ANALYTICAL CHARACTERIZATION OF CHOLINE CHLORIDE IN OILFIELD PROCESS WATERS AND COMMERCIAL SAMPLES BY CAPILLARY ELECTROPHORESIS

By

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ABSTRACT

Shale structures with poor porosity and permeability contain a majority of clay, which can easily swell and slow the production rate of gas recovery by hydraulic fracturing processes. Therefore, a clay stabilizer is an important additive to prevent this problem. Choline chloride, which is replacing many various environmentally-unfriendly clay stabilizers, is considered to have fewer potential impacts on human health and the environment. There are concerns about the purity of choline chloride products for oil and gas companies. The residual choline chloride in oilfield wastewater or drinking water and its effects on marine life have generated additional concerns for many ecologists and environmental regulatory agencies. The current methods involving titration and colorimetry are not sensitive or selective. As a result, a capillary electrophoresis method was developed to unequivocally quantify choline chloride, and possible adulterants such as KCl, NaCl and NH_4Cl which may be present in commercial choline products. The effects of pH and concentration of buffer, type and concentration of organic modifier, detection wavelength and additive on the separation were investigated in order to determine the best conditions for the analysis. The developed method is more sensitive, more robust, faster and simpler than the current methods used in the oil and gas industry to quantify choline chloride in their process waters. To validate the developed CE method, a liquid chromatography-mass spectrometry and the Reinecke salt gravimetric methods were successfully developed and applied on the choline samples. The results obtained from the three methods were found to compare favourably with one another. Using a *t*-test indicated that the three methods are not significantly different and a *F*-test showed that the precisions of the three methods are similar.

Keywords: choline chloride, clay stabilizer, capillary electrophoresis, liquid chromatography/ mass spectrometry, Reinecke salt gravimetry.

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List of Abbreviations

1. CC (Choline Chloride, $C_5H_{14}ClNO$, 2-hydroxyethyl-trimethyl ammonium hydroxide)
2. PB (Hexadimethrine bromide)
3. PVS (Polyvinylsulfonic acid sodium salt)
4. CE (Capillary Electrophoresis)
5. LC/MS (Liquid Chromatography/Mass Spectrometry)
6. LC/MS/MS (Liquid Chromatography/ Tandem Mass Spectrometry)
7. EOF (Electroosmotic Flow)
8. BGE (Background Electrolyte)
9. CZE (Capillary Zone Electrophoresis)
10. UV (Ultraviolet)
11. PDA (Photodiode array)
12. FDA (U.S. Food and Drug Administration)
13. EPA (U.S. Environmental Protection Agency)
14. ESI (Electrospray Ionization)
15. APCI (Atmospheric Pressure Ionization)
16. TOF (Time of Flight)
17. Q-TOF (Quadrupole-Time of Flight)
18. LOD (Limit of Detection)
19. LOQ (Limit of Quantitation)
20. WCSB (Western Canada Sedimentary Basin)
21. Tcf (Trillion Cubic Feet)
22. GIP (Gas in Place)
23. LD_{Lo} (the lowest lethal dose which causes the death of animals or humans)
24. LD50 (the lethal dose that kills 50 percent of a test sample)
25. RSD (Relative Standard Deviation)
26. *m/z* (mass-to-charge ratio)
27. EIC (Extracted Ion Chromatogram)
28. TMAC (Tetramethylammonium chloride)

CHAPTER 1

INTRODUCTION

Petroleum Resources in Canada

Across the world, energy resources have played an important role in the economies of many countries. In other words, modern life has considered energy resources as the lifeblood. In fact, the majority of the energy that Canadians have been consuming comes from crude oil and natural gas. Their uses not only apply to transportation, heating and electricity but also provide raw materials for the pharmaceutical and cosmetic industry, as well as building materials and plastics [1]. Fossil fuel is recognized as the largest source of hydrocarbons in the world, and it can be found across Canada. Three main sources of oil production in Canada are the western Canada sedimentary basin (WCSB), Canada's oil sands (which are in the WCSB) and the offshore oil fields in the Atlantic. Natural gas reserves are concentrated in WCSB and are also found off the coast of Eastern Canada, around Newfoundland and Labrador, Nova Scotia, the Arctic region and off British Columbia's coast. Canada has great prospects of shale gas with at least five basins that accommodate 37.6 trillion m^3 or 1326 tcf (trillion cubic feet) of gas in place (GIP) of which 10 trillion m^3 or 355 tcf has potential for efficient recovery. These five basins illustrated in Figure 1.1 are Horn River basin, Cordova embayment, Laird basin, Deep basin, Colorado groups and these are mainly located in Western Canada [1].

Historically, for over millions of years, sedimentary rocks formed by the accumulation in sedimentary basins of sand, silt and the remains of plants and animals. The schematic geology of natural gas resources is shown in Figure 1.2. They are the places where conventional sources of crude oil and natural gas are discovered. Besides, oil sand, shale oil, and shale gas are considered as Canada's unconventional hydrocarbon resources. Over 97% of Canada's total crude oil reserves are located in the oil sand, and unconventional gas represents about 21% of Canada's recoverable natural gas reserves [1]. Nowadays, even though conventional production is decreasing due to the shortage of conventional resources, the development of technology has encouraged investment, especially in Alberta and British Columbia's unconventional shale resources. Shale is the most

common form of the sedimentary rock which trapped and sealed hydrocarbons into the permeable reservoirs.

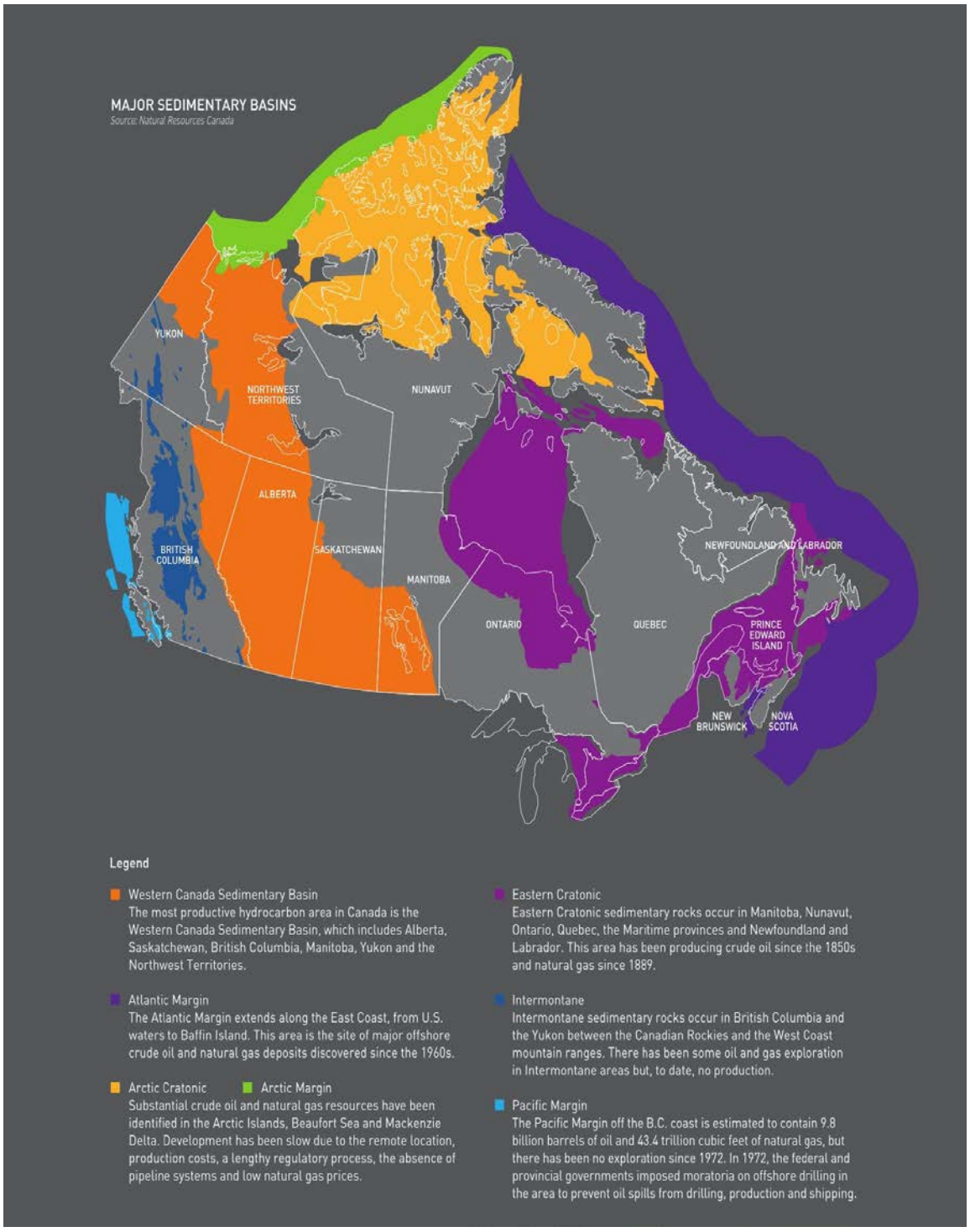

Figure 1.1 - Major sedimentary basins in Canada [1]

Oil and Gas Production Techniques

The demand for fossil fuel is generally going up over time and thus becoming more profitable for the oil and gas industry. Access to these hydrocarbon resources is vital to not only North America but also global countries. The exploration and production of gas has witnessed the developments of many new and efficient techniques to tackle these hydrocarbon resources. Gas recovery from shale reservoirs has been a remarkable breakthrough in North America initially, followed by its wide spread to the global oil and gas industry [2]. Nevertheless, in Canada, only a proportion of the resources in place can be produced economically with existing technology [1].

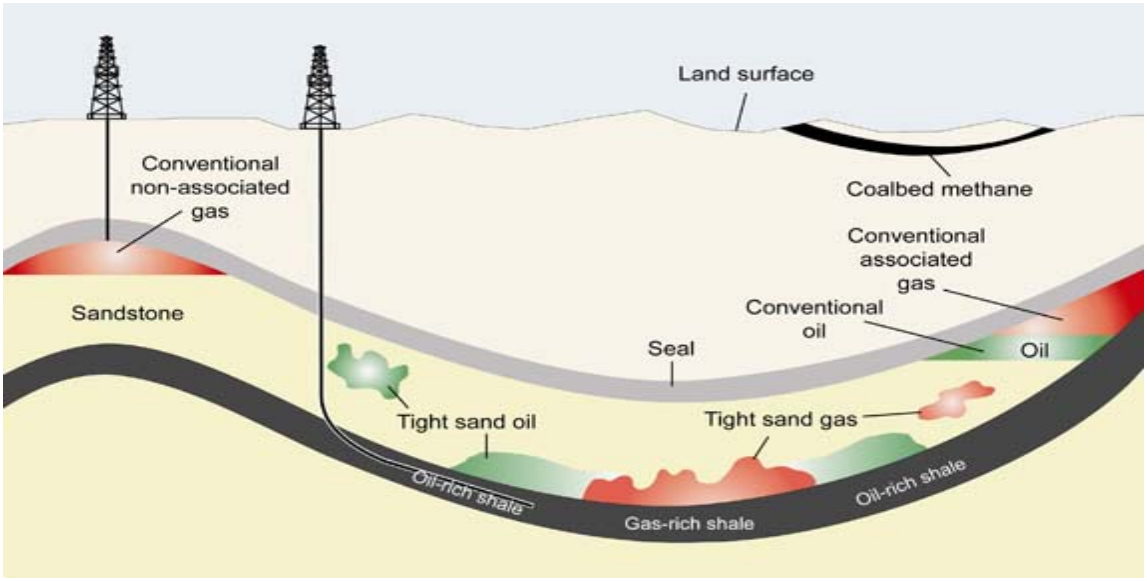

Figure 1.2 - Schematic geology of natural gas resources [3]

The suggested best approach to determine and recover the gas hidden in shale rock underground is the combination of two techniques, fracturing stimulation and horizontal drilling. The hydraulic fracturing stimulation process is the first technology that brought a considerable success to the release of the trapped gas. Undoubtedly, the oil and gas industry has enjoyed a dramatic technological boom time when this approach can support the hydrocarbons to become more permeable in the rock. Vertical wells are generated to fracture shale, providing a very efficient production flow rate at the beginning. However, this efficiency cannot be maintained due to a speedy decrease of this flow rate. Therefore, the effort to overcome this drawback

has led to a second effective technology, horizontal drilling. The horizontal wells can access more gas reservoirs than vertical ones, increasing the efficiency of gas recovery. The conjunction of above-outlined technologies allows the drill extension, bringing undeniable positive outcome for both technical and economic feasibility in shale gas exploration and production [2].

The hydraulic fracturing process, as shown in Figure 1.3, utilizes the mechanical properties of a fracturing liquid to fracture the shale rock. To achieve a pressure high enough to break the rock's hardness, the fluid is pumped into the well with reasonable speed. The water-based fluids are the most popular fracturing agent, allowing large volumes at a feasible cost. In addition to these fracturing treatments, a wide range of chemicals has been innovated to improve the performances of water in more formations. Surfactants and clay-stabilizers have been developed to prevent the emulsions and the collapse of the wellbore. The friction, corrosion or bacterial-growth can be minimized by chemical methods. Another factor contributing to the fracturing is the proppant which supports the permeability by keeping the fissures open. Although there are many special features in shale formations which require many different fluid recipes, the basic fracturing agents always contain three constituents: base fluid (water), additives and proppant [4].

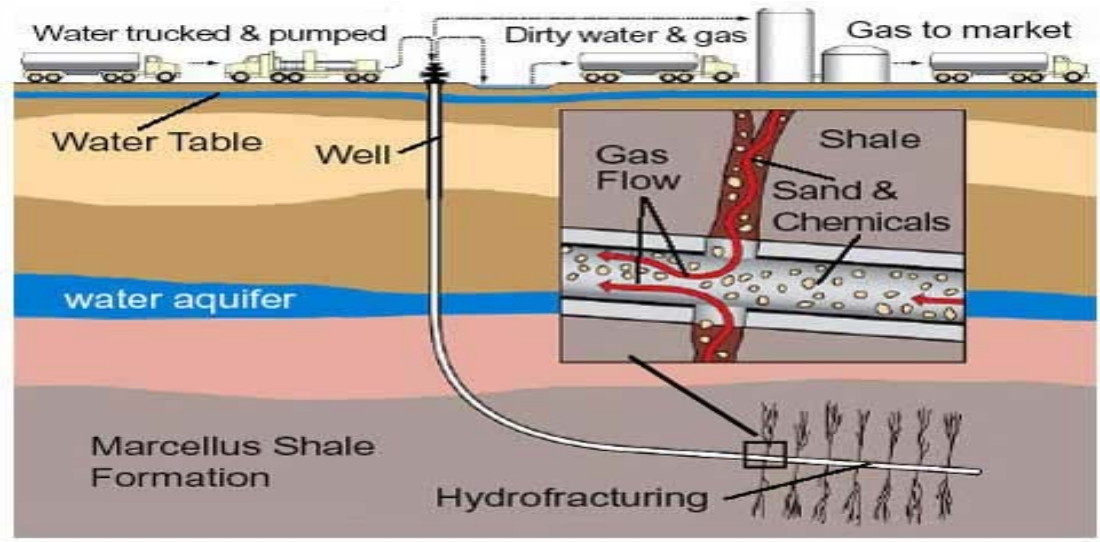

Figure 1.3 - Hydraulic fracturing process [5]

The continuous improvement in technology of hydraulic fracturing undoubtedly accelerates the development of producing shale gas, but meanwhile, causes lots of concerns about environmental impacts. As this technology has been widespread and applied on a large scale worldwide, its drawbacks which can be seen from technical perspectives and environmental problems, cause intense controversy. The water management in the oil and gas industry is illustrated in Figure 1.4. There is an ongoing debate on the possible damages of the hydraulic fracturing caused to the environment by using and losing a large volume of water underground or leaking chemicals into the aquifers as well as the demand to treat flow back and wastewater [6]. Because of the competition within the industry, many chemicals in the drilling fluid were shielded from the public. There are widespread concerns about toxic additives in water contamination and its environmental risk that have raised the pressure of public disclosure of the ingredients in hydraulic fracturing fluid. These concerns led to the recent federal rules that stipulate the disclosure report of all chemicals in drilling fluid [7].

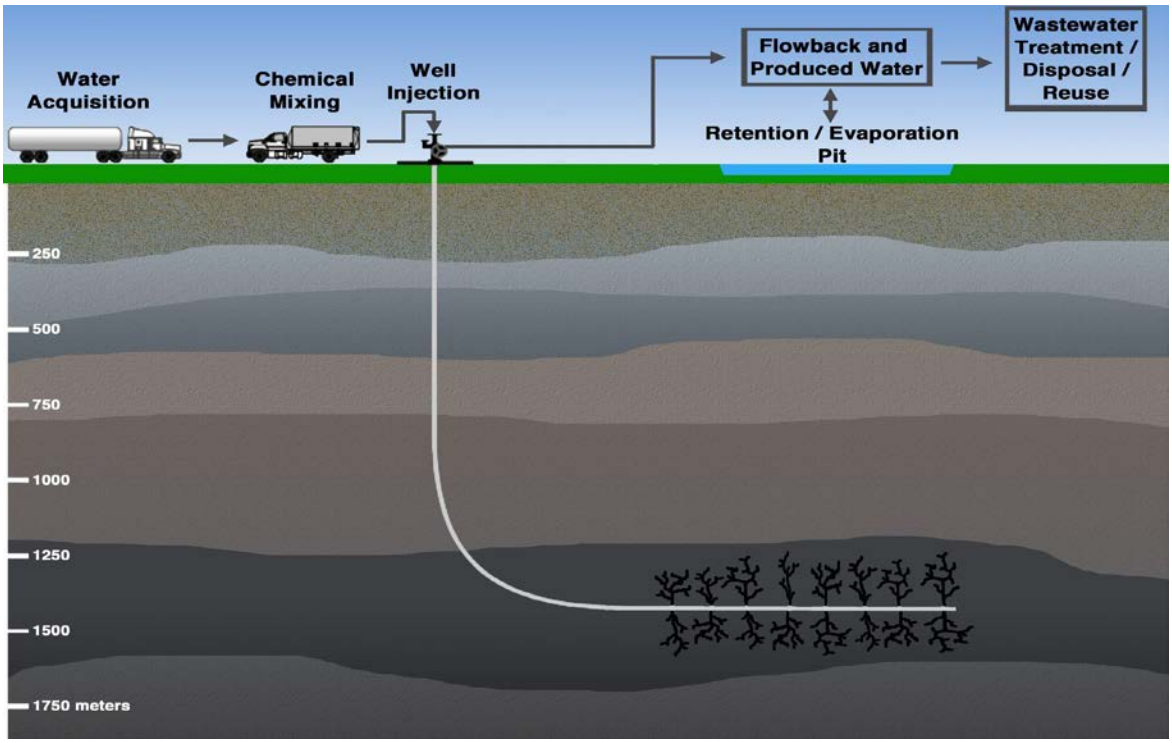

Figure 1.4 - Water management in oil and gas industry [8]

Clay Swelling

The main sedimentary rock structure in Canada is shale with poor porosity and permeability. The stability of shale is an extremely important problem during the drilling process. Some processes have been investigated as the major contributions to the instability of shales such as the fluid's movement between the wellbore and shale, changes in shale-filtrate interaction with stress (and strain), and the softening and erosion due to the mud filtrate's invasion with any chemical changes. Clay hydration is the core reason that leads to these effects. Most of these oil and gas reservoirs contain clays that can attract cations from water or other polar substances. Therefore, there is a tendency for the clays to swell in the presence of water and this may cause a wellbore collapse. Swollen clays are known to be an issue in many engineering problems, such as borehole stability, and foundation stability [6, 9-11]. The clay swelling causes a number of problems with drilling, shale simulation, and hydraulic fracturing in the oil and gas industry. Figure 1.5 shows some of these problems, which might be bit balling, the disintegration of cuttings, instability of the wellbore, migration of the clay fines, reduced permeability of the hydrocarbons in the drilling formation and damage to drilling fluidity. Therefore, clay swelling can cause higher drilling costs and slower production rates [9-11].

