL min^{-1}. As shown in Figure 14. The percentage of recovery at 15 mL min^{-1} was found to be suitable for optimum loading of mercury (II) ions absorbent complex on the column. In order to investigate the effect of flow rate on elution, the flow rate of elution was varied between 2 to 8 mL min^{-1}. As shown in Figure 15, the result showed that the percentage of recovery at 6 mL min^{-1} was found to be suitable for elute mercury (II) ions.

From these results, we chose flow rate of sorption at 15 mL min^{-1} and elution rate at 6 mL min^{-1}.

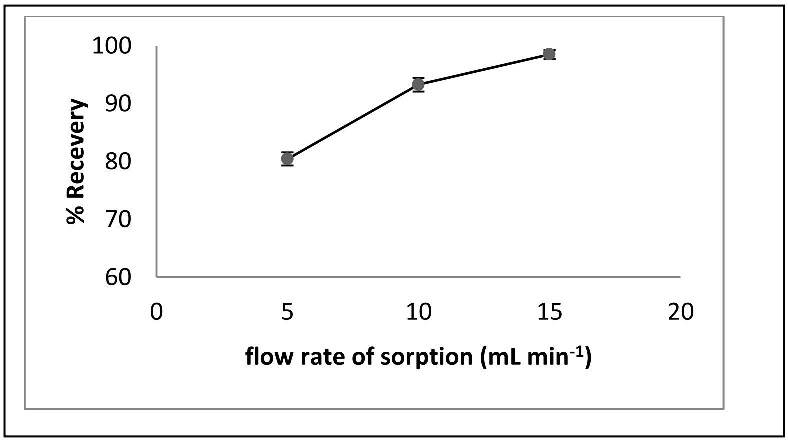

Figure 14 Effect of the flow rate of sorption on the percentage recovery of 10 ng L^{-1} of mercury (II) ions solution.

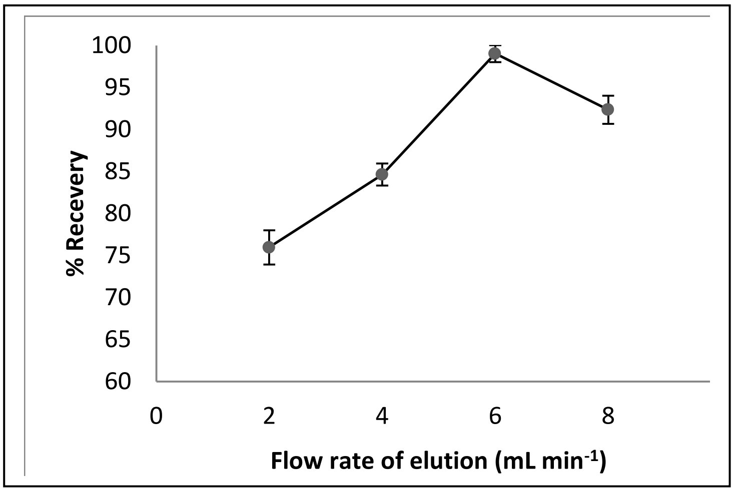

Figure 15 Effect of the flow rate of sorption on the percentage recovery of 10 ng L^{-1} of mercury (II) ions solution.

4.3.3 Effect of the eluent

The eluting solution is the important factor for the elute mercury (II) ions in the column. The effect of the eluent was studied in order to elute the adsorbed complex from the column. In order to, choose the most effective eluent for the quantitative recovery of mercury. The adsorbed complex was eluted with various eluting solution because the hydronium ion (H^+) can be protonated the functional group from the absorbent that mercury (II) ions can exchange and eluting by this method.

It was observed that 3M of hydrochloric acid was useful for quantitative elution of mercury (II) ions. Percentage elution with various eluting reagents are given in Table 6. Therefore, hydrochloric acid concentration of 3M was chosen as optimum condition. The elution was carried out maintaining mercury (II) ions of 10 ng L^{-1} in 250 mL of sample volume.

Table 6 Effect of different the eluent type.