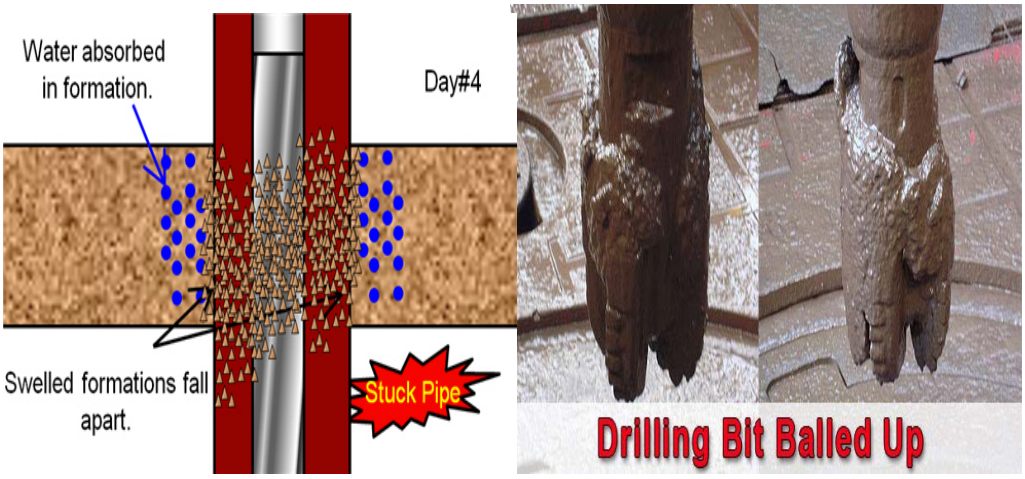

Figure 1.5 - Challenges caused by clay swelling [12]

There have been many types of research done to explain why clays swell due to absorbing the drilling fluid. Generally, clays have many crystal platelets neatly

arranged face to face with very weak attractive force. Different structures of those crystals lead to different properties. Every platelet containing many sheets is a unit layer. The surfaces of the unit layers are basal surfaces. There are two types of sheets: tetrahedral sheet and octahedral sheet as indicated in Figure 1.6. The tetrahedral sheet is composed of silicon atoms tetrahedrally coordinated with oxygen atoms. The octahedral sheet contains aluminum or magnesium atoms, octahedrally coordinated with the oxygen atoms of hydroxyl groups. Within a unit layer, these sheets connect by sharing oxygen atoms. Depending on the number of tetrahedral sheets bonded with one octahedral, the basal surfaces contain exposed atoms or hydroxyl groups. C-spacing is the distance between corresponding planes in adjacent unit layers [11].

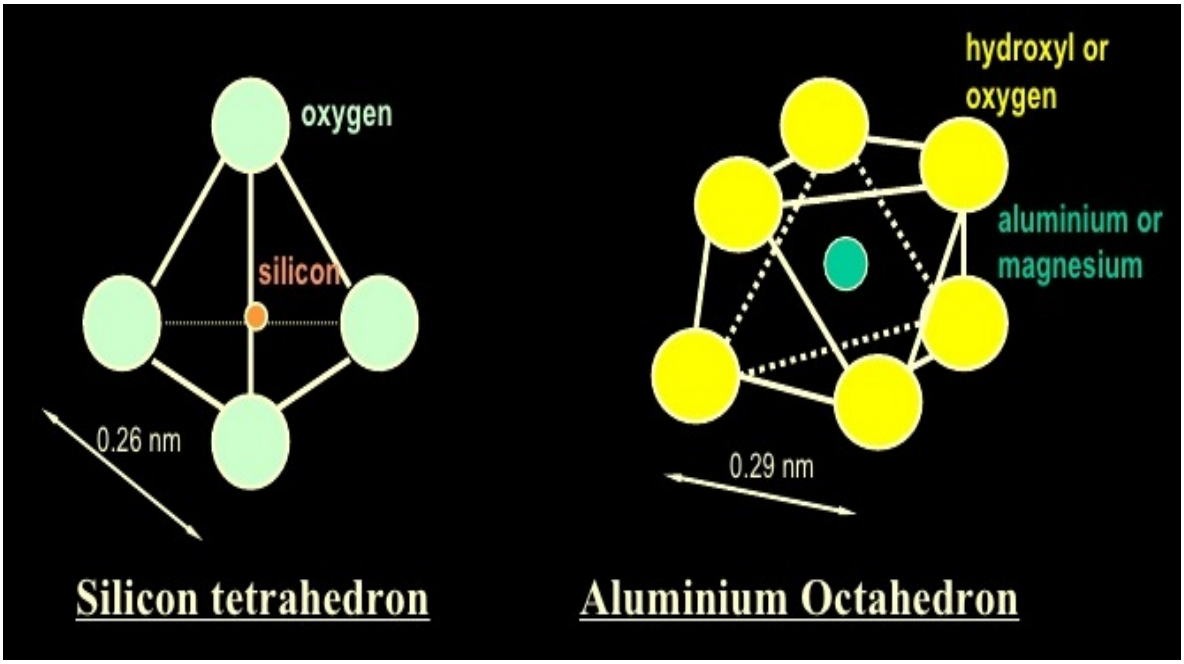

Figure 1.6 - Tetrahedral and octahedral structures of clay [13]

In clay structure, the metal atoms with various valences would be commonly substituted with others within the layer, which results in an overall negative potential at the clay layer's surface. In that case, cations presented in the interlayer region will be adsorbed onto the surface to compensate this charge. Those cations can be freely exchanged when the clay is suspended in water. On the other hand, some cations might also be adsorbed on the edges of clay crystal and exchange with other

ions in the water. These types of substitutions affect clay swelling significantly with the presence of water molecules in drilling fluid, resulting in the increase in c-spacing as well as the volume of clay structures [9-11].

Clay Inhibitors

As a consequence of this swelling, clay inhibitors have been used in every hydraulic fracturing process. In order to maintain the wellbores' stability, many efficient clay stabilizers have been developed to minimize the hydration of clays in water-sensitive shale. The suitable clay swelling inhibitors must not only be capable of reducing the interaction between clay and drilling fluid but also have less impact on the environment. Most studies focus on many inorganic and organic chemical compounds that can hinder the entry of water molecules, resulting in preventing clay swelling. The bond between clay layers is mainly van der Waals linking force. This is a type of physical linking force, which has very small binding energies. Therefore, the bonding of clay layers together is so poor that other molecules can intermix between the clay layers quite easily. In order to make the clays become water-repellent, exchangeable cations in the clay structure are replaced by cationic surfactants such as alkyl ammonium or alkyl phosphate. The most commonly used inorganic cations are Na^+, Ca^{2+}, Mg^{2+}, K^+ and NH_4^+ [11].

Generally, quaternary ammonium compounds are cationic surface active agents and are considered more harmful than anionic or non-ionic surfactants. The positive cationic part is usually the functional group that can generate the irritation effects on the skin. In some case, these compounds can dissolve phospholipids and cholesterol in lipid membranes, resulting in the permeability into cells, causing cell death. Due to the pros and cons of different clay swelling inhibitors, many researchers have been looking for various compounds which can play the role of effective clay stabilizers and also at the same time environmentally friendly [14]. Historically, in the oil and gas industry, tetramethylammonium chloride (TMAC) was used as the main shale inhibitor because of its economy and commercial availability. TMAC prevents the clay in the shale from swelling and blocking the flow of natural gas. However, TMAC has a bad smell, is toxic and can have a serious impact on the

environment. It can also permeate through the soil and underground water during the drilling process [15]. TMAC is very toxic to aquatic organisms and has been proven dangerous on mouse or rat with an LD_{50} of 25-50 mg/kg [16]. Therefore, TMAC must be strictly disposed of as a hazardous waste. With the high concentration of 7.5%, TMAC likely leads to high risks of human exposure such as mucosal and tissue irritation, or major effects like vomiting after ingestion [10,16]. In 2010, TMAC was completely prohibited in petrol drilling fluid by the EPA [15].

Also, potassium chloride (KCl) has been commonly used as a clay stabilizer to prevent the swelling of clay. However, due to the high demand of shale swelling inhibitors, mixing manually a huge amount of powdered KCl with water became inconvenient during the process [17]. Besides, KCl also has some disadvantages in that it is not environmentally biodegradable and it releases a lot of chlorides into the water. Primarily, in drilling there are some environmental concerns as chlorides released from this compound do not degrade and can make their way into the surface water carrying heavy metals such as cadmium and mercury. Moreover, potassium chloride can be incompatible with other materials, negatively impacting other aspects of fracturing fluids such as gelation [1,9].

Choline Chloride

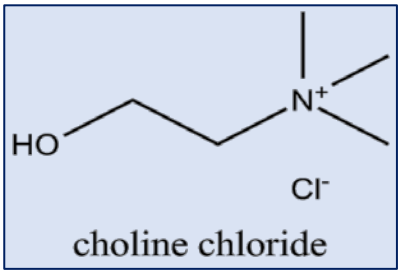

Figure 1.7 - Structure of choline chloride

There has been a huge effort to find suitable replacements for the aforementioned clay stabilizers. Choline chloride, $C_5H_{14}ClNO$, (chemical name is 2-hydroxyethyl-trimethyl ammonium hydroxide) is identified as the best substitute for previous inhibitors. Choline chloride provides an attractive, effective and more environmentally responsible alternative than KCl as a clay stabilizer. Choline chloride is a water-soluble organic compound as a quaternary ammonium salt. The choline portion is the active group that can stabilize the clay swelling. This clay inhibitor is considered environmentally biodegradable, nontoxic, compatible with other drilling auxiliaries and the amount of chloride released is 70-75% less than with KCl [14,18]. The average amount of choline

chloride used in the drilling process might vary depending on the nature of the shale, from 500 to 2,000 ppm or 0.05% - 0.2% of the total fluid pumped [17]. With the huge amount of this additive used in the water-based drilling process, it is critical to have a comprehensive understanding of this compound.

While the efficacy of choline chloride is recognized, its impact on the environment is considered safe because it is used as a nutritional supplement in animal feed or human supplement. Choline is one of the vitamin-like necessary nutrients and a methyl contributor related to many physiological processes as well as normal metabolism. Moderate utilization of choline (≥7,500 mg) has been associated with lower blood pressure, sweating, fishy body smell, and gastrointestinal side impacts. For choline level, infants just need around 125 mg/day and the children's demand is 550 mg/day. The adequate upper intake level (UL) for healthy adults is 3,500 mg/day. With a choline level near the UL, people with diseases related to liver or kidney or Parkinson's symptom might face side effects. High doses (10,000 to 16,000 mg/day) of choline can result in fishy body smell, retching, salivation, and expanded sweating. The fishy body odor has been associated with production and excretion of too much trimethylamine, also known as a metabolite of choline. In the acquired condition called principal trimethylaminuria, an inadequate flavin containing monooxygenase 3 (FMO3) enzyme leads to debilitated oxidation of trimethylamine in the liver. Disease management consists of the utilization of choline-restricted diet plans in affected people [19-23].

Because of potential adverse effects when choline chloride contacts with the eye or skin or over exposure, it should be handled with care to prevent any irritation, pain or dizziness. There are some reports about the toxicity level of choline chloride as follows: [22-23]

- LD_{50} (ingestion): 3400 mg/kg (rat)
- LD_{50} (intraperitoneal): 320 mg/kg (rat)
- LD_{50} (intravenous): 53 mg/kg (mouse)
- LD_{Lo} (intraperitoneal): 500 mg/kg (rabbit)

- LD_{Lo} (intravenous): 1100 µg/kg (rabbit)
- LD_{Lo} (subcutaneous): 735 mg/kg (mouse)
- TD_{Lo} (ingestion): 331 mg/kg/14 weeks-continuous (rat)
- TD_{Lo} (intraperitoneal): 3564 mg/kg/5 weeks-intermittent (rat)

For ecotoxicity, although choline chloride is considered safe for the environment, the limitation of data does not provide an adequate report and knowledge about its potential impacts [23-24]. The concern has also been raised by environmental specialists and ecologists because of the availability of this limited ecotoxicity data of choline. On the other hand, there is no proper research about any effect of choline on marine life and human health or the potential effect on the quality of drinking water resources. To answer these concerns, the industry really needs effective analytical methods to quantify the content of choline chloride.

Choline chloride has all the features of an effective clay stabilizer based on the information below. Rule-based criteria of powerful clay-swelling preventers were designed by a group of researchers, resulting in significant characteristics that the cationic inhibitors should have. For example, they should be able to replace ions in the interlayer of clay, possess a water-soluble as well as hydrophobic backbone, have primary di-amine or mono quaternary amine functionality and have little alcohol functionality. In addition, the hydrophobic backbone of the cationic inhibitor should be long enough to form a dense monolayer in the interlayer [25]. The mechanism by which choline chloride stabilizes clay is demonstrated in Figure 1.8. The surface of clay has a negative charge which always attracts the cation H^+ from the water. Then the clay will be very wet and swollen, causing stress on dry shale which makes it unstable. When choline chloride is applied, the positive ammonium part of choline is attracted to the surface of the clay. Then the alkyl chains of choline would behave as a hydrophobic agent, preventing water attack into the clay. Therefore, the shale will be dry and stable [10].

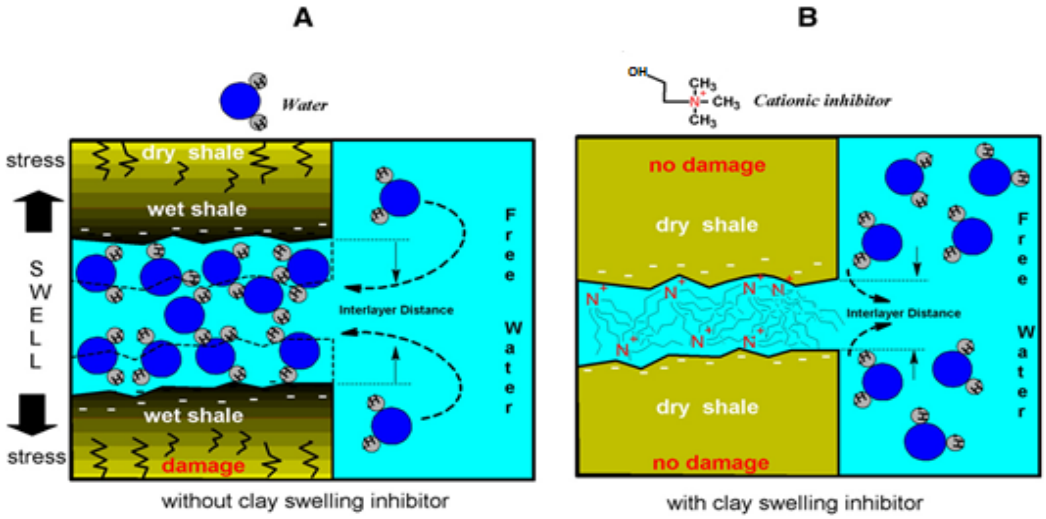

Figure 1.8 - Improved clay with choline chloride [10]
(A) Without cationic inhibitors' action, and (B) Under the cationic inhibitors' action

Because of the prominent features of choline chloride as a clay inhibitor, the consumption of choline chloride has been increasing sharply in the oil and gas industry. Its growth creates concerns for many different subjects, including suppliers, oil and gas companies and environmental specialists. The first company that marketed choline chloride is Balchem in the US. Nowadays, there are more competitors around the world, especially from China. Some of them provide a lower quality of choline chloride with a lower price. The good products usually contain 60-100% content of choline chloride. Some companies might add different salts such as ammonium chloride (NH_4Cl) and potassium chloride (KCl) to simulate the content of choline chloride. Some current analytical methods did not identify this adulteration. For example, Balchem company did a test on a product that has 35% choline chloride + added 16% NH_4Cl. The results by ion chromatography showed as 60% choline chloride [26]. Because of this reason, the suppliers are concerned with how to determine the content of choline chloride in a more accurate way. For the oil and gas companies, they worry that they have overpaid for low-quality products. If they bought the products with low content of choline chloride, they might encounter significant risks during the production because of an overestimated amount of clay stabilizer.

Previous and Current Analytical Methods

Choline is present in many different kinds of food and supplement as free choline or esterified form. It is considered as an important micronutrient which is critical for proper function of the human body [27]. A variety of analytical approaches with different instrumentations have been developed for determination of choline in food and dietary supplements. Nuclear magnetic resonance (NMR) spectroscopy was used to determine level of total choline in milk and infant formula [28]. In this work, free choline with other choline esters were extracted by perchloric acid or chloroform and methanol based on aqueous or lipid fraction before using proton nuclear magnetic resonance spectroscopy. The separation of free choline and other choline esters still showed some overlapped signals at certain experimental pHs, therefore multiple pH levels were applied for the analysis [28]. Tsai *et al* and Szilagyi *et al* used gas chromatography – mass spectrometry (GC/MS) to determine choline and choline esters concentrations. The constraint of this method is that choline compounds must be converted into volatile tertiary amines by pyrolysis GC–FID before injection into GC [29-30]. In the work by Pomfret and coworkers, choline in rat's organ, blood, plasma and in human plasma was also converted to volatile derivatives such as propionyl ester or demethylated compounds and then analyzed by gas chromatography [31]. Another popular method for choline analysis is hydrophilic interaction liquid chromatography (HILIC) coupled to tandem mass spectrometer. Lewis *et al* used HILIC to analyze choline in commercially available pulses [32], and Wang and coworkers determined the choline contents in human liver tissues by HILIC [33]. Wang showed that hydrophilic interaction chromatography (HILIC) improved the retention of the strong polar quaternary ammoniums on the column compared to reversed-phase liquid chromatographic columns, resulting in enhanced separation [33]. In both HILIC works, mobile phase A was aqueous ammonium formate with 0.1% formic acid (v/v) while mobile phase B was acetonitrile [32-33]. Holm et al used similar mobile phases in a liquid chromatography – tandem mass spectrometry (LC/MS/MS) for the analysis of choline in plasma with normal-phase column packed with particles of Hypersil silica [34]. In another work by Airs and Archer, liquid chromatography – mass

spectrometry (LC/MS) with reversed phase as a Spherisorb S5 ODS1 column in the positive ion mode was employed to quantify choline in seawater particulates from ocean waters [35].

In addition to the above analytical methods, capillary electrophoresis has also been utilized for choline analysis. Capillary electrophoresis with UV detection has been developed to determine choline in pharmaceutical preparations [36-38], infant formula and selected foods [38], Chinese traditional medicine [39], and plant leaves [40]. In the works by Lambert *et al*, Chernoy'vants et al and Carter *et al*, indirect UV detection with uncoated capillary was mainly used to separate choline with other compounds because choline did not absorb in most UV range [36-38]. There are few studies in which direct UV detection with phosphate buffers was used for the analysis [39-40]. The background electrolytes used in the various CE studies were 5 mM creatine at pH 3.2 under the wavelength of 210 nm [36]; mixture of 6 mM benzimidazole and 2.5 mM tartaric acid at pH 5.25 and wavelength of 253.7 nm [37]; 5 mM 1-methylimidazole at 214 nm [38]; 30 mM phosphate at pH 9.25 at a wavelength of 200 nm [39]; 100 mM sodium phosphate/phosphoric acid at pH 2.0 at same wavelength [40]. Wahby and coworkers developed a capillary electrophoresis-electrospray mass spectrometry to enhance the qualitative and quantitative analysis of choline in hairy root extract of marijuana using a background electrolyte of 20 mM ammonium acetate at pH 8.5 with a wavelength of 200 nm [41]. Capillary electrophoresis with indirect electrochemical detection has also been applied to detect choline and acetylcholine but it was reported that these compounds must be converted to choline oxidase and acetylcholinesterase, respectively, by selective enzymatic reaction before the CE analysis [42-44]. The applied background electrolytes were mainly 50 mM 2-aminoethane-sulfonic acid (TES) at pH 8.0 [42-44] and 20 mM phosphate at pH 7.0 [43].

Although choline chloride has been consumed in large amounts in the oil and gas industry, not many researchers pay attention to the determination of its content in oilfield waters. Choline chloride concentration in oilfield waters could be determined by different methods that have their own benefits and drawbacks. The nature of the

sample and the purpose of the analysis determine which technique should be applied in practice. Unfortunately, the current methods are usually time-consuming and not sensitive and selective for choline [26,45]. Moreover, some protocols have individually defined choline but they have not provided simultaneous separation among choline with other compounds in oil and gas processing waters [29-31]. Most current methods to determine choline in oil and gas industry relate to titrations, ion chromatography or gravimetric analysis.

Mohr/Volhard titrations are traditional methods that measure the amount of chloride in the sample solution through a silver nitrate ($AgNO_3$) titrant, where the amount of choline chloride is then calculated. This titration results in the formation of silver chloride precipitate. Subsequently, the residue of titrant will be titrated again with indicator ions such as ammonium thiocyanate and the ferric ion, Fe^{3+} or the chromate ion, CrO_4^{2-} to form visible endpoint with color changes [46-47]. This method is specific for chloride only and not for choline. Unfortunately, it has become all too common whereby some production companies simulate higher choline chloride content by adding other chloride salts to a choline chloride product, such as KCl, $MgCl_2$ and NH_4Cl [48]. Therefore, the current methods cannot identify the adulteration. Another titration method used relates to perchloric acid in acetic acid. This is a non-aqueous titration to measure choline salts through getting a precise endpoint. It also suffers the same drawbacks as the Mohr/Volhardt method because it cannot detect the effect of NH_4Cl, other ammonium salts and other amine compounds on the result [48]. Ion chromatography is another method used to measure all positively charged ions, including choline. Without good separation, the result can be influenced by the presence of some cations in the product such as potassium and ammonium. Though this method can reveal possible adulteration of the choline chloride content, the drawbacks are that it is not specific for choline and the instrument is quite expensive [26,49].

Gravimetric analysis is another option to determine choline chloride content. This method is based on the quantitative isolation of choline in the sample by forming and weighing an insoluble combined complex or precipitate. The content of choline in the

original sample can be calculated based on the weights of sample and precipitate. Sodium tetraphenylboron, $NaB(C_6H_5)_4$ solution, is one of the agents that precipitate choline [50]. This method is effective, but not specific among choline, potassium and ammonium ion [48]. Reinecke salt gravimetric method is an improvement of traditional gravimetric analysis because it is selective for amine compounds such as choline. This method measures choline through formation of a choline reinecke, which then is quantified gravimetrically. This choline reinecke can be also dissolved in acetone for colorimetric determination. Although Reinecke salt gravimetric method is quite difficult, time-consuming, and involves a huge amount of labour work [26,45], it is the most common protocol used to quantify choline content in the oil and gas industry.

In addition to the drawbacks mentioned above for the current methods, none of the current methods provides a reliable single test to quantify choline chloride unequivocally. In addition, the adulterants that may have been added cannot be easily identified by the above methods. Sometimes, different methods need to be combined to confirm the content of choline chloride. This approach is therefore difficult, slow, and not effective.

Research Goals and Importance of the Research Project

In view of the high demand of choline chloride in the oil and gas industry, it is very important to monitor its concentration for manufacturers, oil and gas companies and ecologists. The developed method might become a powerful tool to detect and quantify choline content in the oil and gas industry. This research focuses on the following goals:

- Develop a sensitive, rapid, robust and efficient analytical method to separate choline from other compounds that might be present in oilfield process waters using capillary electrophoresis (CE).
- Develop an analytical protocol to detect and determine the concentration of choline chloride in oilfield process waters using capillary electrophoresis (CE).
- Validate the method by comparing the result with other analytical methods such as LC/MS and the Reinecke salt gravimetric method.

CHAPTER 2

ANALYTICAL TECHNIQUES

Techniques and Instruments Used

Capillary Electrophoresis

Basic Theory

Capillary electrophoresis (CE) is a modern analytical technique with high separation efficiency, excellent sensitivity and rapid analysis. In addition, CE technique can minimize the amount of sample, solvent consumption and hazardous waste production. Capillary electrophoresis is a separation technique based on the different movement rates of various electrically charged ions in solution under the influence of an electrical field [39,51-52].

Electrophoresis is the migration of charged ions in a conductive liquid medium under the influence of an electric field [51]. Arnes Tiselius is the father of the electrophoresis technique when he developed the system to separate proteins in 1930. This work earned him the Nobel Prize in 1948 [52]. His work has been developed and improved by many different scientists since then. Nowadays, to increase the efficiency of capillary electrophoresis, a capillary with very small diameter is commonly used to eliminate overheating at high applied voltages [51,53]. Nowadays, CE uses a fused silica capillary with typically a 25-100 μm inner diameter [51]. To generate an electric field through a capillary, the conductive liquid medium will transfer the flow of electric current. This liquid is usually called a background electrolyte (BGE). The mobility of ions toward a cathode depends on the electrophoretic mobility and electroosmotic flow [40,51].

Electrophoretic mobility is an ions' ability to move through a buffer solution in the capillary to the opposite charged electrods with different electrophoretic velocity (v_{EP}) and direction in an electric field, depending on their charge and size. Neutral particles stay static because they do not react to an electric field [51]. The electrophoretic mobility of an ion is proportional to the charge-to-size ratio, and inversely proportional to the viscosity of the buffer. The electrophoresis velocity of the ions is directly proportional to applied electrical field and electrophoretic mobility.

The electrophoretic velocity (v_{EP}) can be calculated by the equation below [51]:

$$v_{EP} = \mu_{EP}E$$

where,

$v_{EP:}$ is electrophoretic velocity (cm/s)

E is applied electrical filed

μ_{EP} is electrophoretic mobility (cm^2/V), given by:

$$\mu_{EP} = q/6\pi\eta r$$

where,

q is the charge of ionized solute

η is the viscosity of the buffer

r is the radius of the ion

The electroosmotic mobility is electroosmotic flow (EOF), generally moving from anode to cathode. EOF is generated by applying a high voltage to a capillary filled with an electrolyte. The movement of background electrolyte (BGE) in the applied electric field is the electroosmotic mobility. As demonstrated in Figure 2.1, EOF is generated because of the ionization on the capillary wall and the adsorption of BGE ions on the charged surface after that.

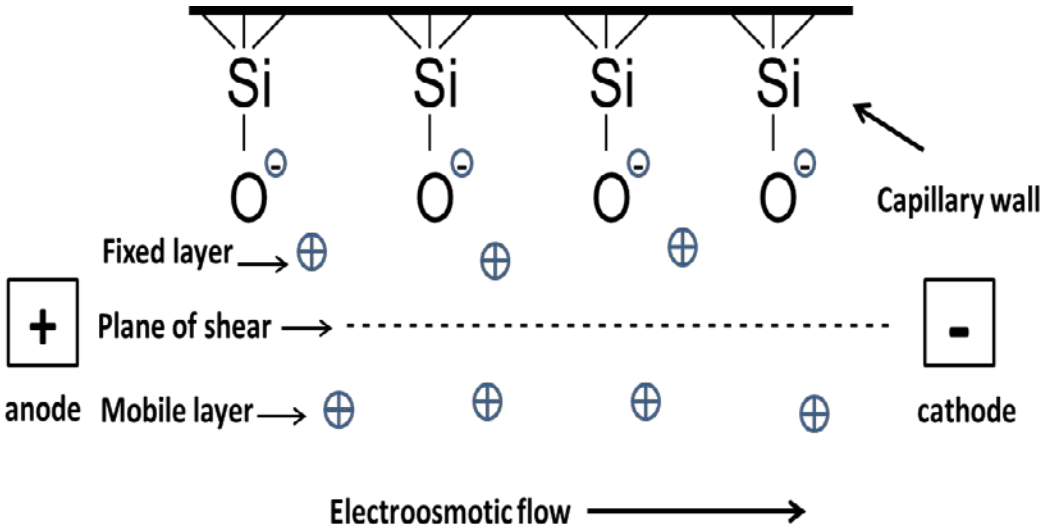

Figure 2.1 - Representation of electroosmotic flow in the capillary [4]

With a pH over 3, the silanol groups (SiOH) of capillary inner wall are deprotonated into silanoate ions (SiO^-). The cations from buffer solutions with opposite charges get attracted to the negatively charged surface of the capillary to create a fixed layer at the wall. This layer cannot neutralize all silanoate groups, resulting in a second cation layer being formed which is more mobile. There is an electrical imbalance between two layers, a plane of shear, which generates potential difference across the layers. It is called the zeta potential ζ. The outer layer can move through the capillary under the electric field, generating a strong flow to drag all solutes and buffer solutions toward the cathode. This flow is electroosmotic flow (EOF) [40,51]. The electrophoretic velocity (v_{EP}) can be calculated by the equation below [51]:

$$v_{EP} = \mu_{EP} E$$

where,

$v_{EP:}$ is electrophoretic velocity (cm/s)

E is applied electrical filed

μ_{EP} is electrophoretic mobility (cm^2/V), given by:

$$\mu_{EP} = q/6\pi\eta r$$

where,

q is the charge of ionized solute

η is the viscosity of the buffer

r is the radius of the ion

The electroosmotic mobility depends on buffer characteristics such as dielectric constant, viscosity, pH and concentration that can influence the zeta potential. The electroosmotic velocity is proportional to the electroosmotic mobility and the applied electrical field.

Generally, the buffer and neutral components will move to the cathode under the effect of EOF and with the same rate of the EOF. With the combination of the two kinds of mobility, the cations will migrate faster to the cathode. Because the EOF is usually stronger than the electrophoretic mobility, an anion will still move “upstream” to the cathode. Therefore, the order of ions that reach the cathode is cations first, neutral then, and finally anions as shown in Figure 2.2. Their migration depends on

their charge-to-size ratios. The migration time refers to the time that takes a solute to move from the capillary inlet to the detector window [51].

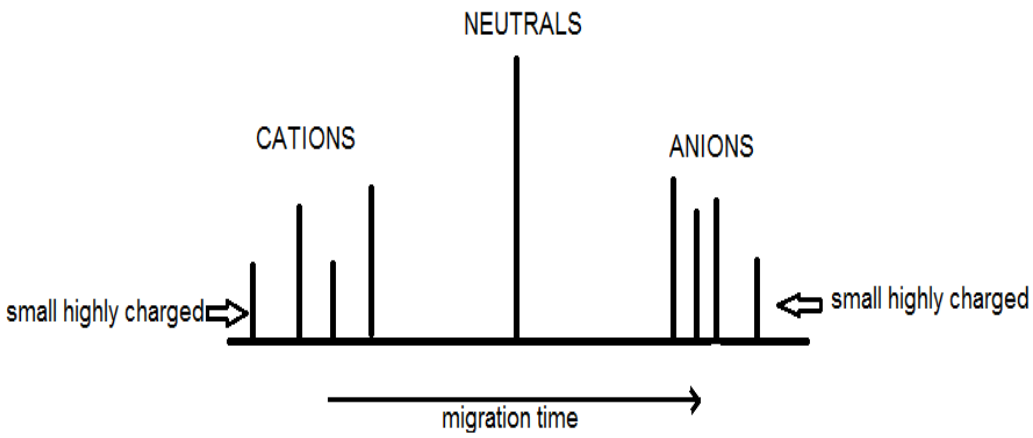

Figure 2.2 - Elution order in a capillary zone electrophoresis [51]

Capillary zone electrophoresis (CZE) is the most basic and popular technique to detect analytes based on the differences in mobility. Theoretically, in zone electrophoresis, the solutes will move through the buffer as zones without any diffusion or spreading to prevent blending into each other. In electrophoresis, while there is diffusion that is parallel to the migration direction of the samples, there is also radial molecular diffusion in a perpendicular direction. These diffusions are minor compared to the velocity with which the analytes migrate through the buffer, so the zone spreading in this situation is insignificant. The diffusion that has notable impact on zone spreading is thermal or convective diffusion, which is caused by Joule heating generated when the electric current moves through conductive solution. The heat motivates molecules in the centre of the capillary with warmer temperature to move faster compared with those near the cooler wall, resulting in zone spreading. To prevent this problem, it is necessary to minimize and dissipate Joule heat, and keep zone electrophoresis narrow. One of the widely used approaches to minimize the thermal diffusion is reducing the diameter of the capillary tubing [51-52].

CE System

A basic CE system as shown in Figure 2.3 consists of several main components such as a controllable high voltage power supply, inlet and outlet buffer vials, a capillary with optical viewing window, a detector, and a data display device like a computer. Most systems also have a cooling system to control or dissipate heat inside the capillary [51-52]. The power supply can be set at a constant voltage up to 30 kV or a constant current up to 300 µA as well as a constant power up to 6 W. The polarity can be also reversed. Because the migration time varies if the voltage changes, it is very common to operate at constant voltage. The most important variable in CE is the composition of the buffer, which influences the EOF. Any small change in pH or concentration of buffer can affect the migration time of solutes. Capillaries in CE system usually have very small inner diameters, around 25-100 µm. It is very common to use a fused silica capillary instead of the glass ones because they are still transparent at shorter wavelengths than 280 nm. An external coating of polyimide covers the capillary, making them stronger, more flexible, durable and not easily broken [51].

The light intensity I_{in} through the capillary comes out with smaller intensity Iout

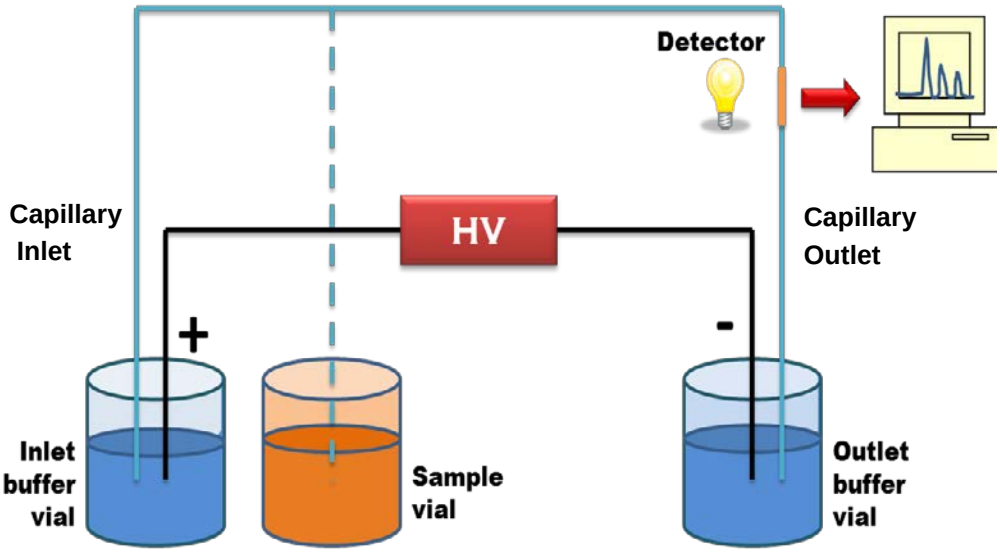

Figure 2.3 - Schematic diagram of capillary electrophoresis [Personal communication, Donkor's lab, TRU)

because of the absorbance by the solutions as shown in Figure 2.4. The photodetector, a photodiode, detects the intensity of light out [51].

The transmittance, T is calculated by [51]:

$$T = \frac{I\ out}{I\ in}$$

UV detection measures the absorbance, A, of solutions through a capillary having a certain path length, giving the output in absorbance unit (AU). The concentration of absorbing solutes is proportional to their absorbance, corresponding to Beer's law as follows [51]:

$$A = \log\frac{1}{T} = abC$$

where

a is the extinction coefficient or molar absorptivity.

b is pathlength of cell in cm (the capillary i. d.)

C is the concentration of solute

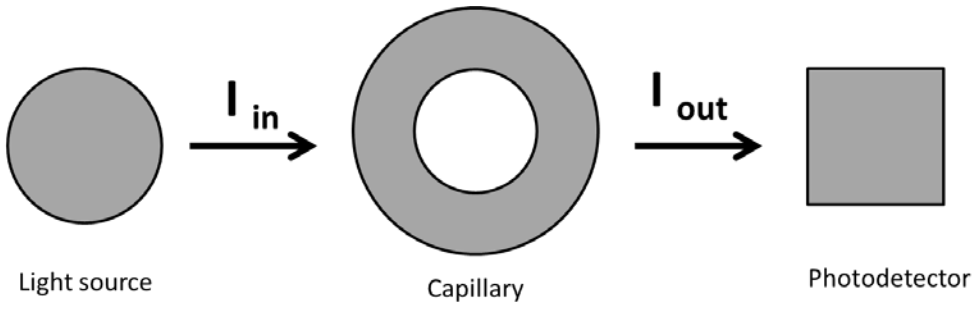

Figure 2.4 - UV absorbance detection [4]

The chromophore of the analytes, the wavelength of UV light as well as the pH and composition of the background electrolyte have significant impacts on the absorptivity. The chromophore is the light absorbing part of a molecule, which gives maximum absorbance at a certain wavelength. Various chromophores absorb light strongly at different wavelengths, but most components have some absorbance at 190 nm. Thus, it is advisable to use the longest wavelength which can provide the best selectivity. The different absorptivity of compounds also affects the peak heights as well as the sensitivity, leading to different limits of detection. The PDA detector is another type of detector that can detect samples at different wavelengths

with one injection. Therefore, a PDA detector can provide spectral analysis of the samples. The spectrum of a standard can become the signatures that can be compared with sample peaks to identify unknowns. With a PDA, the optimum wavelength can be investigated for different solutes [51].

Either UV detection or PDA detection can be used for direct or indirect detection. Direct detection can be applied for the normal compounds that can absorb UV light strongly, resulting in high positive peaks in the electropherogram without any software adjustment. Nevertheless, for some solutes that do not have strong UV absorbance, indirect UV detection can be an option. In most of these cases, the buffers will contain UV absorbing compounds, chromophoric ions, as visualization agents to make the background electrolyte absorbent with indirect UV detection. In the electropherogram, the absorbance of buffer with those UV absorbing compounds will show up as the baseline with a high background signal. When the non-UV absorbing solutes pass through the detector, their peaks will be a negative dip compared with the baseline. These negative peaks will be flipped to become positive in the electropherogram by a software that revers detector's output polarity [37-38,51].

Generally, an analysis by CE includes some basic steps. Firstly, the capillary is pretreated if necessary before it is filled with buffer solutions at a fixed pH. Then, a few nanoliters of sample are injected into the capillary, followed by its separation upon application of a voltage. The high voltage also creates a flow of buffer through the capillary. This movement of the buffer is electroosmotic flow (EOF), dragging sample components to the detector with different speeds based on their charge and mass. The solutes are detected by a detector and displayed as peaks in an electropherogram. The location of a peak indicates the identity of component and the peak area is a measure of the amount of the component [51].

Factors Affecting CE Analysis

- Capillary Inner Diameter

As mentioned above, capillary diameter has an influence on the convective diffusion which is desirable to be minimized in the analysis. The smaller the capillary radius

is, the less convective diffusion is generated due to the smaller temperature difference, leading to narrower zones, and better separation of the zones. ΔT, the temperature difference between the center and the wall of the capillary, is given by the equation shown [51]:

$$\Delta T = (\frac{0.239Q}{4k})r^2$$

Q: power density (Watt/m^3)

k: thermal conductivity

r: capillary radius

In other words, reduced capillary diameter provides higher electrical resistance and less current for the applied voltage, resulting in less Joule heat. Moreover, a small capillary dissipates heat faster because of a larger inner surface area-to-volume ratio. In a smaller capillary, the solutes also tend to move to the detector as single zones. In a larger capillary, the solutes around the warm center will move through the tube with different speeds compared to the ones at the cooler outer wall, leading to two distinct zones for the same solute. Therefore, for CE analysis, it is desirable to use a capillary with the smallest diameter possible, as it can provide good separation as well as reduce Joule heat and increase heat dissipation [51-53].

- Chemical Modification of the Capillary Wall (Coating)

The capillary wall can be modified by coating or chemical bonding to reduce or eliminate the EOF. These modifications can reduce the zeta potential by shielding the surface charges on the wall, resulting in a reduced or eliminated EOF. They also increase the viscous drag on the buffer at the wall, thus reducing the EOF rate. A coating can also minimize the solute adsorption to the wall, providing better results in some cases. When applying a chemical treatment to a capillary, in order to assure reasonable detection time, enough EOF is still a requirement, the amount required depending on the analytes [51-52].

- Voltage

High voltages of up to 30 kV can be used because the capillary has a small diameter and can dissipate heat quickly. The EOF is proportional to the electrical field which depends on the applied voltage. The higher the voltage applied, the shorter the migration time of samples because of the increase in EOF. High voltage also provides faster separation and better efficiencies. Theoretically, it is better to use the highest voltage because of faster analysis time and narrow peaks. Nevertheless, Joule heat will be increased by the higher voltage, a decrease of the buffer's viscosity or an increase of flow rate. The Joule heat may cause broader peaks, unstable migration times, solution decomposition or denaturation, or buffer boiling which is a cause of electrical discontinuity. Therefore, the applied voltage should be reasonable so that the heat can dissipate well in capillary. The maximum optimized voltage can be investigated and determined based on a plot of Ohm's law, which is a plot of the observed currents versus the applied voltages as shown in Figure 2.5. The point at which nonlinearity starts represents the maximum voltage that can be applied to prevent Joule heat [51].

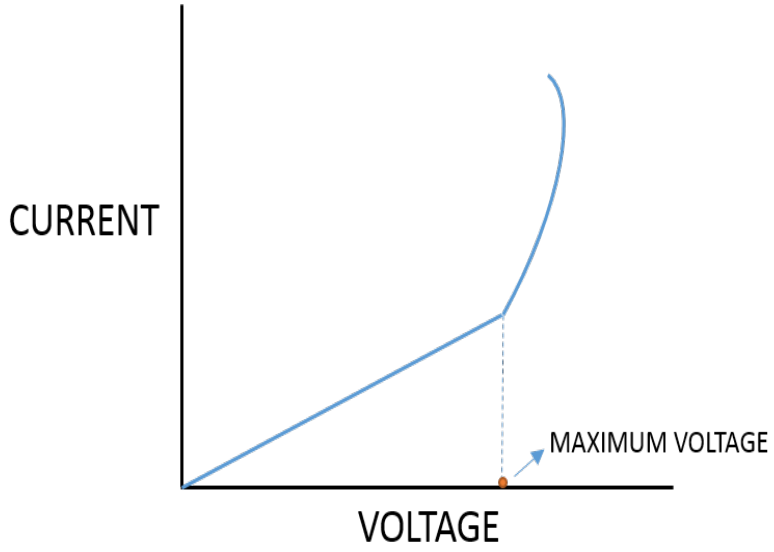

Figure 2.5 - Ohm's law plot [4]

- Source and Destination Vials Buffers

Source and destination vials are filled with the same buffers with even levels to prevent changes in migration time or laminar flow due to the siphoning of the buffer. Even with that, repeated analysis might also change the concentration and pH of the buffer because of the electrolysis of water, where hydrogen ions are formed at the cathode and the hydroxyl ions at the anode. Moreover, after repeated analysis, the buffers in outlet vial may have a different composition because solute ions in the capillary might elute and accumulate into the outlet buffer vial, which can change the electrical field and lead to non-reproducible migration times. Therefore, buffer replenishment is very important by rinsing and refilling the vials and capillary to achieve good reproducibility. Especially in indirect absorbance detection, any change in the chromophore's concentration or composition might lead to a drifting baseline and more noise [51].