Elute type	Ints	RSD. (%)
3M HCl	0.1176	3.768
2M HCl	0.1129	4.128
1M H_2SO_4	0.973	7.809
1M HNO_3	0.0895	1.416

4.3.4 Effect of salinity

The salinity of solution would affect the sorption capacity. The effect of salinity was studied by varying synthetic seawater in range of 0 - 28 psu. The solution containing 10 ng L^{-1} of mercury (II) ions was used as a sample. The recoveries were found in the range of 93 – 114 %. It can be seen from Table 7, it is evident that there was no salinity effect in the recovery of mercury.

Table 7 Effect of the salinity on the percentage recovery of mercury (II) ions solution.

Salinity (psu)	Hg^{2+} (ng L^{-1})	Recovery (%)
0	10	113.6
	20	97.9
14	10	94.6
	20	98.5
28	10	93.5
	20	113.3

Table 8 Effect of the salinity on the percentage recovery of total mercury solution.

Salinity (psu)	Total mercury (ng L^{-1})	Recovery (%)
0	10	91.6
	20	93.6
14	10	112.0
	20	95.2
28	10	92.3
	20	104.6

4.4 Sorption capacity

Sorption capacity represented to the maximum amount of metal adsorbed per gram of the absorbent.

In this section, study of the effect of mercury (II) ions concentration on the sorption capacity of the absorbent by using Langmuir adsorption isotherm. The

Langmuir equation was y = 39.526x + 5E-12 with R^2 = 0.9997. It was found that the exchange capacity increase with increasing concentration of mercury. The maximum capacity was 0.0253 mmol g^{-1}.

4.5 Quality control

The method validation was evaluated after obtained optimum conditions. The method was validated by relating to the accuracy and precision, that was reported in term of percentage of recovery and percentage of relative standard deviation (RSD), respectively. The lowest concentration of mercury that can be detected by this method was represented in term of detection limit (LOD).

4.5.1 Calibration curve and linearity

The calibration curve was done by using different concentration of mercury (II) ions in the range of 0 – 20 ng L^{-1} under optimum conditions. The result show in Figure 16 the linear regression equation was Y = 0.0062X + 0.0552, with r = 0.9919. where Y is the absorbance and X is concentration of mercury in ng L^{-1}, respectively.

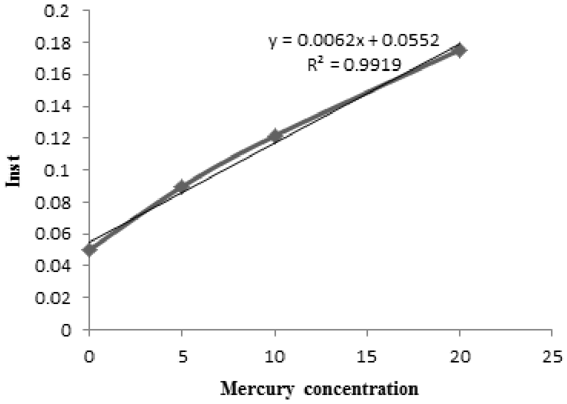

Figure 16 The calibration curve.

4.5.2 Limit of Detection

The limit of detection was obtained by measuring three times of the standard deviation (3 SD) of blank (sample without mercury (II) ions). It was found that, the lowest concentration of mercury (II) ions was determined at 3.18 ng L^{-1} for this method.

4.5.3Accuracy

The accuracy of this proposed preconcentration method was represented in term of percentage of recovery. The accuracy was evaluated by measuring different concentration of mercury (II) ions in 5, 10 and 20 ng L^{-1}. The result showed that the percentage of recovery was found in the range 93 – 119%.

4.6 Application for natural sample

The proposed of this method was applied to determine the amount of mercury in the natural sample. The sample was collected from Chao Phraya river and Pranburi river. To obtain the accuracy of this proposed method, sample were spiked the different of mercury (II) ions concentration 5 and 10 ng into 250 mL of natural water. The recovery of the spiked standard solutions to the sample solution before applying solid phase extraction procedure are shown in Table 9. The percentage recoveries of mercury (II) ions concentration from spike samples at the different concentration were acceptable, which show that this method is well to operative for solid phase extraction of mercury (II) ions from seawater.

Table 9 Analysis of mercury in real sample.