There are some criteria for selecting the buffer or chromophoric ion in CE analysis. Firstly, the mobility of a chromophoric ion or any ions in the buffers should be similar to the solute's mobility to prevent asymmetrical peaks. Secondly, in indirect absorbance detection, the chromophore should have a high molar absorptivity at the specific wavelength so that when non-absorbing solutes pass through the detector, there will be a large decrease, indicated by a negative dip peak. In other words, the chromophoric ions should absorb light strongly at the wavelength that the solutes cannot absorb. Lastly, the buffer or chromophoric ion must be stable and nonreactive with the capillary or any components in the samples [51].

- pH of the Buffer

Buffer pH can change the zeta potential, thus affecting strongly the EOF. At higher pH, the inner wall of the capillary will be highly negatively-charged because of the high dissociation of Si-OH to $Si\text{-}O^-$. The surface's high charge is proportional to the zeta potential, resulting in increased EOF or electroosmotic velocity. In contrast, at low pH (below 2), the EOF is eliminated because most of the silanol groups cannot be deprotonated. In addition, buffer pH will also affect the electrophoretic mobility of

the solutes. Depending on the analysis, the required buffer pH can be selected by providing the best separation or optimum EOF flow [40,51-52].

- Buffer Concentration

With the stable capillary temperature, a high buffer concentration will decrease the zeta potential, leading to lower EOF. Analysis time will be shorter with lower concentration of buffers, but unreasonably low concentration may lead to broader and asymmetric peaks as well as cause adsorption of solutes. Another noticeable distortion of electrical field can occur when the buffer concentration is not higher than the solute concentration. This results in broad, skewed peaks. As a general rule, the run buffer concentrations are in the range of 10-100 mM [51].

- Temperature

Temperature is one of the important factors in CE analysis. Unstable temperature can lead to various migration times, zone spreading, and sample decomposition. The EOF rate also increases when the temperature elevates. It is always advisable to control the temperature. There are many different cooling systems to help the heat dissipate quickly and keep the temperature stable, either by air or liquid cooling. The most common method is liquid cooling in which a coolant is circulated through a jacket placed around the capillary [51].

Liquid Chromatography - Mass Spectrometry (LC/MS)

Basic Theory

Liquid chromatography-mass spectrometry (LC/MS) is a powerful separation technique which combines the advantages of a high performance liquid chromatography (HPLC) system with a mass spectrometer. This technique can separate and quantify the analytes in a complex mixture based on the separation power of HPLC and detection power of mass spectrometry [54-55]. Under high pressure, an HPLC system can quickly differentiate many compounds in a liquid phase, depending on their physical-chemical properties [54]. These properties affect their relative affinity between the compounds with stationary phase of the column. Compounds which interact strongly with the stationary phase will stay longer in the column. The longer a compound spends on the stationary phase, the slower it will

travel through the column and elute. Retention time is the time an analyte will pass through the column to the detector. It can be also determined from the time when sample is injected to the point the maximum peak height of that compound shows up in the chromatogram [54-55].

With a matrix effect from a complex mixture, HPLC might not always provide good peak resolution or precise quantitative analysis because of the interference from other sample components [54-56]. It also may give uncertain identification of the compounds that have similar retention times [56]. Therefore, a mass spectrometer is coupled to the LC system so that it can enhance the compound identity by their mass-to charge-ratio (*m/z*). A mass spectrometer itself cannot be sufficient to determine analytes because there are many different compounds that could have same molecular mass. Thus, it is always supported by another technique like HPLC to separate components. The masses of all components shown in the chromatogram can be used to enhance the identity of the compound [54]. This combination of two techniques therefore becomes a powerful tool to resolve the above disadvantages.

LC/MS System

A system of liquid chromatography coupled with a mass spectrometer is shown in Figure 2.6. A liquid chromatography system basically consists of four main components: a sample injector, a detector, a mobile phase (solvent) and a stationary phase (column). A mass spectrometer can be also considered to consist of the following major parts: an ion source and interface, an ion separation (mass analyzer), an ion detector and data system [56]. In HPLC, the loop injector, also known as the valve injector, is set up with a conventional syringe to introduce a liquid sample into a flowing liquid stream. This stream is generated by the mobile phase pumped at a certain flow rate through the valve to the column, keeping the column in equilibrium with the flowing stream for stable chromatographic performance. Another important component in the system is the detector because it can contribute to the success of HPLC analysis. There are many different types of detector such as the UV, fluorescence, electrochemical, conductivity and refractive

index detectors. Each type has its own advantages and disadvantages, but the UV detector is the most common which is used in routine analysis [56]. The most important parts in liquid chromatography are the stationary phase and the mobile phase because they extremely influence the separation. The stationary phase can be categorized into two types: a normal-phase column and a reversed-phase column [56]. Because both of them are packed with various stationary phase adsorbents, the chosen column must depend on the characteristic of the analytes to provide the best separation. Moreover, the column should have inert reactions with the eluent, analytes, or matrix of the samples. Following the column options, the mobile phase are the solvents, which are the eluents that dissolve and carry analytes to pass through the column [54,56]. In general, normal phase columns use higher polar rigid silica, or silica-based compositions [54-56]. A typical column usually has a length of 150 to 250 mm with an internal diameter of less than 4.6 mm. The mobile phases in normal-phase chromatography are nonpolar, non-aqueous solvents such as chloroform and hexane. The least-polar components will be easily soluble in non-polar solvents and have weak affinity with the polar column, hence they will elute first. The higher polar analytes engage the polar column by hydrogen-bonding or dipole-dipole interactions and stay longer on the column [56]. Normal-phase HPLC is not commonly used [54,56]. In contrast, reversed-phase chromatography (RPC) has a non-polar stationary phase and polar mobile phase. The column has the same size but its surface is modified to become non-polar by a long hydrocarbon chain with 8 or 18 carbon atoms. This non-polar surface on the column will attract non-polar compounds in the eluent due to van der Waals dispersion forces, hence retain them longer in the column. The polar compounds that are soluble in the polar mobile phase will pass through the column faster [57]. The polar mobile phase using a buffered aqueous phase with the buffer pH less than 7.5 is advised because of the degradation of the non-polar silica column in basic solutions [56]. There are two channels of mobile phase, A and B, by convention installed on the HPLC system. Aqueous solvent such as water by convention is the A solvent while the B solvent is always the organic solvent like acetonitrile, methanol or propanol. This convention is important because the A solvent and the B

solvent refer to the aqueous and organic solvents, respectively. In reversed phase LC/MS, 0.1% acid is usually added in both solvents to improve the peak shape and supply a source of protons. The added acids can be formic acid, trifluoroacetic acid, or acetic acid [32-34]. An ideal mobile phase has many necessary characteristics to perform many different tasks. It should not only dissolve and carry the analytes to elute with good peak separation, but also be volatile and inert with the analyte. It must also supply adequate ion source in the ionization interface. It seems impossible to find one perfect solvent for all analyses, hence a gradient of multiple solvents and ionization agents are employed [54-56]. Because reversed phase chromatography can effectively analyze various polar compounds, it is the most common and robust technique used form of HPLC [56].

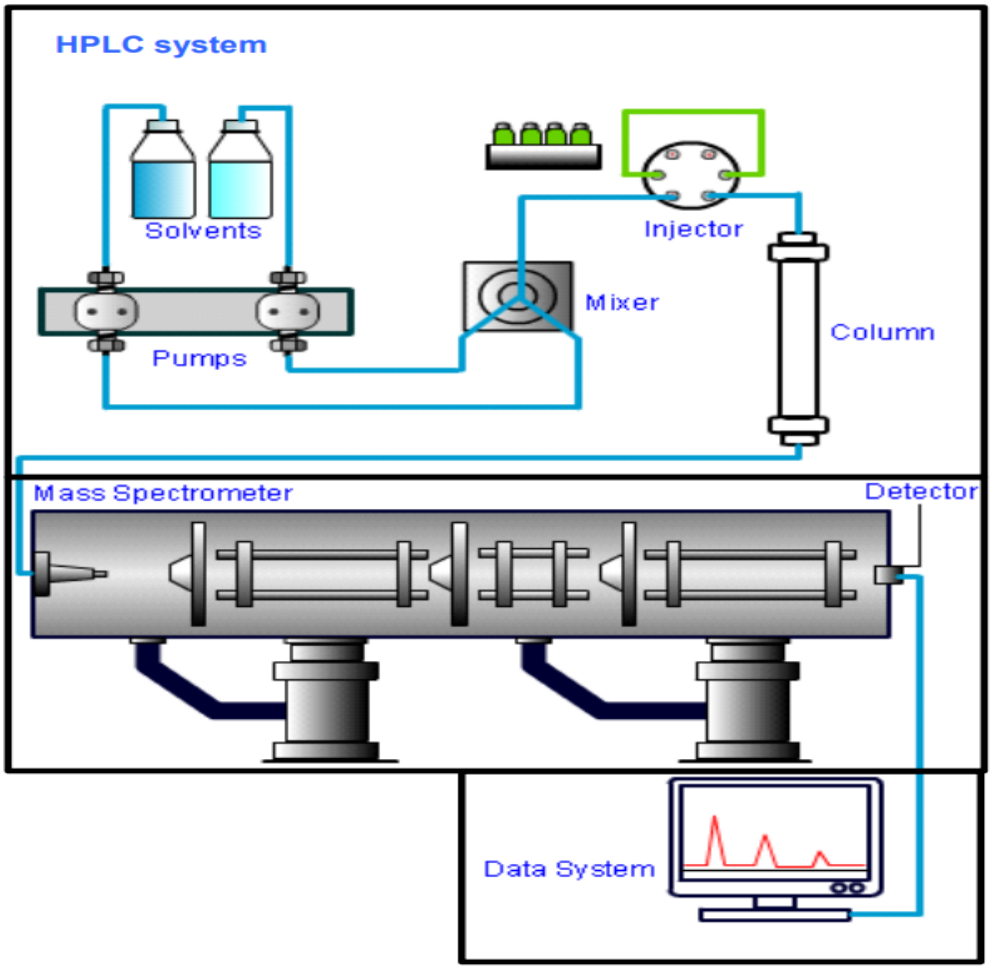

Figure 2.6 - A schematic diagram of LC/MS [10]

It is impossible to pump the eluate from an HPLC column directly into the source of the mass spectrometer because of the limitation of flow rate [54-56]. A block schematic diagram of a mass spectrometer is illustrated in Figure 2.7. The mass spectrometer therefore is connected with the HPLC system by a sample introduction component, including ion source and interface part. This component is responsible for removing a large portion of unnecessary mobile phase but still passing a maximum amount of analyte into the mass spectrometer [56]. Generally, the separated analytes in eluate are injected into an ion source to convert them into ions in the gas phase while the eluent is pumped to waste. Most analytes separated by HPLC are not volatile or thermally labile, thus many ionization methods have been developed [56]. Generally, after the eluate is injected into an ion source, the ionization is considered to consist of four basic steps: the formation of droplets, charging of droplets, desolvation of the droplets, and the formation of ions from analyte [56]. The most common ionization techniques in LC/MS analysis are the electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) [54,56,58].

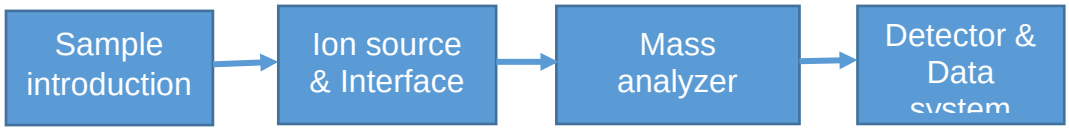

Figure 2.7 - A block schematic diagram of mass spectrometer [54,64]

The electrospray ionization (ESI) is the popular ionization technique which involves spraying eluent into a strong electrical field with desolvation as shown in Figure 2.8. Basically,the ionization process in this method takes place in the liquid phase [54]. The analyte eluted from HPLC with the solvent is pumped through a metal capillary needle at a rate of few microliters per minute. The nebulizer will spray eluent flow when exiting the electrospray capillary to form droplets as an aerosol plume. These droplets acquire a charge when passing through the atmospheric-pressure region where there is a large potential difference (2-5 kV) between the electrospray capillary needle and a counter cylindrical electrode. These charged fine droplets then pass through a desolvating capillary to reduce the droplet size by evaporating

the solvent with the support of a heated drying gas maintained at high voltage. With smaller size of the droplets, the surface charge density increases and those ions start to desorb into the ambient gas. This charge density generates an electric repulsion, resulting in droplet fission. At the point it exceeds the surface tension of the droplet, Coulomb fission will occur to make original droplet explode into many smaller stable droplets [54,56,61]. During the fission, the droplet loses a small percentage of its mass (1.0–2.3%) along with a relatively large percentage of its charge (10–18%) [59-60]. Gas-phase multiple charged ions are formed as the droplet ions then pass through two differentially pumped regions into the mass analyzer. The advantage of the ESI technique is the majority of ions carry multiple charges, reducing their mass-to-charge (*m/z*) ratio compared to a singly charged ion. Therefore, ESI technique can analyze mass spectra for large molecules [56]. Besides, the ionization happens directly from solution, thus the thermally labile molecules can be ionized without degradation [56]. Nevertheless, the matrix effect with co-eluting analytes or matrix component sometimes causes an uncertainty for this method [54,56]. The flow rate and analyte concentration can influence the ESI ionization efficiencies. Even though the lower flow rates through the ESI interface can improve the analyte's detection, it should be also based on retention time, peak resolution and analyte concentration. The higher concentration of analyte might

increase detected ion intensity [54,56,59-60]. ESI is not really effective for non-polar or low polarity analytes [56].

Atmospheric pressure chemical ionization (APCI) is a technique suitable for the compounds which are non or less polar, and normally not ionized by ESI. APCI is a gas-phase ionization, producing protonated or deprotonated ions from the analytes by a proton transfer or abstraction [54-56]. When the eluent is injected into a silica capillary, the solvent will be heated and evaporated by a heater to the gaseous phase in the capillary. A discharge corona's needle placed in an ion source which can generate electron and ionize above gaseous solvent into solvent ions. The analytes are also vaporized in the heated nebulizer and sprayed into droplets. These analyte droplets emerge into the solvent ion's cloud, transferring or abstracting protons with those ionized solvents. APCI is not suitable for volatile samples and ionizing compounds with low vapor pressures. It also requires a larger content of additives in the solvents to monitor the chemical ionization in the gaseous phase [54-56]. The additives can be added into mobile phase for both ionization techniques as ionization agents to increase the efficiency of ionization of the analyte. Depending on positive ion or negative ion mode, typical ionization agents could be an acidic ionization agent such as formic acid, a weakly acidic ionization agent like ammonium formate, or a basic ionization agent such as ammonium carbonate.

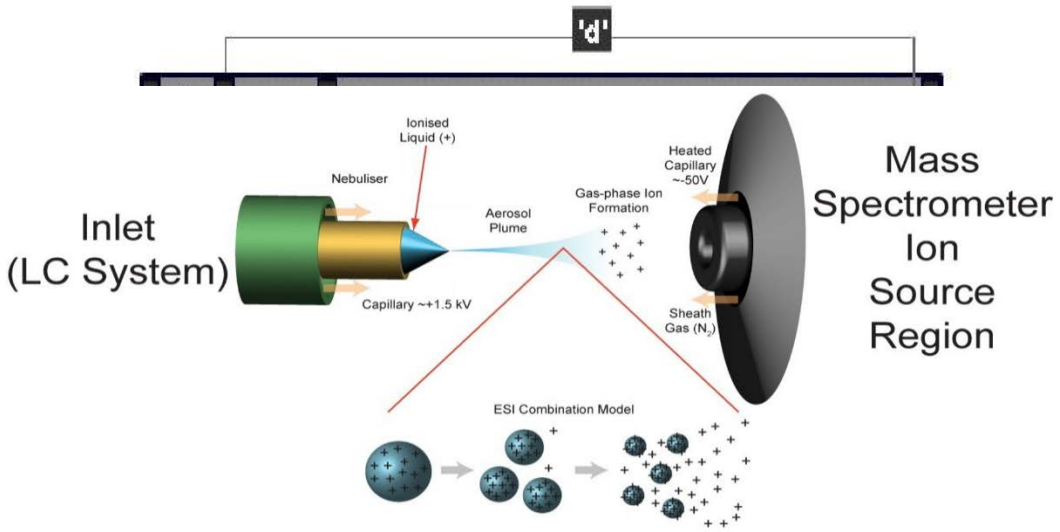

Figure 2.8 - Electrospray ionization (ESI) and ion source [61]

A mass analyzer is the significant component to separate ions on a mass-to-charge ratio basis in mass spectrometry analysis. There are many types of mass analyzers. The selection of mass analyzer depends on its application, cost, and performance. The most popular mass analyzer in LC/MS systems are Time-of-Flight analyzers and Quadrupole analyzers [56,62]. The Time-Of-Flight (TOF) mass analyzer is the simplest mass analyzer system. As shown in Figure 2.9, the ions are separated based on their velocities or time of flight. The velocity of different ions will be inversely proportional to the square root of their masses. The time for ions to travel the whole flight tube is also inversely proportional to its velocity and directly proportional to its mass [56]. The ions produced from the ion source will drift under an accelerating voltage through straight tube. These ions will reach the detector with different velocities, depending on their mass (*m*) and charge (*z*) [56,62]. A quadrupole mass analyzer is another type of analyzer, consisting of four cylindrical metallic rods as a mass filter as illustrated in Figure 2.10 [54]. Two rods have positive electrical potential and the other two are at negative potential. Each set consists of both radiofrequency (RF) and direct-current (DC) [54,56]. The ion source produces an accelerated ionic beam through the space between the four rods. The positive ions are attracted to the negatively charged rods while negative ions are attracted to the rods with positive charge. Therefore, the positive pair of rods works as a high mass filter and negative pair filters the low mass [54]. The continuous changing of the relative charge on the pair of rods generates the electrostatic potentials that separate ions with selected *m/z* ratio. Only those ions with given *m/z* can resonate and pass through the space between the rods to the detector, whereas

the rest of the ions will be destabilized to strike the rod and consequently are not detected [54-56].

Nowadays, hybrid mass spectrometers, which can be employed for either LC/MS or LC/MS/MS analysis, have been developed to enhance the ion separation. This system, also called tandem mass spectrometry, is the combination of two or more different types of *m/z* separation devices [63]. Quadrupole-Time of Flight (Q-TOF) mass spectrometer is one type consisting of a quadrupole analyzer coupled with a time-of-flight analyzer. This device involves multiple steps of *m/z* selection, with some forms of fragmentation occurring between the stages. In this system, the ions are generated in the ion source and separated by *m/z* ratio in the first stage of mass spectrometry (MS1). The ions with selected mass-to-charge ratio (precursor ions or father ions) are filtered and broken into fragment ions (product ions or daughter ions) by collision-induced dissociation, ion-molecule reaction or other processes. Consequently, the fragment ions are split up and detected in a second stage of mass spectrometry (MS2) [63,65]. As demonstrated in Figure 2.11, if the analysis operated in the “MS” mode of Q-TOF analyzer, the quadrupole analyzer simply works as a lens to focus the ion beam into the second analyzer (TOF) that separates the ions based on their mass-to-charge ratio only. In this case, the collision chamber also does not create any fragment ions. On the contrary, when the Q-TOF spectrometer is switched into "MS/MS" mode, the ions out from ion source can be

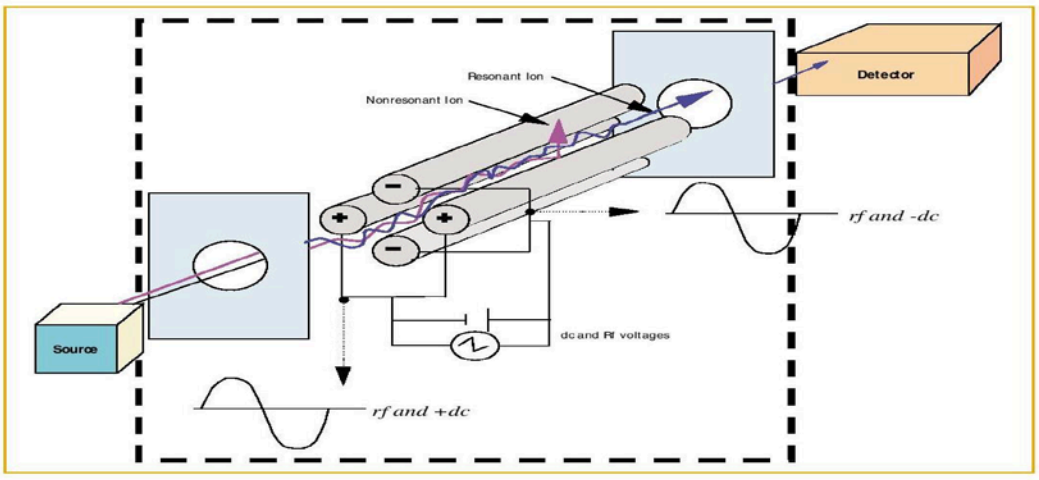

Figure 2.10 - Schematic of Quadrupole mass analyzer [64]

separated and detected through the quadrupole analyzer. The selected ions with desired *m/z* ratio are transmitted into the collision chamber, where an introduced inert gas (argon) bombards those ions into smaller fragments. These fragments then enter the TOF analyzer and get detected [65-66]. Their signals are interpreted by a computer to identify the analytes.

Comparison between HPLC and CE

Theoretically, both HPLC and CE can be employed for qualitative and quantitative analysis because they can determine the presence of a component in a mixture as well as its amount. The comparisons of retention time (in HPLC) or migration time (in CE) of pure sample's peak and the desired component's peak in the mixture can yield its identity. Unfortunately, these comparisons are not always conclusive evidence due to other compounds or contaminants in the mixture that can influence the migration time (CE) or retention time (HPLC) of the analyte. Therefore, the analyte can be isolated after eluting from the HPLC or CE instrument and identified by mass spectrometer or infrared spectroscopy. Besides, "spiking" is also an effective method to determine a compound in a mixture, by comparing the peak area that is increasing in the electropherogram of the mixture before and after adding pure sample of the same analyte. CE or HPLC are also suitable for quantitative analysis because their detectors are mostly concentration dependent. An external or internal calibration method or standard addition method can be used to determine the analyte concentration [51].

There is a basic difference in the peak shape between HPLC and CE. In HPLC, with equal solute concentrations and detector responses, the longer the retention time of

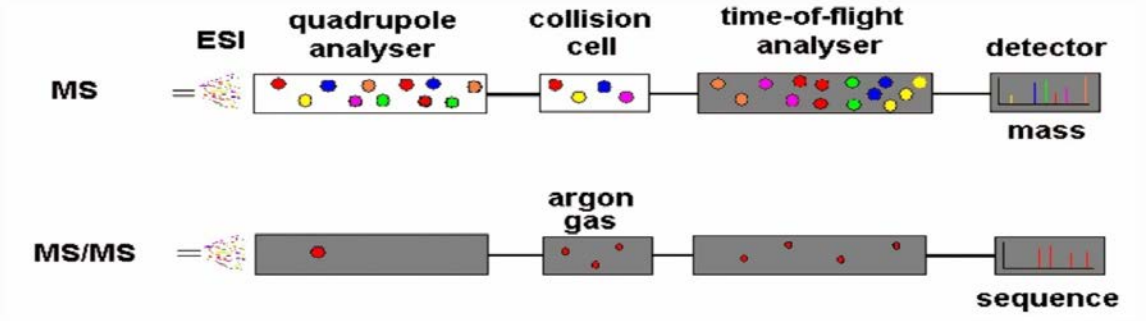

Figure 2.11 - Q-TOF mass spectrometer operating in MS (upper) and MS/MS (lower) modes [66]

the solutes is, the broader and shorter the peaks become but peak areas remain stable. The solutes move to the detector with the same velocity, but when they stay longer inside the column, the later eluted peaks are more dilute, causing broader and shorter peaks. With CE, the peak heights remain stable even when the solutes stay in the capillary longer because the solutes migrate to the detector in zones with

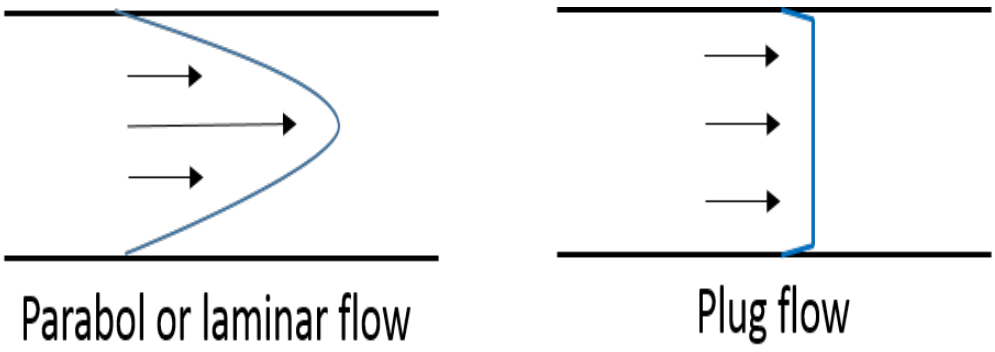

Figure 2.12 - Laminar flow in HPLC and plug flow in CE [51]

the same length and, thus the same amount. Another explanation for this difference is the behaviour of flows in HPLC and CE as shown in Figure 2.12. While HPLC has parabolic or laminar flow, electroosmotic flow in CE has a relatively flat profile. The frictional drag might make the electroosmotic flow at the wall surface slower than the flow through the rest of the capillary, but this effect is minor because the area near the wall is very tiny. This flat flow profile of EOF supports the solute molecules having the same velocity and moving to the detector as narrow bands, resulting in narrow peaks. In contrast, the laminar flow in HPLC makes the solutes in the centre of the tube move significantly faster than the ones near the wall, creating an uneven flow profile and broadening the peaks. Moreover, the connective tubing in HPLC among the injector, the column, and detector can also cause peak broadening [51].

Reinecke Salt Gravimetric Analysis

Reinecke salt gravimetric analysis is a traditional method based on the precipitation of choline as a reinecke precipitate. The principle of this method was first applied by Kapfhammer and Bischoff in 1930 by forming choline reinecke and quantifying

choline from tissues based on choline reinecke color [26,45,50]. This method has been applied through many decades because it can operate without any complex systems. Nowadays, many oil and gas companies still quantify choline content in clay swelling inhibitor by forming and weighing choline reinecke [45]. Choline reinecke has a glistening appearance and is insoluble in water and alcohol but is easily soluble in acetone giving a bright red solution [26,45,50]. A gravimetric method for choline chloride utilizes ammonium reinecke [i.e**.,** ammonium tetrathiocyanato-diamminechromate(III)] as an analytical reagent to form choline reinecke precipitate. It is then filtered off, dried, and weighed. This method is convenient over many other gravimetric methods because it does not require the use of anhydrous solutions, nor does the precipitation need to be done in an anhydrous system [45]. Choline is a strong base which can be decomposed into trimethylamine and glycol when heated in a strong basic solution. Therefore, before precipitating choline with ammonium reinecke, the pH is adjusted to neutral or slightly acidic with acid [67]. The precipitate of choline reinecke also takes a long time to be formed completely [45,67]. The Reinecke salt gravimetric method for choline chloride is applied widely but it is not considered sensitive enough. It is mainly employed for the determination of larger concentrations of choline [67].

CHAPTER 3

MATERIALS AND METHODS

Overview of Methodology

Firstly, to develop an analytical method using capillary electrophoresis to analyze choline cation, the determination of optimized experimental conditions is the most important step so that this method can provide stable and more effective data. As mentioned in Chapter 2, there are many factors in the capillary electrophoresis method which can influence the analytical data. Therefore, in this research, to determine the optimized condition, some major factors were investigated including the inner diameter of the capillary, the applied voltage, the wavelength of the detector light source, as well as the buffer composition, concentration and pH of the background electrolyte (BGE). The capillary was coated with a bilayer coating of hexadimethrine bromide (PB) and polyvinylsulfonic acid sodium salt (PVS). The capillary sizes with diameters: 25 µm, 50 µm and 75 µm were investigated. The purpose was to pick the narrow-bore capillary which provides the best resolution. Three different applied voltages were also investigated at 15 kV, 20 kV and 25 kV to determine the most suitable one. The background electrolyte (BGE) i.e., buffer is the most important factor required to carry components to the detector. Five buffer types were investigated: sodium phosphate (Na_2HPO_4); sodium phosphate with sodium dodecyl sulfate (SDS); sodium tetraborate ($Na_2B_4O_7{\cdot}10H_2O$); sodium tetraborate with SDS; and imidazole with 18-crown-6 and 1-propanol. Their concentrations and pH were also studied. Each different buffer was tested at a different wavelength. A photodiode array (PDA) detector was used initially to identify the best wavelength for the above buffers as PDA can provide information at all wavelengths simultaneously. After determining the optimized wavelength for the buffers, a UV detector was applied for further investigation at the optimized wavelength. Secondly, the analysis of choline concentrations in standard solutions and real samples was performed under optimized conditions. The analysis targeted the separation of choline and various cations such as ammonium, potassium and sodium that are usually present in oilfield waters. The linearity of standard solutions was also established. Choline standard solutions from 100 ppm to 1000 ppm were analyzed to determine the linear dependence of peak area with increasing

concentrations of the standards and how good the linearity was by way of the R^2 value. The quantification of choline content in real oilfield water samples was done by using the External Calibration method and the Standard Addition method. Finally, method validation was conducted to verify the precision, limit of detection (LOD), limit of quantitation (LOQ) and the accuracy of the developed capillary electrophoresis method. Precision was established by intraday and interday analyses. The LOD and LOQ were also calculated to determine the sensitivity of the method. Accuracy was determined by percent recovery studies using the CE method and comparison with data obtained from LC/MS and Reinecke gravimetry.

Capillary Electrophoresis (CE)

Instrumentation and Reagents

A Beckman P/ACE™ System MDQ capillary electrophoresis unit (Fullerton, CA) installed with a PDA or UV detector is the main instrument used for CE analysis in this research, as shown in Figure 3.1. The separations were carried out using fused silica capillary was purchased from Polymicro Technologies (Phoenix, AZ). The capillary temperature was controlled by the circulating liquid perfluorocarbon coolant, which was purchased from Ideal Vacuum Products, LLC. (Albuquerque, New Mexico). The pH meter used in all experiments to check the pH was a Symphony SB90M5 pH meter from VWR (Buffalo Grove, IL, USA). Nylon® syringe filters (0.45 µm) obtained from Canadian Life Science Inc. (ON, Canada) were used to filter solutions before they were used for CE analysis. Reagents were obtained from various suppliers in Canada and the USA. Choline chloride, hexadimethrine bromide (PB), polyvinylsulfonic acid sodium salt (PVS), 18-crown-6, imidazole, 1-propanol, sodium tetraborate, ammonium chloride and potassium chloride, were purchased from Sigma Aldrich Canada Ltd. (Oakville, Ontario, Canada). Hydrochloric acid (HCl) and sodium hydroxide (NaOH) were purchased from Caledon Laboratory Ltd. (Georgetown, Ontario, Canada). Sodium phosphate dibasic (Na_2HPO_4) was purchased from Fisher Scientific Company (Fairlawn, New Jersey, USA). Sodium dodecyl sulphate (SDS) was purchased from VWR (Mississauga, Ontario, Canada).

Procedure

Preparation of Solution

- Standard Stock Solution Preparation

A standard stock solution at 10,000 ppm was prepared by dissolving 0.5 g choline chloride (solid form) in 18 MΩ water in a 50 mL total solution. All stock solutions were filtered using 0.45 µm Nylon® syringe filters and stored in a fridge. The stock solution was diluted into different standard solutions with various concentrations at 100 ppm, 200 ppm, 400 ppm, 600 ppm, 800 ppm, 1000 ppm, 1250 ppm, 1500 ppm, 1750 ppm and 2000 ppm for the experiments.

- Sample Preparation

Eleven samples comprising of oilfield process waters and commercial choline chloride products were kindly provided by Dr. Kenneth Schmidt (Wilson Analytical Services Inc., Sherwood Park, AB) and Dr. Neil Warrender (Engenium Chemicals Corp., Calgary, Alberta). They were labelled as 11241, 11242, 11243, 11244, 11245, 11411, 11412, 11413, 11414, 11415 and 11421. These samples were diluted 100 times into unknown concentrations (ppm) and labelled as diluted samples. All samples were filtered using 0.45-µm Nylon® syringe filters.

- Solutions of Choline Chloride and Other Salts Preparation

Five different solutions of different salts were prepared, including ammonium chloride (NH_4Cl) solution, potassium chloride (KCl) solution, a mixture of NH_4Cl and KCl, a mixture of NH_4Cl, KCl and sodium hydroxide (NaOH), and a mixture of NH_4Cl, KCl, NaOH and choline chloride.

- Conditioning Solutions Preparation

Sodium hydroxide (NaOH) solutions of 0.1 M and 1.0 M were prepared in a volumetric flask using 18 MΩ water. A hexadimethrine bromide (PB) 5% solution (w/v) was prepared in a volumetric flask by dissolving 2.52 g of PB in 18 MΩ water in a 50 mL total solution. A PVS 5% solution (v/v) was also prepared by mixing 20 mL of PVS 25% with 18 MΩ water in a 100 mL total solution. All solutions were

filtered using 0.45-µm Nylon® syringe filters. The PB solution was stored in fridge as it is not stable at room temperature.

- Background Electrolyte (BGE) Preparation

Five different types of buffers were prepared to find the optimized buffer. They were prepared with 18 MΩ water in a 50-mL volumetric flask. The desired pH of the solutions was obtained by adjusting with 0.1 M HCl and 0.1 M NaOH. The solutions were filtered using a 0.45-µm Nylon® syringe filter. The five buffers were:

- Sodium phosphate (Na_2HPO_4) solutions at 50 mM at pH 5.0, 6.0, 7.0, 8.0, 9.0, 10.0
- Sodium phosphate (Na_2HPO_4) solutions at 50 mM and 25 mM sodium dodecyl sulphate (SDS) solution at pH 5.0, 6.0, 7.0, 8.0, 9.0
- Sodium tetraborate ($Na_2B_4O_7 \cdot 10H_2O$) solutions at 50 mM at pH 5.0, 6.0, 7.0, 8.0, 9.0, 10.0
- Sodium tetraborate ($Na_2B_4O_7 \cdot 10H_2O$) solutions at 50 mM and 25 mM sodium dodecyl sulphate (SDS) solution at pH 5.0, 6.0, 7.0, 8.0, 9.0
- Imidazole solutions at 30 mM, 40 mM, 50 mM imidazole and 3 mM 18-Crown-6 with 10% 1-propanol (v/v) at pH 4.0, 5.0, 6.0, 7.0.

Capillary Preparation and Coating

A capillary with the length of 65 cm was prepared and placed inside a cartridge as shown in Figure 3.2. At the black window on the cartridge, approximately 0.3 - 0.5 cm of capillary's polyimide coating was removed so that the light could go through the transparent silica to detect the components. In this experiment, the capillary was conditioned and coated to effectively minimize adverse ion adsorption on the capillary inner wall and also to provide a stable electroosmotic flow.

For a new fused-silica capillary, it was first rinsed with methanol at 30 psi for 30 min to remove any debris or particulates. Then the capillary was rinsed with 1 M NaOH for 30 min. Following that, the capillary was rinsed with water for 5 min. The capillary was then rinsed with 0.1 M NaOH for 30 min, followed by rinsing with water for 5 min again. These steps are aimed to ensure that the silanol groups (SiOH) of the

capillary inner wall were deprotonated into silanoate ions (SiO^-), generating the negatively charged inner surface of the capillary. After that, the inner wall of the capillary was coated with a bilayer as follows. It was rinsed with 5% hexadimethrine bromide (PB) (w/v) solution for 30 min, then it was flushed with water for 5 min. The capillary was rinsed with 5% polyvinylsulfonic acid sodium salt (PVS) (v/v) for 30 min before rinsing in water again for 5 min. This coating procedure was done at 20 psi. These conditioning and coating steps were repeated every week to ensure that the capillary was properly coated for the analysis. The capillary was filled with 18 MΩ water and the ends immersed in vials of water when not in use.

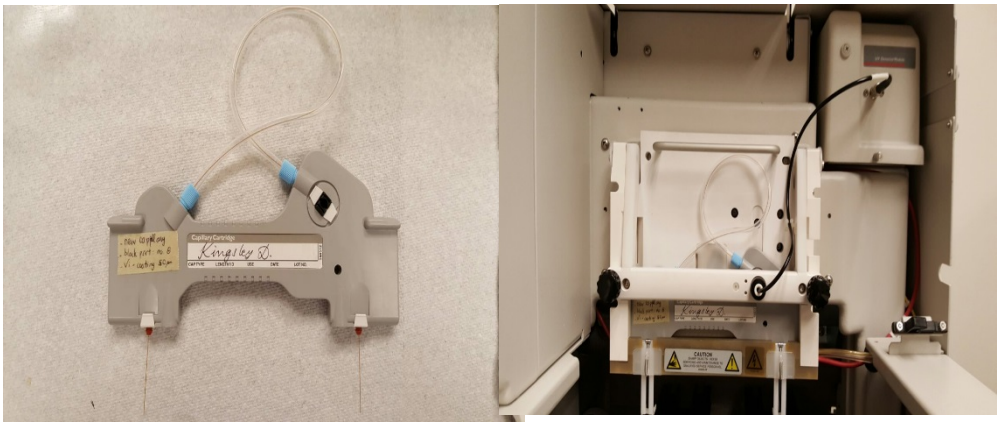

Figure 3.1 - Capillary cartridge and its position in CE system

The capillary with a bilayer coating is illustrated in Figure 3.3. Choline chloride is a quaternary ammonium salt. The positive choline portion therefore tends to adsorb onto the negative inner wall of the capillary. Hexadimethrine bromide (PB) is considered as a cationic polymer (or surfactant) which can get attracted to the negatively charged surface of the capillary wall. The alkyl chains of PB can attract each other, creating a positively charged layer as the cationic part. Polyvinylsulfonic acid sodium salt (PVS) being an anionic polymer can then attach to this positive layer, generating a second layer which is negatively charged. Consequently, this bilayer coating can prevent choline's adsorption onto the capillary wall.

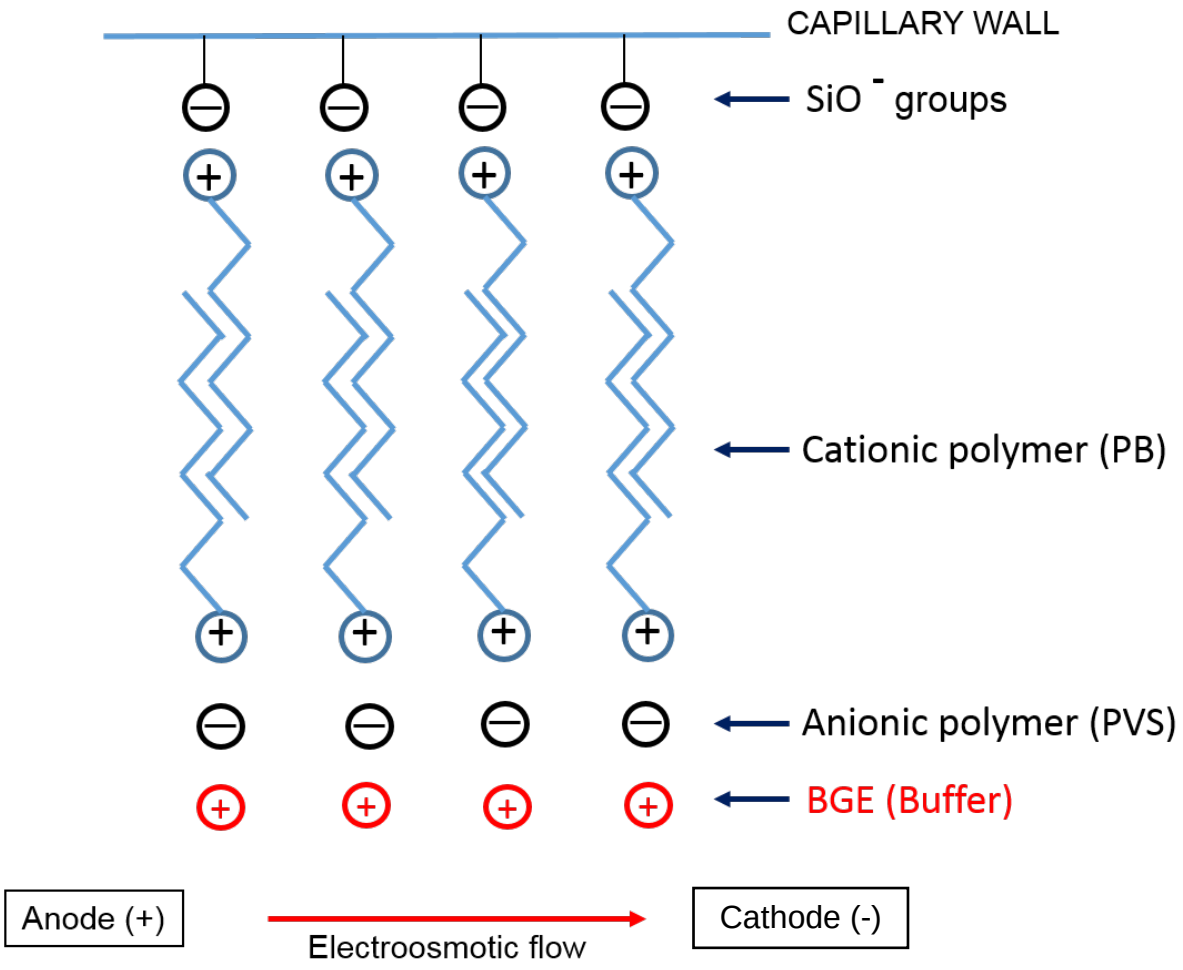

Figure 3.2 - The capillary with bilayer coating of PB/PVS [51]

Procedure for Experimental Conditions Optimization and Sample Analysis

Choline does not absorb in the ultraviolet (UV) range i.e., above 200 nm [38]. Therefore, indirect UV detection is considered more suitable for analysis of choline ion by CE. Before every injection, the coated capillary was rinsed at 20 psi with water for 5 min, then rinsed with 0.01% PVS solution (v/v) for 5 min to support the negatively charged PVS layer. After that, the capillary was rinsed with water again for 3 min. Then, before separation, the capillary was rinsed with buffer (BGE) for 5 min at 20 psi. For optimization of the buffer, the separation was performed for 15 min at 20 kV using normal polarity and at a constant temperature of 25 °C. The standard choline chloride solutions from 100 ppm to 2000 ppm were used in the

optimization. Samples were injected at a 10 s interval and a pressure of 0.5 psi. All experiments for optimization were performed using the same capillary with 50 µm diameter. Four different types of buffers as mentioned above with different pHs and concentrations were tested at different wavelengths by a PDA detector to find the suitable one for the analysis. After the suitable buffer was determined, other parameters such as capillary diameters and the applied voltages were also investigated to determine the optimal conditions.