Sample	Amount of Hg(II)added (ng)	Amount of Hg(II)found (ng)	Recovery (%)
Pranburi river	0	16.4 ± 5.0	-
	5	21.6 ±1.6	100.9
	10	25.5 ±4.7	96.6
Chao Phraya	0	ND	
	5	6.16 ±2.3	100.8
	10	11.13 ±3.3	100.6

Moreover, this method was applied to studies the speciation of mercury. The sample collected from Pranburi and Samaesarn river. The sample was filtrated by online filtration. After that, the samples divide for studied of mercury (II) ions and total mercury. For total mercury determination added Bromine monochloride. After pre-reduction of excess oxidant by hydroxylamine chloride before applying solid phase extraction procedure. As shown in Table 10, it can be assumed that this method can apply for determination of mercury species by indirect way.

Table 10 Analysis of mercury in natural sample.

Sample	Total mercury	Mercury (II) ions
Pranburi	16.4 ± 5.0	11.9 ±3.2

CHAPTER V

CONCLUSION

5.1 Conclusion

N-(2-chloro benzoyl)-N'-phenyl thiolcarbamide ligand was successfully synthesized and confirm by NMR sprectrum. Modified sulfur powder absorbent was prepared for preconcentration mercury from water sample. The sorption capacity was studied in order to evaluate the maximum capacity for solid phase extraction. The maximum capacity was 0.0253 mmol g^{-1}. According to the sorption capacity the suitable for column conditions were 4 mg g^{-1} for ligand quantity and 2 g of amount of modified sulfur column perform a highest recovery. Citrate buffer was used for conditioning an absorbent. According to experimental condition optimization, 3M HCl with flow rate of sorption and elution were 15 and 6 mL min^{-1} found to be suitable for elution of mercury (II) ions from column**.** Mercury concentrations were determination by CV-AFS. The calibration curve was constructed at a wavelength of 253.7 nm. Method validation was obtained by the calibration curve. Linear range was 5 to 20 ng L^{-1}. The limit of detection was estimated to be 3.18 ng L^{-1}, with a relative standard deviation 1.024%. The acceptable recoveries of spiked samples were found in the range 93-114%.

Overall, the modified solid phase extraction was successfully tested, validated and applied to study the mercury (II) ions and total mercury in natural sample water in the lowest concentration of 3.18 ng.

5.2 Suggestion for further work

- The linear range should be improved in order to be able to determination mercury in high concentration.
- The method should be test with standard methyl mercury.
- The sample should be test by the other company to confirm the accuracy of the method
- Detection limit should be improved concentration of mercury in natural seawater is as low as 1 ng L^{-1}

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APPENDIX

Effect of ligand quantity

Column 1 Ligand (mg g^{-1})		PA			PH		
		Ints	**SD**	**%RSD**	**Ints**	**SD**	**%RSD**
2	Pro	0.11661	0.0008	1.957	0.0328	0.0002	2.114
	Post	0.089	0.0027	1.862	0.025	0.0007	1.702
4	Pro	0.1134	5E-05	0.26	0.0319	1E-06	0.036
	Post	0.1102	7E-06	0.054	0.031	2E-05	0.488
6	Pro	0.1024	0.0001	0.132	0.02898	0.0002	0.652
	Post	0.0712	0.0013	0.959	0.02014	0.0003	0.858
8	Pro	0.1189	0.0014	1.52	0.035	0.0019	6.32
	Post	0.0694	0.012	1.42	0.0204	0.0024	0.89

Effect of ligand quantity (Cont.)

Column 2 Ligand (mg g^{-1})		PA			PH		
		Ints	**SD**	**%RSD**	**Ints**	**SD**	**%RSD**
2	Pro	0.1358	7E-06	0.054	0.03826	2E-05	0.488
	Post	0.102	0.005	4.21	0.0288	0.0012	3.21
4	Pro	0.1142	0.0009	0.789	0.03559	0.0002	0.523
	Post	0.1129	0.0005	0.448	0.0351	0.0001	0.372
6	Pro	0.1129	0.0029	6.269	0.03215	0.0008	6.299
	Post	0.0802	0.0021	3.35	0.0229	0.0006	6.034
8	Pro	0.11968	5E-05	0.26	0.03367	1E-06	0.036
	Post	0.0732	0.0008	1.957	0.0205	0.0002	2.114

Effect of ligand quantity (Cont.)