After getting the optimized conditions, the separation time was adjusted to 10 min depending on the migration time of choline to shorten the analysis time. The separation between choline and other ions like ammonium, sodium and potassium was explored by injecting five solutions of the salts and choline chloride. Each prepared solution of choline and other salts was analyzed in the same way to determine the peak's identity. Each component of the optimized buffer was also investigated for its role in the separation.

Under optimized conditions, the oilfield process water samples were also analyzed. Two methods were applied to quantify choline concentrations. The first one was the External Calibration method. A calibration curve was set up by plotting the peak areas of choline ion corresponding to the standard choline chloride solutions at 100 ppm, 200 ppm, 400 ppm, 600 ppm, 800 ppm and 1000 ppm. A linear regression plot of the peak area (y-axis) and the standard choline chloride solution concentration in units of ppm (x-axis) was constructed to yield the calibration curve. The peak area of choline in the oilfield water samples was used to infer choline chloride concentration based on the resulting calibration curve. These oilfield water samples were prepared by mixing 20 µL diluted samples with 18 MΩ water and made up to 200 µL. Sample solution concentrations were calculated using the constructed calibration curves.

The second method used for analysis of samples was the Standard Addition method. For each sample, five different vials containing 20 µL of diluted sample were prepared. Then 10,000 ppm choline stock solution with different amounts: 0 µL, 5 µL, 15 µL, 25 µL and 40 µL were added to the five vials. Each sample vial was then topped up with 18 MΩ water to make the volume up to 200 µL. The

solutions were then analyzed. For each set of the sample, a linear regression plot of the peak area and added concentrations was constructed. The choline chloride concentration in the sample vial without spiking (y = 0) could be calculated based on the linear regression equation and used to determine the concentration of the choline in the original samples.

Procedure for Method Validation

A validation of the developed method was carried out. The precision was investigated through intraday and interday analyses. Three standard choline chloride solutions at 200 ppm, 600 ppm and 1000 ppm were analyzed three times in a day, and on three consecutive days. The LOD and LOQ were determined by analyzing the 100 ppm standard choline chloride solution several times. The standard deviation (SD) of these peak areas of choline peaks was used to calculate the LOD and LOQ as follows:

LOD = 3 x SD / Slope of the calibration curve

LOQ = 10 x SD / Slope of the calibration curve

Percent recovery was also studied and calculated by the percentage of how much of the original substance was obtained at the end of the analysis. It is the amount of how much we obtained divided by the initial amount we started multiplied by 100. The recovery of choline in samples was determined after spiking 750 ppm, 1250 ppm and 2000 ppm stock choline chloride solutions.

$$\% \text{ Recovery} = \frac{Spike\ (exp)}{Spike\ (true)}\ x\ 100$$

where,

Spiked (true) is known spiked concentration

Spike (exp) is spiked concentration calculated by the difference of the choline peak areas before and after spiking using the calibration equation.

Liquid Chromatography - Mass Spectrometry (LC/MS)

Instrumentation and Reagents

The LC/MS system employed in this research consists of an Agilent 1200 series HPLC system (Agilent Technologies, Mississauga, ON, Canada) connected to an Agilent 6530 Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) spectrometer equipped with an electrospray ionization (ESI) source. The instrument is shown in Figure 3.4. The column in the HPLC instrument was a Zorbax Extend-C18 column (100 mm × 2.1 mm; 1.8 µm particle size) from Agilent (Canada). All the solutions used in LC/MS were filtered with 0.45-µm Nylon® syringe filters which were purchased from Canadian Life Science Inc. (ON, Canada).

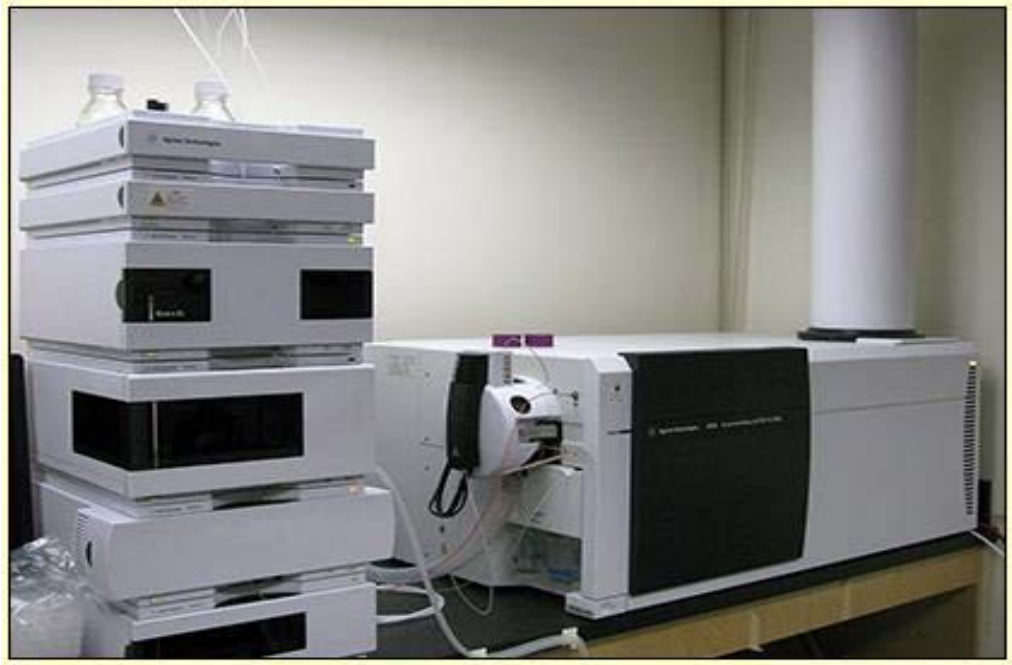

Figure 3.3 - Liquid Chromatography-Mass Spectrometry (LC/MS) system

Most reagents used for the LC/MS analysis were HPLC grade. Choline chloride and formic acid were purchased from Sigma Aldrich Canada Ltd. (Oakville, Ontario, Canada). LC/MS grade acetonitrile and LC/MS grade water were purchased from Caledon Laboratory Ltd. (Georgetown, Ontario, Canada).

Procedure

Preparation of Solutions

- Standard Stock Solution Preparation

A standard stock solution at 10,000 ppm was prepared in a 50-mL volumetric flask by dissolving 0.5 g of choline chloride (solid form) in 18 MΩ water. All stock solutions were filtered by using 0.45 µm Nylon® syringe filters and stored in the fridge. This stock solution was diluted into different standard solutions at various concentrations as follows: 50 ppm, 100 ppm, 250 ppm, 500 ppm, 750 ppm and 1000 ppm for the experiments.

- Sample Preparation

Eleven samples of oilfield waters were provided by Wilson Analytical Services Inc. (Edmonton, AB). They were labelled as 11241, 11242, 11243, 11244, 11245, 11411, 11412, 11413, 11414, 11415, 11421. These samples were diluted 1000 times into unknown concentrations (ppm) and used for the analysis. All samples were filtered using 0.45 µm Nylon® syringe filters.

- Mobile Phase Solvents Preparation

Mobile phase solvents were prepared, consisting of solvent A (LC/MS grade water + 0.1% formic acid) and solvent B (acetonitrile + 0.1% formic acid) by adding 0.5 mL formic acid in 500 mL 18MΩ water and in 500 mL acetonitrile, respectively. All solutions were filtered using 0.45 µm Nylon® filters.

Procedure for Calibration and Sample Analysis

The mobile phase solvents were initially set at 0% solvent A and 100% solvent B and flushed through the column at a flow rate of 4 mL/min for 2 min. After that, it was switched to 100% solvent A and 0% solvent B and flushed through the column at flow rate of 4 mL/min for 2 min. Then, the percentage composition was changed to 75% solvent A and 25% solvent B and flushed through for two more minutes. After these rinsing steps, the flow rate was reduced to 0.2 mL/min while the pressure increased and stabilized at around 200 bar. A linear mobile phase gradient program was used as shown in Table 3.1, and a flow rate of 0.2 mL/min was used for the analysis.

Table 3.1 - Mobile Phase Gradient Program [68]

Time (min.)	%A	%B
0.00	75	25
0.20	75	25
4.00	100	0
6.00	100	0
6.01	75	25
9.00	75	25
9.01	75	25
10.00	75	25

The run time was set for 10 min per injection. The stationary phase (column) was kept at a constant temperature of 40 ± 0.2°C. The injection volume for sample and calibration standard solutions was 2 µL with the flow rate set at 0.2 mL/min. Choline ion was analyzed with positive ion polarity and mass spectra were acquired between 80 and 500 mass-to-charge ratio (*m/z*). The choline peak shows at an *m/z* close to 104. The gas temperature was 300°C, drying gas flow was 8 L/min, nebulizer was at 10 psig, sheath gas temperature was 350°C; sheath gas flow was 8 L/min; and the voltage of the capillary was 3500 V. A list of LC/MS optimized parameters is shown in Table 3.2. Under optimized conditions, a calibration curve was developed by plotting the peak areas of choline versus choline chloride concentrations, using external calibration standard solutions of 50, 100, 250, 500, 750 and 1000 ppm choline chloride. After obtaining the calibration curve, the concentration of choline chloride in diluted oilfield water samples were determined. The peak areas of choline from the chromatograms were used to calculate choline chloride concentration in eleven samples based on the external calibration curve.

Table 3.2 - Optimized LC/MS parameters used in the analysis of choline ion [68]

Parameters	Method
Injection Volume	2 µL
Flow Rate	0.2 mL/min
Solvents	A (H2O + 0.1% FA); B (ACN + 0.1% FA)
Run Time	10 min
Column Temperature	40 ºC
Gas Temperature	300 ºC
Ion Source	ESI
Ion Polarity	Positive
Drying Gas	8 L/min
Capillary Voltage	3500 V

Reinecke Salt Gravimetric Method

Reagents

Choline chloride and ammonium reinecke salt were purchased from Sigma Aldrich Canada Ltd. (Oakville, Ontario, Canada). Citric acid was obtained from Alfa Aesar (Ward Hill, MA, USA).

Procedure

Preparation of Solutions

The precipitating agent solution was made by mixing 3.0 g of ammonium reinecke salt with 100 mL of 18 MΩ water in a volumetric flask. This mixture was shaken until saturated and then filtered by gravity filtration as shown in Figure 3.5.

The stock choline chloride was prepared as follows. A mixture of 0.25 g of choline chloride and 2 g of citric acid added into a 100 mL volumetric flask and diluted to mark with 18 MΩ water. This flask contained 250 mg choline chloride in 100 mL of solution. A pH paper was used to ensure that the solution's pH was around 6 - 7.

Each sample solution was prepared by mixing 1 mL of original oilfield water sample with 2 g citric acid and diluted with 18 MΩ water to a 100 mL total solution. A pH paper was again used to ensure that the solution's pH was around 6 - 7.

The wash solution was prepared by mixing 2 mL of saturated ammonium reinecke salt solution with one liter of 18 MΩ water.

Figure 3.4 - The saturated ammonium Reinecke salt solution

Procedure for Sample Analysis

From the stock standard solution, 20 mL, 15 mL, 8 mL and 4mL (containing 50 mg, 37.5 mg, 20 mg and 10 mg of choline chloride, respectively) were pipetted into four different beakers containing 25 mL of 18 MΩ water. To these solutions, 15 mL, 10 mL, 10 mL and 10 mL of ammonium reinecke salt solutions were added into these beakers, respectively. Then, disposable pipets were used to add reinecke solution dropwisely and slowly down the inner side of beaker for at least one minute. The purpose was to form a layer under the choline chloride layer without any turbulence. The mixture in the beaker changed from clear to the pink color of a reinecke salt as shown in Figure 3.6.

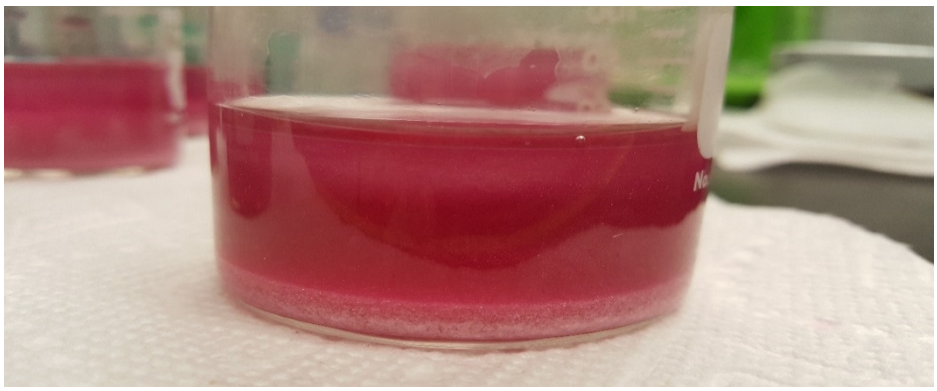

Figure 3.5 - Formed choline reinecke precipitates in the mixture

The beakers were then rotated gently to mix the solution before leaving them for at least one hour in order to allow more pink precipitate to form. After that, the precipitates were filtered off by vacuum filtration on the pre-weighed glass filter crucibles. The precipitates were rinsed with three 15 mL portions of wash solution. This step also prevented the precipitates getting completely dry during washing because the dry precipitates were almost impenetrable. As demonstrated in Figure 3.7, these crystals were left to dry on the vacuum after the last washing time. Then, the filter crucibles were put in the oven at 100 ^{o}C for at least 1 h before cooling in a desiccator. The glass filter crucibles with pink choline reinecke precipitates were weighed to determine the yield.

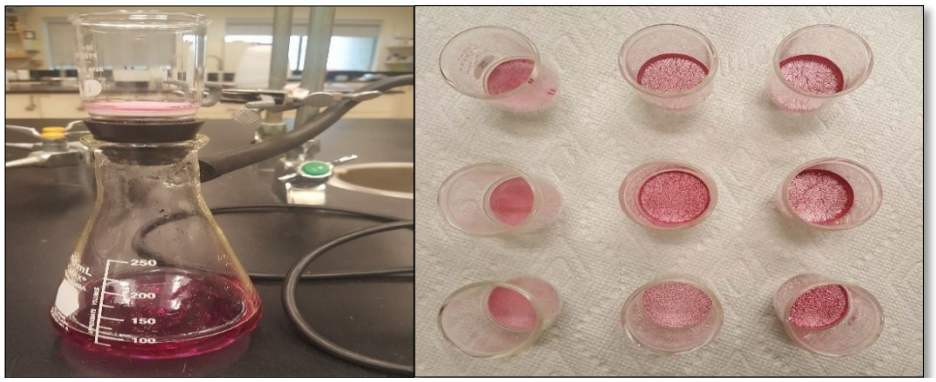

Figure 3.6 - Vacuum filtration and collected choline reinecke precipitates

For the prepared samples, each set of 1 mL, 1mL, 1mL and 2mL of each sample was pipetted into four beakers containing 25 mL 18 MΩ water. A 10 mL ammonium reinecke salt solution was added into each beaker. The rest of the procedure was the same as mentioned earlier. The amount in gram of choline chloride in each beaker was calculated based on the weight of choline reinecke precipitate according to the following formula: [45]

Weight of choline reinecke X 0.3304 = Grams of choline chloride

where,

Molar mass of choline chloride (CC): 139.62

Molar mass of choline reinecke (CR): 422.56

Ratio of CC/CR = 0.03304

CHAPTER 4

RESULTS AND DISCUSSION

Capillary Electrophoresis

Results of Optimization

Capillary Inner Diameter

Theoretically, the capillary with smaller inner diameter (i.d.) would provide a better separation but poorer sensitivity. The capillary with a bigger i.d. would generate larger Joule heat which might affect the analysis. The Joule heat which can be generated by the capillaries was controlled by the cooling system of the CE instrument which prevented the increase of temperature in the capillaries. In this research, capillaries with i.d. ranging from 25 - 75 µm were investigated for analysis. The capillary with 25 µm inner diameter provided a decent resolution but periodically got clogged. Capillaries with 50 µm and 75 µm i.d. provided good resolutions without any problem. Therefore, either a 50 µm or 75 µm i.d. capillary was suitable for the analysis. In the end, the analysis was carried out in 50 µm i.d. capillary as the smaller narrow bore capillary would limit the Joule heat.

Buffer Type

Many different types of buffers were investigated using 500 ppm and 1000 ppm choline chloride solutions. The 50 mM sodium phosphate (Na_2HPO_4) solutions with a pH range of 5.0 - 10.0 were the first background electrolytes (BGEs) used for the direct UV analysis of choline. The next investigated BGEs were 50 mM sodium tetraborate ($Na_2B_4O_7 \cdot 10H_2O$) solutions with a pH range of 5.0 - 10.0. Both types of buffer solutions at pH 8.0 - 10.0 produced peaks in the electropherograms at a detection of 280 nm. However, the peak areas did not change much for 500 ppm and 1000 ppm choline chloride solutions. Therefore, it was uncertain if those peaks represent choline ion. Following that, sodium dodecyl sulfate (SDS) was added into sodium phosphate BGE and sodium tetraborate BGE for the analysis of choline ion. The purpose was to check if SDS would enhance the quality of the separation by stacking with choline ion. However, the electropherograms also did not show any good peak. Moreover, the results showed very poor baseline with lots of noise. After testing many different buffer solutions, a direct UV analysis with these BGEs did not work for choline ion analysis. As a result, another BGE was tested using indirect UV

analysis of choline. It was a mixture of imidazole, 18-crown-6 and 1-propanol. Imidazole, the main component in the buffer, is a cationic visualization agent, known as a probe, to make the electrolyte absorbent with indirect UV detection. The 18-crown-6 is the complexing agent used to enhance the selectivity in separation of cations [69] and the 1-propanol is an organic modifier. The role of each component in this BGE was investigated later in this research for the separation of choline ion and other cations. The BGE consisting of a mixture of imidazole, 18-crown-6 and 1-propanol provided better results for the analysis compared to the earlier set of BGEs. With this imidazole BGE, a good peak of choline ion was obtained as shown in the top electropherogram of Figure 4.1. The number of theoretical plates (N) for the choline peak obtained with the imidazole BGE was calculated to be of the order of 10^8.

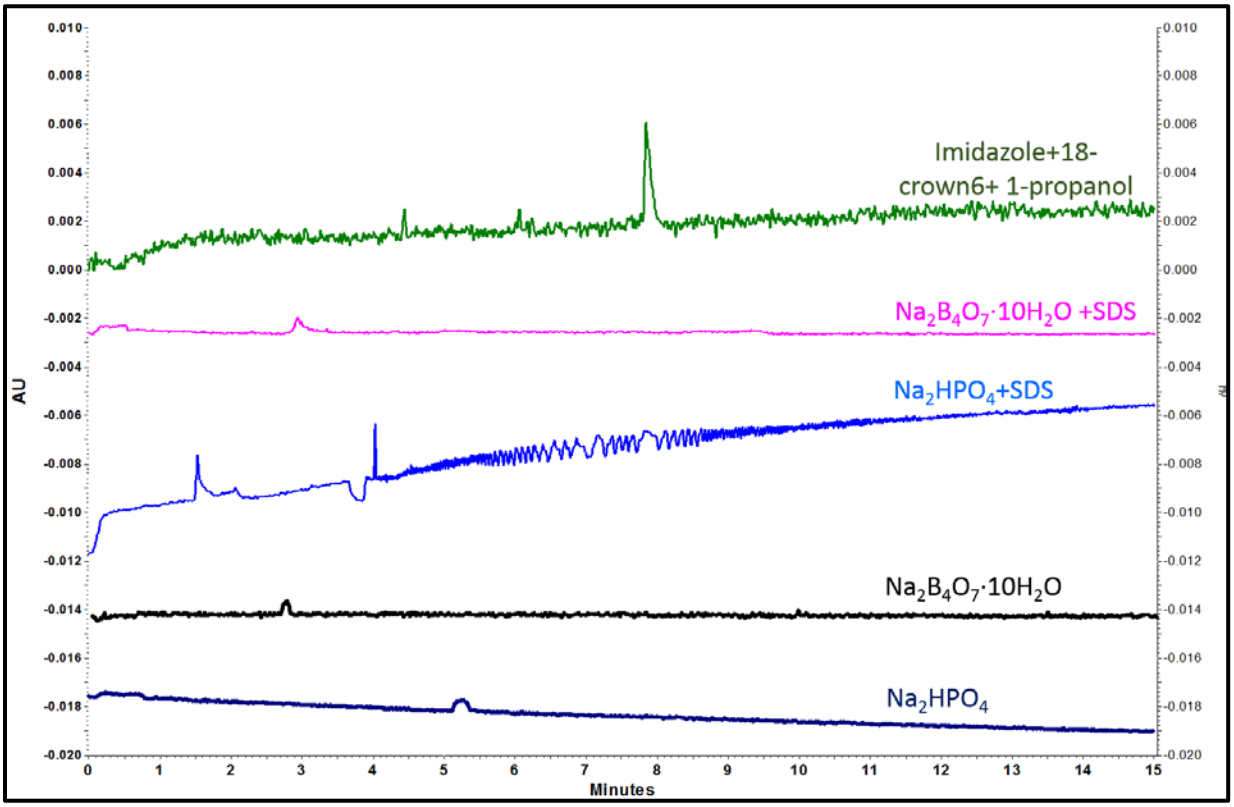

Figure 4.1 - Electropherograms of 1000 ppm choline chloride solution with different background electrolytes (BGEs)

Wavelength

Each BGE might have different UV absorbance at different wavelengths. Therefore, to determine the best wavelength where the selected BGE absorbs UV strongly, a photodiode array (PDA) detector was used. The electropherograms showed that the BGE which is made up of imidazole and 18-crown-6 in 1-propanol provided the best results with the largest peak area of choline ion at 214 nm as shown in Figure 4.2. The results are also shown in 3D and contour in Figure 4.3.