Column 3		PA			PH		
Ligand (mg g^{-1})		Ints	SD	%RSD	Ints	SD	%RSD
2	Pro	0.119	0.0011	6.006	0.0337	0.0002	4.894
	Post	0.0894	0.0001	0.934	0.0253	0.0001	2.464
4	Pro	0.1016	0.0029	6.269	0.0269	0.0008	6.299
	Post	0.0998	0.0011	3.538	0.0264	0.0004	4.229
6	Pro	0.1196	0.0012	5.949	0.0257	0.0004	6.461
	Post	0.084	0.0021	3.35	0.0181	0.0006	6.034
8	Pro	0.1157	5E-05	0.093	0.03282	7E-05	0.48
	Post	0.0887	0.0001	0.105	0.0195	1E-05	0.041

Effect of amount of absorbent

Column 1		PA			PH		
Absorbent (g)		Ints	SD	%RSD	Ints	SD	%RSD
0.5	Pro	0.11732	0.0032	5.426	0.03312	0.0009	5.541
	Post	0.0235	0.0008	0.446	0.00664	0.0003	0.516
1.0	Pro	0.1118	0.0037	5.965	0.03167	0.0011	6.121
	Post	0.0582	0.0036	1.195	0.0165	0.001	1.217
2.0	Pro	0.1123	0.002	3.272	0.03177	0.0006	3.204
	Post	0.111	0.0037	1.745	0.0314	0.0013	2.125
3.0	Pro	0.1162	0.0002	0.98	0.0329	0.0004	0.052
	Post	0.0859	0.0013	1.855	0.0243	0.0207	2.143

Effect of amount of absorbent (Cont.)

Column 2		PA			PH		
Absorbent (g)		Ints	SD	%RSD	Ints	SD	%RSD
0.5	Pro	0.1023	0.0005	0.807	0.028	0.0001	0.81
	Post	0.02077	0.0001	0.918	0.00568	5E-05	1.491
1.0	Pro	0.1116	0.0042	7.536	0.0313	0.0012	7.461
	Post	0.062	0.0048	3.005	0.0174	0.0014	3.108
2.0	Pro	0.1076	0.0039	1.812	0.02967	0.0011	1.91
	Post	0.1038	0.0009	0.789	0.02863	0.0002	0.523
3.0	Pro	0.1126	0.0005	0.448	0.03212	0.0001	0.372
	Post	0.0827	0.004	5.98	0.0236	0.021	6.012

Effect of amount of absorbent (Cont.)

Column 3		PA			PH		
Absorbent (g)		Ints	SD	%RSD	Ints	SD	%RSD
0.5	Pro	0.10698	0.0005	2.714	0.03039	0.0001	2.256
	Post	0.0223	0.0001	0.661	0.0063	1E-05	0.274
1.0	Pro	0.1141	0.0002	1.176	0.0325	8E-05	1.635
	Post	0.0605	7E-05	0.405	0.0173	1E-05	0.309
2.0	Pro	0.11382	5E-05	0.293	0.03192	2E-05	0.468
	Post	0.1099	0.0003	1.731	0.03083	5E-05	1.075
3.0	Pro	0.1166	9E-05	0.523	0.0365	3E-06	0.362
	Post	0.09188	0.0001	0.549	0.02877	2E-06	0.075

Table Effect of flow rate on sorption

Column 1 (mL min^{-1})		PA			PH		
		Ints	SD	%RSD	Ints	SD	%RSD
5	Pro	0.1023	7E-05	0.058	0.0293	0	0.001
	Post	0.0833	0.0047	6.251	0.02386	0.0014	6.487
10	Pro	0.1155	0.0017	1.511	0.03312	0.0005	1.572
	Post	0.1076	0.0004	0.558	0.03086	0.0001	0.552
15	Pro	0.1196	0.0002	1.374	0.0382	5E-05	0.978
	Post	0.1182	0.0001	0.664	0.0378	7E-05	1.475

Effect of flow rate on sorption (Cont.)

Column 2 (mL min^{-1})		PA			PH		
		Ints	SD	%RSD	Ints	SD	%RSD
5	Pro	0.1036	0.0002	0.277	0.0289	0.0001	0.609
	Post	0.0835	0.0005	0.963	0.02329	0.0001	0.821
10	Pro	0.10258	0.0002	0.277	0.02874	0.0001	0.609
	Post	0.0969	0.007	1.031	0.02715	0.0002	0.821
15	Pro	0.1132	0.0009	0.767	0.03359	0.0001	0.493
	Post	0.112	0.0005	0.448	0.03325	0.0001	0.372

Effect of flow rate on sorption (Cont.)

Column 3 (mL min^{-1})		PA			PH		
		Ints	SD	%RSD	Ints	SD	%RSD
5	Pro	0.10925	0.000447	2.047	0.02928	0.000136	2.333
	Post	0.0865	0.000233	1.206	0.02319	0.000084	1.58
10	Pro	0.103994	0.000306	1.708	0.02826	0.000075	1.558
	Post	0.09578	0.000201	0.897	0.02603	0.000027	0.433
15	Pro	0.1099	0.000202	0.277	0.03079	0.000125	0.609
	Post	0.10726	0.000617	1.149	0.03005	0.000263	1.747

Effect of flow rate on elution

Column 1 (mL min^{-1})		PA			PH		
		Ints	SD	%RSD	Ints	SD	%RSD
2	Pro	0.11272	0.0016	3.598	0.03167	0.000459	3.63
	Post	0.08815	0.0009	2.147	0.02477	0.000285	2.535
4	Pro	0.115991	0.0012	1.822	0.03274	0.000352	1.828
	Post	0.09948	0.0013	0.326	0.02808	0.000109	0.534
6	Pro	0.11166	0.0014	1.846	0.03188	0.000342	1.609
	Post	0.1104	0.0013	1.57	0.03152	0.00035	1.523
8	Pro	0.1024	0.0005	0.465	0.02925	0.000072	0.249
	Post	0.0964	0.0019	2.559	0.02753	0.00056	2.696

Effect of flow rate on elution (Cont.)

Column 2 (mL min^{-1})		PA			PH		
		Ints	SD	%RSD	Ints	SD	%RSD
2	Pro	0.11932	0.0003205	1.075	0.03312	0.0000963	1.163
	Post	0.08854	0.0000515	2.211	0.0246	0.000178	2.736
4	Pro	0.1129	0.000035	0.056	0.031734	0.000031	0.18
	Post	0.0939	0.001389	2.599	0.0264	0.000417	2.779
6	Pro	0.1142	0.000901	0.789	0.03559	0.000173	0.523
	Post	0.112	0.000506	0.448	0.0346	0.000119	0.372
8	Pro	0.0982	0.003135	5.241	0.01677	0.000869	5.188
	Post	0.09044	0.00241	2.511	0.0154	0.000752	2.774

Effect of flow rate on elution (Cont.)

Column 3 (mL min^{-1})		PA			PH		
		Ints	SD	%RSD	Ints	SD	%RSD
2	Pro	0.10932	0.007	1.031	0.030704	0.000157	0.821
	Post	0.0825	0.0052	2.177	0.02318	0.0001499	2.247
4	Pro	0.11731	0.0014	3.769	0.0329	0.000316	3.079
	Post	0.09957	0.001	2.112	0.0279	0.000255	1.986
6	Pro	0.1085	0.0005	0.963	0.031	0.000116	0.821
	Post	0.1086	4E-05	0.234	0.0317	0.000017	0.349
8	Pro	0.11988	0.0009	2.147	0.03375	0.000285	2.535
	Post	0.10885	0.0004	0.675	0.03064	0.000143	0.889

Effect of elute type

	PA			PH			
	Ints	**SD**	**RSD**	**Ints**	**SD**	**RSD**	**cone**
3M HCl	0.1176	0.0004	3.768	0.0033	1E-04	3.031	10.065
2M HCl	0.1129	0.0005	4.128	0.032	0.0001	3.839	9.3065
1M HCl	0.3191	0.0062	19.42	0.0897	0.0018	19.66	42.56
1MH_2SO_4	0.0973	0.0008	7.809	0.0271	0.0002	7.793	6.7854
1MHNO_3	0.0895	0.0001	1.416	0.0249	4E-05	1.7	5.35

Effect of pH stability

Column 1		**PA**			**PH**		
		Ints	**SD**	**%RSD**	**Ints**	**SD**	**%RSD**
pH2	Pro	0.1373	0.0006	1.149	0.03009	0.0003	1.747
	Post	0.1032	0.007	1.031	0.0225	0.0002	0.821
pH2.5	Pro	0.1193	0.0052	2.17	0.03335	0.0001	2.24
	Post	0.1182	0.0014	3.769	0.033	0.0003	3.079
pH3	Pro	0.1145	0.001	2.112	0.0322	0.0003	1.986
	Post	0.1069	0.0005	0.963	0.0301	0.0001	0.821
pH4	Pro	0.1103	0.0064	3.552	0.03128	0.0013	2.602
	Post	0.0913	0.0014	3.81	0.0259	0.0038	3.644

Effect of pH stability (Cont.)