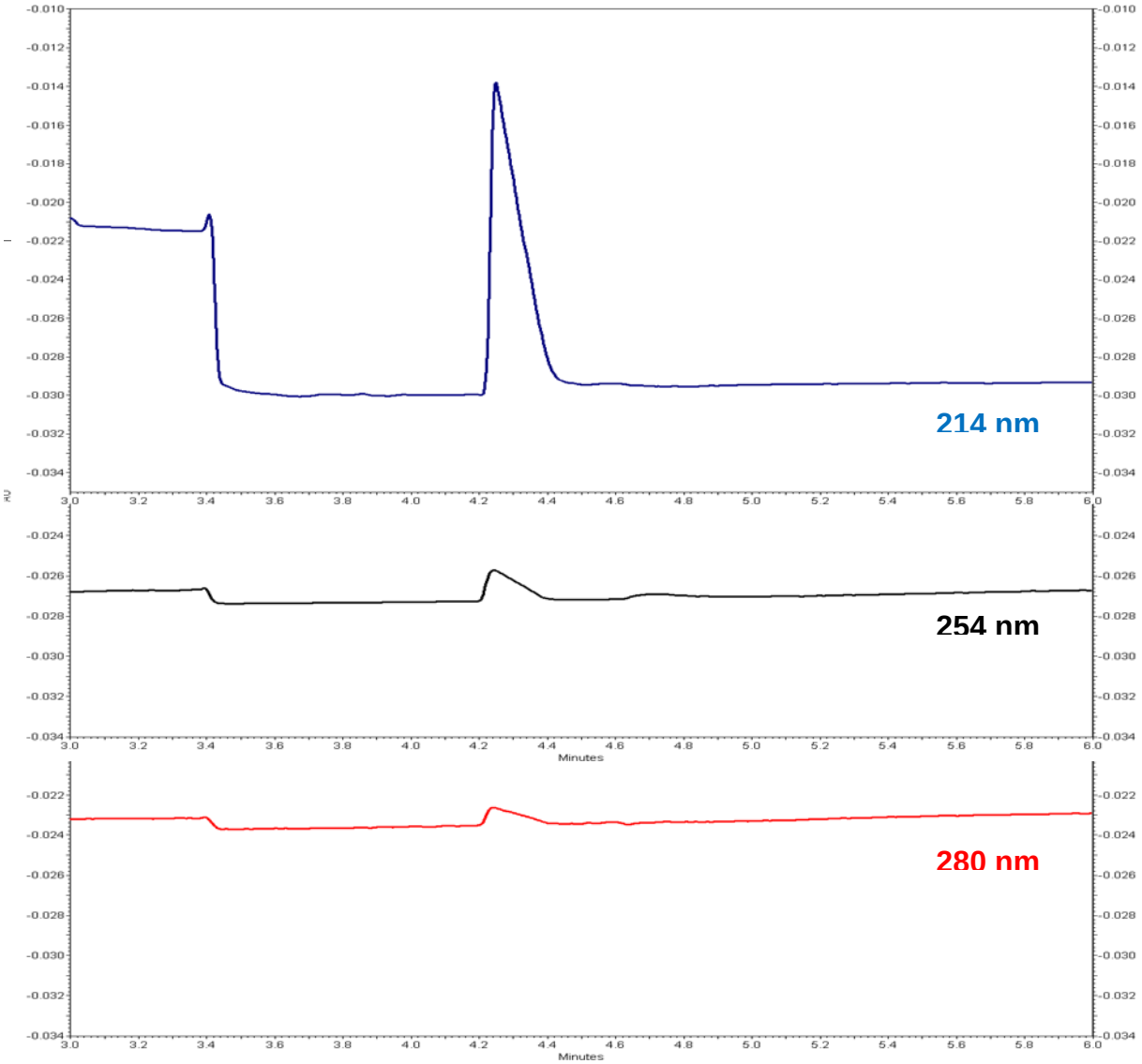

Figure 4.2 - Electropherograms of choline ion at the wavelengths: 214 nm, 254 nm and 280 nm

Buffer Concentration

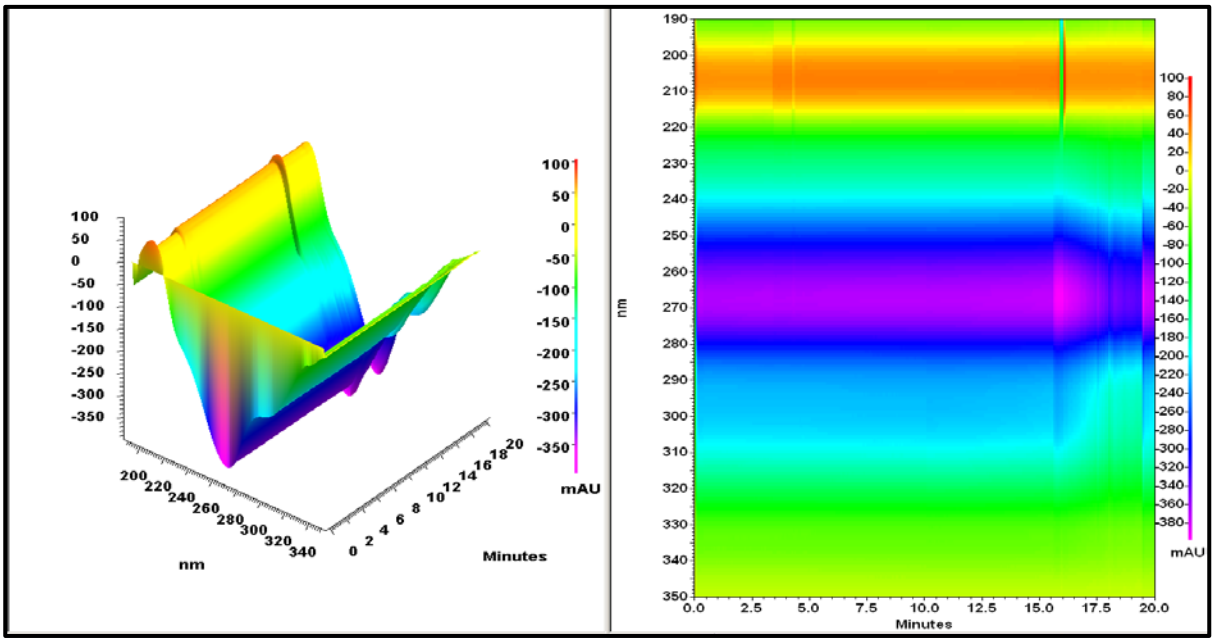

Figure 4.3 - Electropherogram's 3D and contour of choline ion analysis with PDA detector

A high buffer concentration would slow the EOF while a low concentration of buffer might result in broad and skewed peaks. In this work, the concentration of imidazole, the main component in the BGE, was investigated in the range of 30 - 50 mM. Because 18-Crown-6 was used in a very small amount as additive, its concentration remained constant at 3 mM for all the analysis. 1-propanol concentration was also unchanged. The results demonstrated that a lower concentration of imidazole at 30 mM reduced the migration time of choline ion a little bit but the base of the peak shape was broader. Therefore, 40 mM or 50 mM imidazole provided the better peak shape of choline. In addition, with quite similar migration times of choline in both cases, 40 mM imidazole BGE showed a bigger peak area of choline compared to 50 mM imidazole BGE. The number of theoretical plates (N) and the asymmetry factors were calculated for the peaks at the three imidazole concentrations of 30 mM, 40 mM, and 50 mM and the values are shown in Appendix A.1. As can be seen from the table in Appendix A.1 the 30 mM had the highest N (8.54×10^7), however its asymmetry factor is the highest with a value of 23.1. The 40 mM peak had a reasonably good N value (8.02×10^7) as well but had the smallest asymmetry factor

of 6.3. Thus, 40 mM imidazole solution was selected as optimized buffer concentration for the analysis. The electropherograms are shown in Figure 4.4.

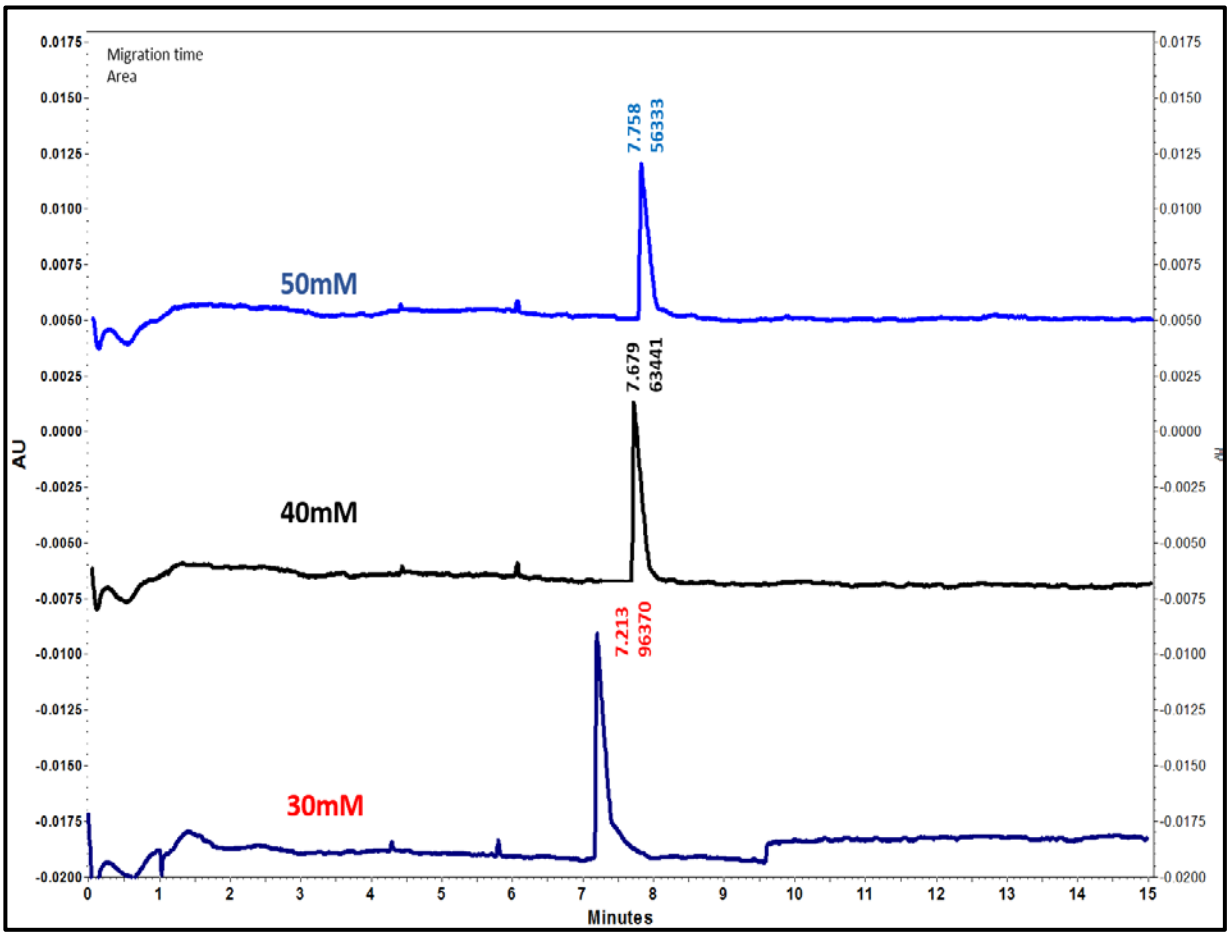

Figure 4.4 - Electropherograms of choline ion with the BGE of imidazole concentrations at 30 mM, 40 mM, 50 mM

Buffer (BGE) pH

The buffer pH influences the EOF and changes the migration time of the analyte. In this work, the pH of the selected BGE was investigated at 4.0, 5.0 and 6.0. The electropherograms in Figure 4.5 shows that choline ion was detected successfully at that pH range but the migration times were slightly different. The migration time of choline ion was slower for the lower buffer pH. With pH 4.0, the baseline in the electropherogram was not as good as those with pH 5.0 or 6.0. Besides, the peak shape at pH 6.0 was broader at the base. The electropherogram at pH 5.0 had good baseline, good resolution and reasonable migration time.

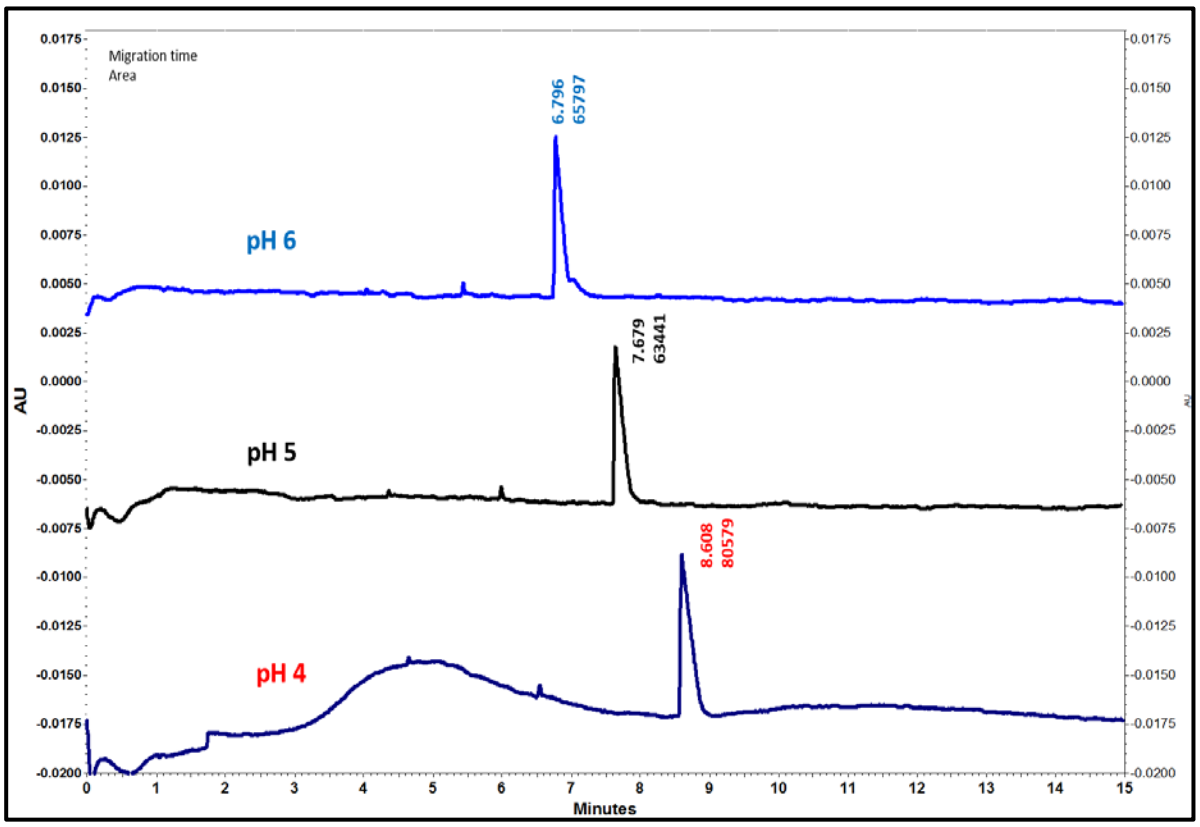

Figure 4.5 - Electropherograms of choline ion with the BGE at pH 4.0, 5.0, 6.0

Table 4.1 - The peak areas of choline ion at varying choline chloride concentrations at pH 4.0, 5.0, 6.0

Standard CC Concentration (ppm)	pH 4	pH 5	pH 6
	Peak Area	Peak Area	Peak Area
100	11552	6956	8882
200	26731	19577	19366
400	59957	38519	54179
600	93165	64718	67631
800	117484	82316	79458
1000	161622	106742	124367
1250	208581	153008	174996
1500	264244	160027	197773
1750	409009	199866	198552
2000	417611	229951	272796

Standard choline chloride solutions from 100 ppm to 2000 ppm were run with buffer pH from 4.0 to 6.0 to investigate the linearity. The results shown in Table 4.1 proved that in this pH range, the peak area of choline ion was directly proportional to the concentration of choline chloride. In Figure 4.6, the best linear regression was achieved at pH 5.0 (0.9985). Again, the number of theoretical plates (N) and the asymmetry factors were calculated for the peaks at the three pH values for the 40 mM. The values are shown in the table in Appendix A.2. As can be seen from the table in Appendix A.2, the pH 5 had a reasonably good N (8.02 x 10^7), and its asymmetry factor is the lowest at 6.3. Therefore, pH 5.0 was selected as the optimized buffer pH to use for the analysis.

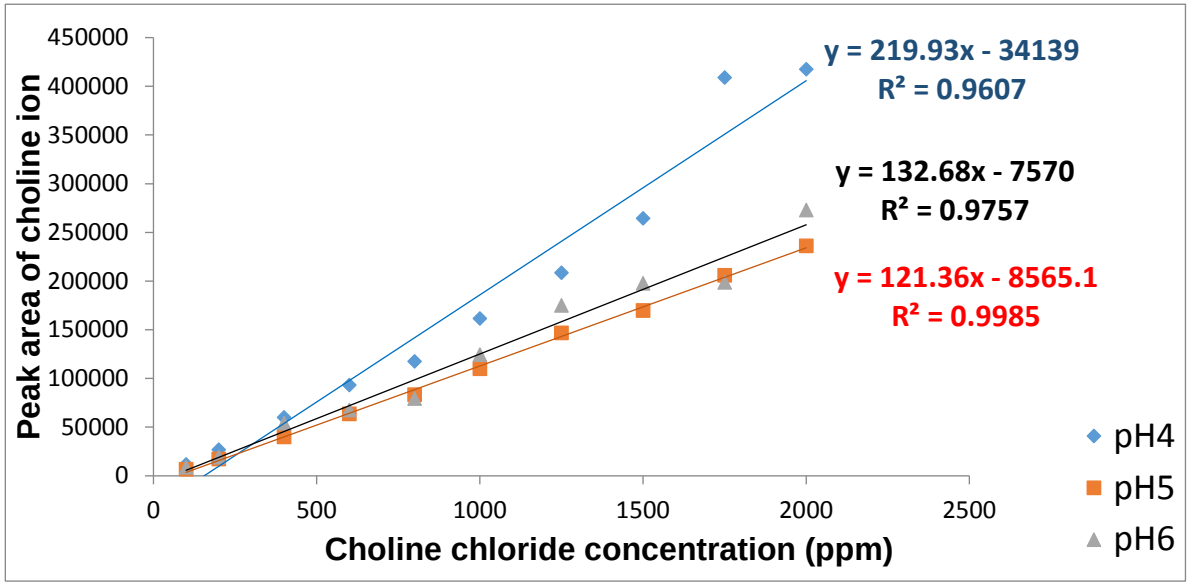

Figure 4.6 - The calibration curve of standard choline chloride solutions at concentrations from 100 ppm to 2000 ppm at different pH 4.0, 5.0, 6.0

Applied Voltage

High applied voltage would result in a short migration time and a narrow peak but it would also generate more Joule heat. Experimentally, different voltages were used for the analysis of choline chloride solutions at 500 ppm and 1000 ppm. The currents at the middle of the separation time were recorded at 10, 15, 20 and 25 kV as shown in Table 4.2. Based on the Ohm's law, the stable linearity of observed currents corresponding to the applied voltage in Figure 4.7 showed that Joule heat was not

generated at high voltage in this case. Therefore, the applied voltage of up to 25 kV could be used if it still provided good separation. The results shown in Figure 4.8 indicated that the migration time of choline ion was increased with lower applied voltage. Nevertheless, with the applied voltage at 25 kV, the capillary routinely broke. Therefore, the voltage at 20kV was selected as the optimal voltage to use for all subsequent work.