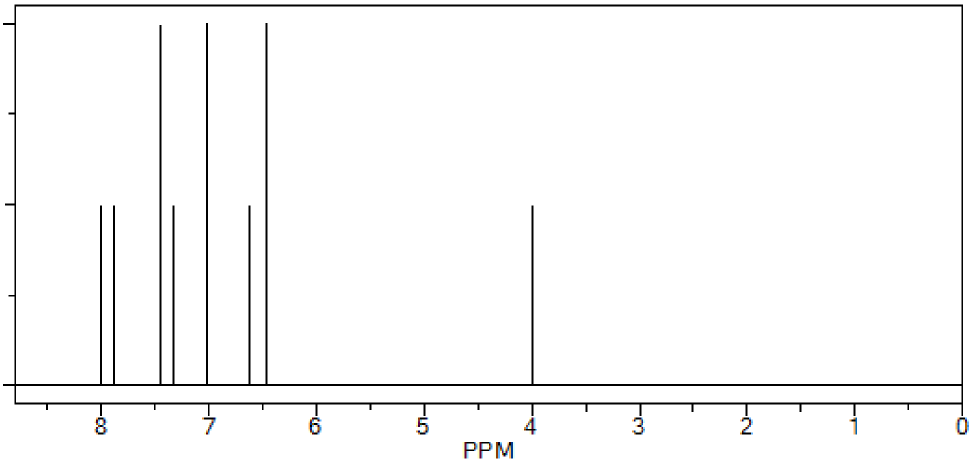
Column 2		**PA**			**PH**		
		Ints	**SD**	**%RSD**	**Ints**	**SD**	**%RSD**
pH2	Pro	0.1175	0.0004	0.373	0.03338	0.0002	0.538
	Post	0.09024	0.002	3.551	0.02594	0.0005	3.053
pH2.5	Pro	0.1142	0.0009	0.789	0.0325	0.0002	0.523
	Post	0.1129	0.0005	0.448	0.03215	0.0001	0.372
pH3	Pro	0.1164	0.0023	1.186	0.02394	0.0006	1.035
	Post	0.1115	0.0008	1.066	0.02506	0.0002	1.201
pH4	Pro	0.128	0.0262	1.46	0.03659	0.0075	1.061
	Post	0.1083	0.0064	1.03	0.3087	0.0017	0.975

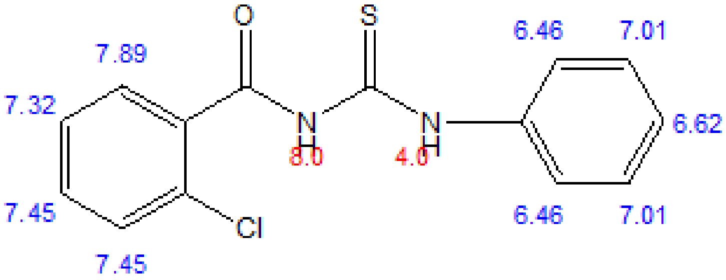
Effect of pH stability (Cont.)

Column 3		**PA**			**PH**		
		Ints	**SD**	**%RSD**	**Ints**	**SD**	**%RSD**
pH2	Pro	0.11761	0.0064	1.3	0.326	0.0017	0.974
	Post	0.898	0.0023	1.186	0.02606	0.0006	1.035
pH2.5	Pro	0.108	0.0025	2.11	0.03459	0.0072	2.46
	Post	0.107	0.0012	1.302	0.03245	0.0003	1.136
pH3	Pro	0.109	0.0009	1.106	0.0312	0.0002	1.096
	Post	0.1026	0.0006	0.837	0.0294	6E-05	0.286
pH4	Pro	0.1044	2E-05	0.031	0.0298	5E-05	0.276
	Post	0.0878	0.0011	2.301	0.0251	0.0004	2.807

Online Filtration

H NMR spectrum





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