Table 4.2 - The currents at different applied voltage for analysis of 500 ppm and 1000 ppm choline chloride (CC)

Applied voltage (kV)	500 ppm CC Current (µA)	1000 ppm CC Current (µA)
0	0	0
10	16.94	16.99
15	24.73	24.66
20	31.26	32.13
25	39.6	40.087

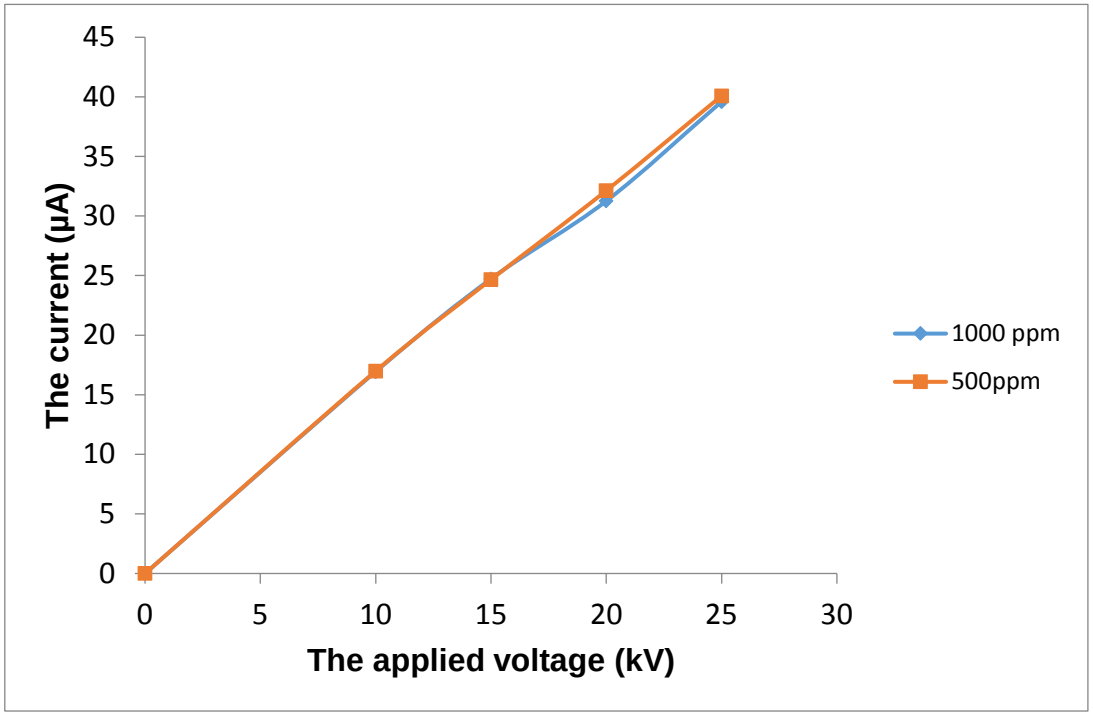

Figure 4.7 - The Ohm's law plot of choline chloride analysis at different applied voltages

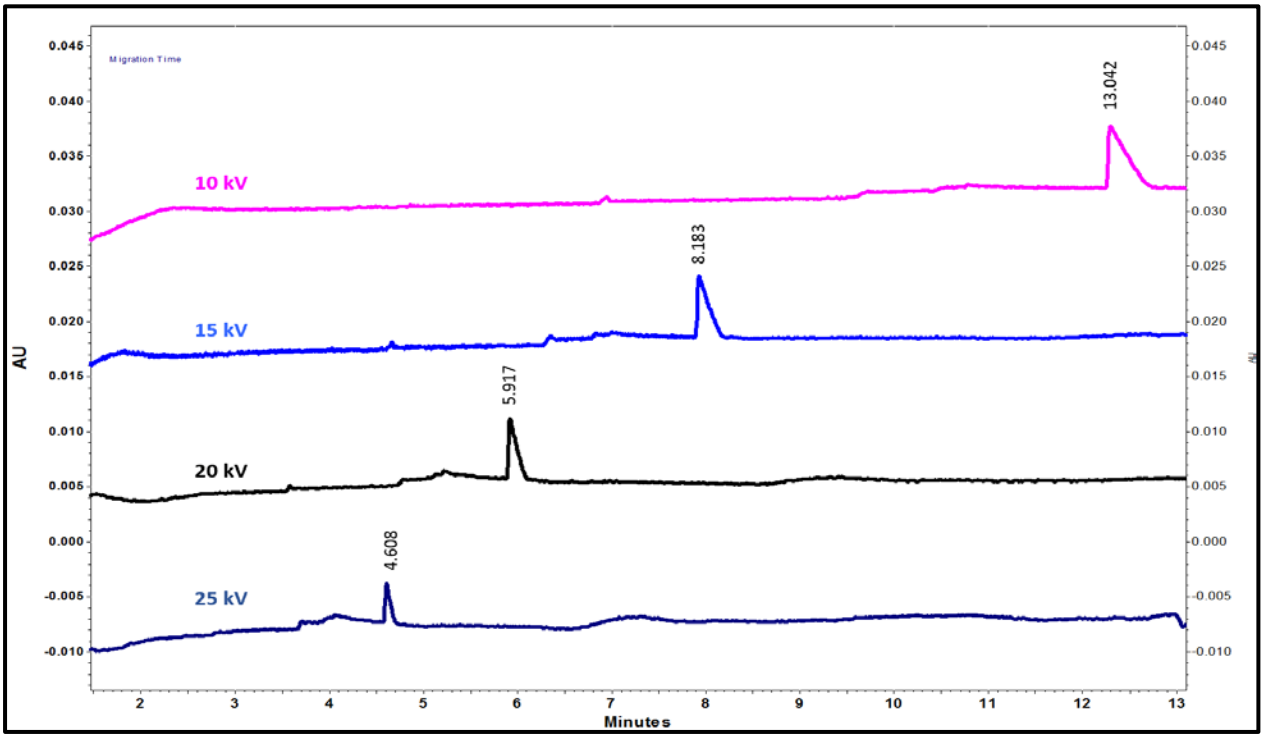

Figure 4.8- Electropherograms of choline peaks at different applied voltages: 10 kV, 15 kV, 20 kV, 25 kV

Summary of CE Optimized Conditions

The optimized conditions of the capillary electrophoresis method to analyze choline chloride are summarized below in Table 4.3.

Table 4.3 – Optimized conditions of choline chloride analysis by CE

Optimized Conditions	
UV Detector Absorbance	214 nm
Inner Diameter of Capillary	50 µm
Experimental Temperature	25°C
Buffer Type & Concentration	40mM Imidazole 3mM 18-crown-6 5% 1-propanol
Buffer pH	5.00 ± 0.01
Applied voltage	20kV
Bilayer coating capillary	5%PB / 5%PVS

Separation of Choline Ion and Other Cations

The separation of choline ion and other cations such as ammonium, potassium, and sodium was investigated under optimized conditions. Each component in in BGE was also explored for its role in the separation. With the BGE containing only 40 mM imidazole solution, the peaks of these ions were detected. However, ammonium and potassium ions did not separate well in the mixture as shown in Figure 4.9.

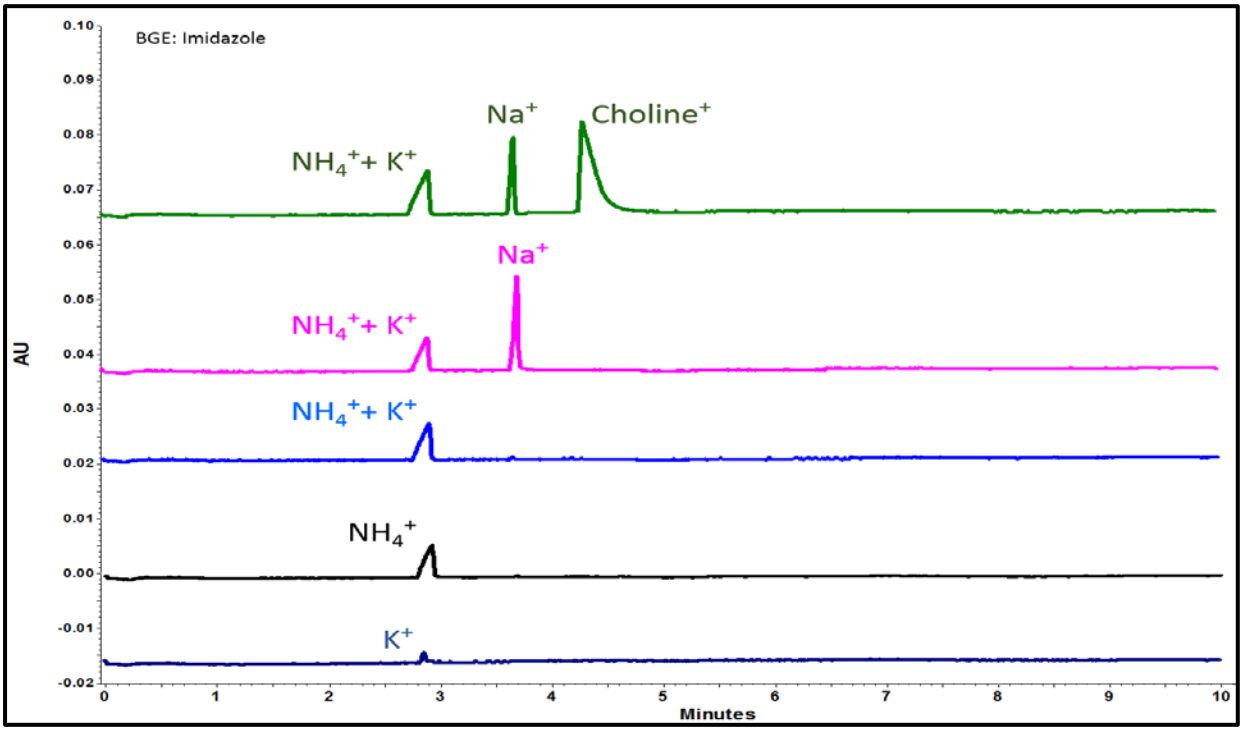

Figure 4.9 - Electropherograms of the separation of choline ion and ammonium, potassium, sodium ions by capillary electrophoresis with BGE containing imidazole

When 1-propanol was added into the BGE, the migration time of the cations was longer probably due to the increase in higher viscosity. The ammonium and potassium peaks were not well separated as illustrated in Figure 4.10. With the presence of 18-crown-6, all four cations were separated as shown in Figure 4.11.

As an added confirmation of the separation, the resolution values for the various pairs were calculated and the values are shown in the table in the Appendix A.3. As can be observed in the table, all the values for the separation of the various pairs of cations are greater than 1.5, which indicate baseline separation as seen in the electropherogram.

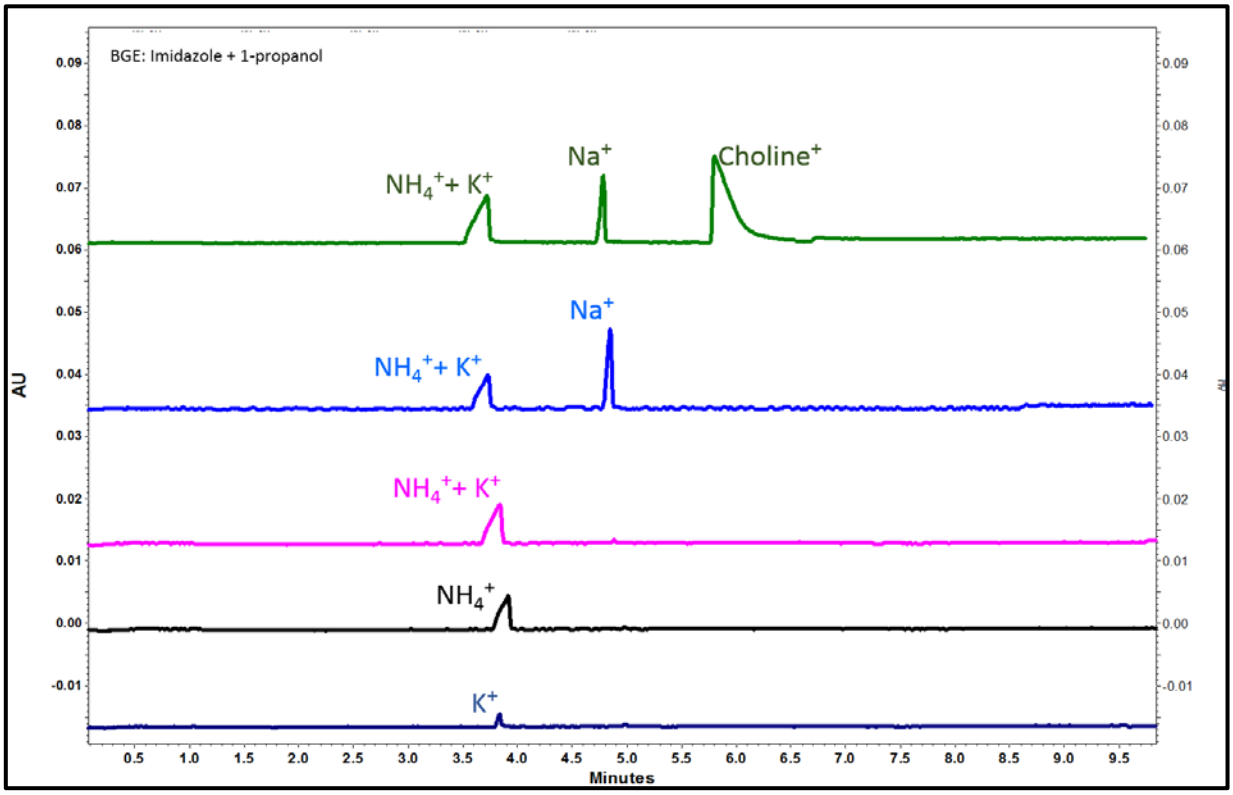

Figure 4.11 - Electropherograms of the separation of choline ion and ammonium, potassium, sodium ions by capillary electrophoresis with BGE containing imidazole and 1-propanol

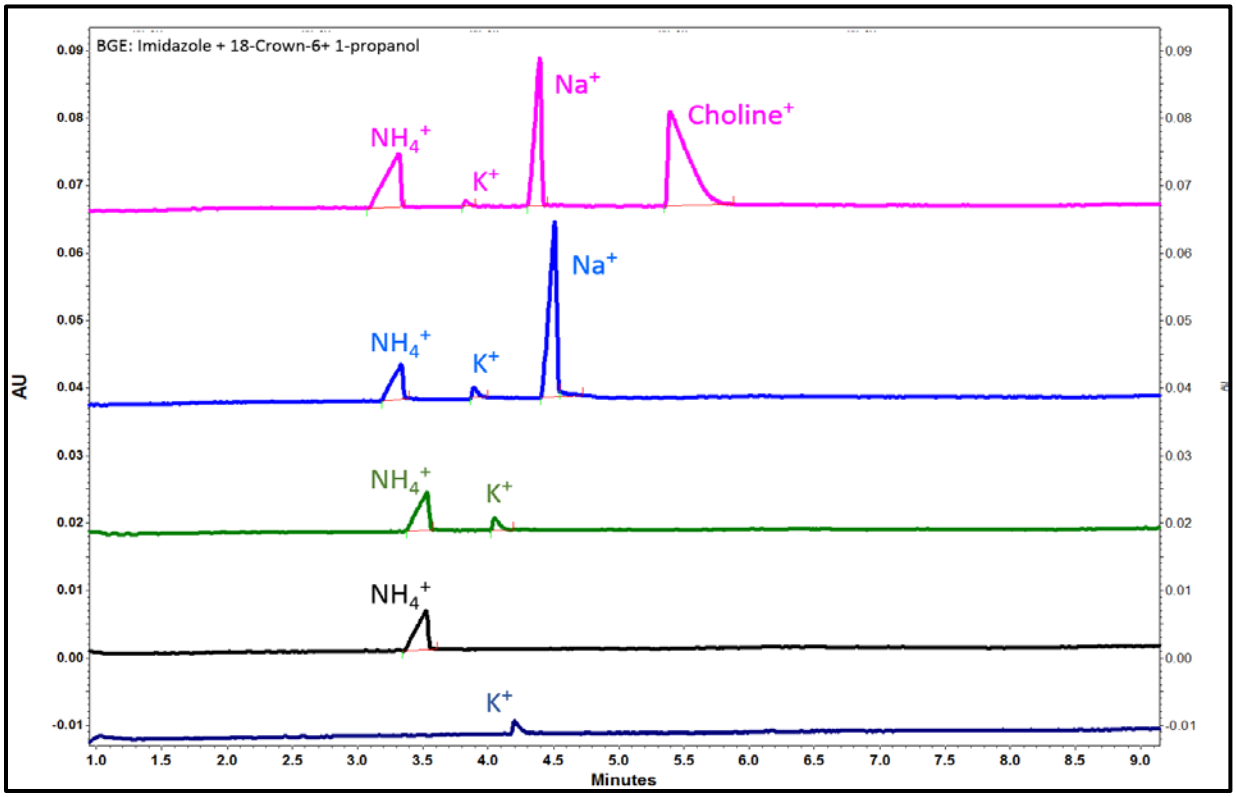

Figure 4.10 - Electropherograms of the separation of choline ion and ammonium, potassium, sodium ions by capillary electrophoresis with BGE containing imidazole, 18-crown-6 and 1-propanol

Calibration of Standard Solutions

Under optimized conditions, the standard choline chloride solutions with various concentrations from 100 ppm to 2000 ppm were analyzed. The data is shown in Table 4.4 and the plot is shown in Figure 4.12. The electropherograms are shown in Figure 4.13. The peak area of choline ion increased with increase in choline chloride concentrations, giving a very good linear relationship.

Table 4.4 - Peak area of choline ion versus the concentration of choline chloride from 100 ppm to 2000 ppm

Standard choline chloride solutions	
CC Concentration (ppm)	**Peak Area**
100	6956
200	19577
400	38519
600	64718
800	82316
1000	106742
1250	153008
1500	160027
1750	199866
2000	229951

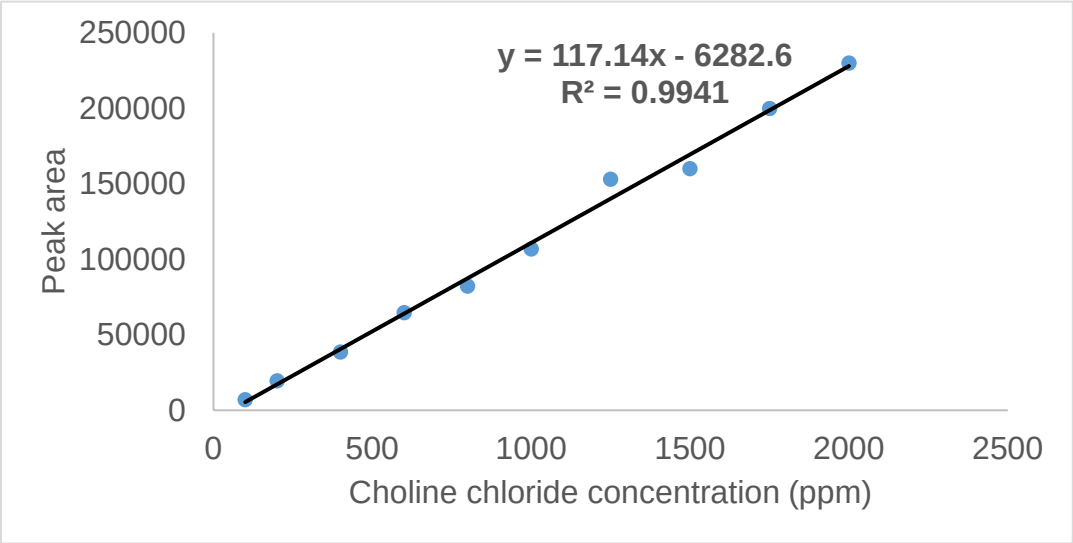

Figure 4.12 - Calibration curve of peak area of choline ion versus choline chloride concentration

Analysis of Oilfield Water Samples

Eleven choline samples consisting of oilfield process waters and commercial products were analyzed. These samples were grouped into two sets because they were sent at different times from the oil and gas company. The first set was five (5) samples while the other had six (6) samples. All original samples were filtered and diluted 100 times. For every analysis, 20 µL of the diluted samples were put into a vial and diluted with 18 MΩ water until the total volume was always 200 µL. Thus, the dilution factor was 1,000 compared to the original samples. The choline chloride concentrations (ppm) calculated from the peak areas in the electropherograms were therefore multiplied by 1,000 to obtain the amount of choline chloride in the original samples. The choline chloride in the samples were converted from ppm to percentage choline chloride (% CC) by dividing by 10,000.

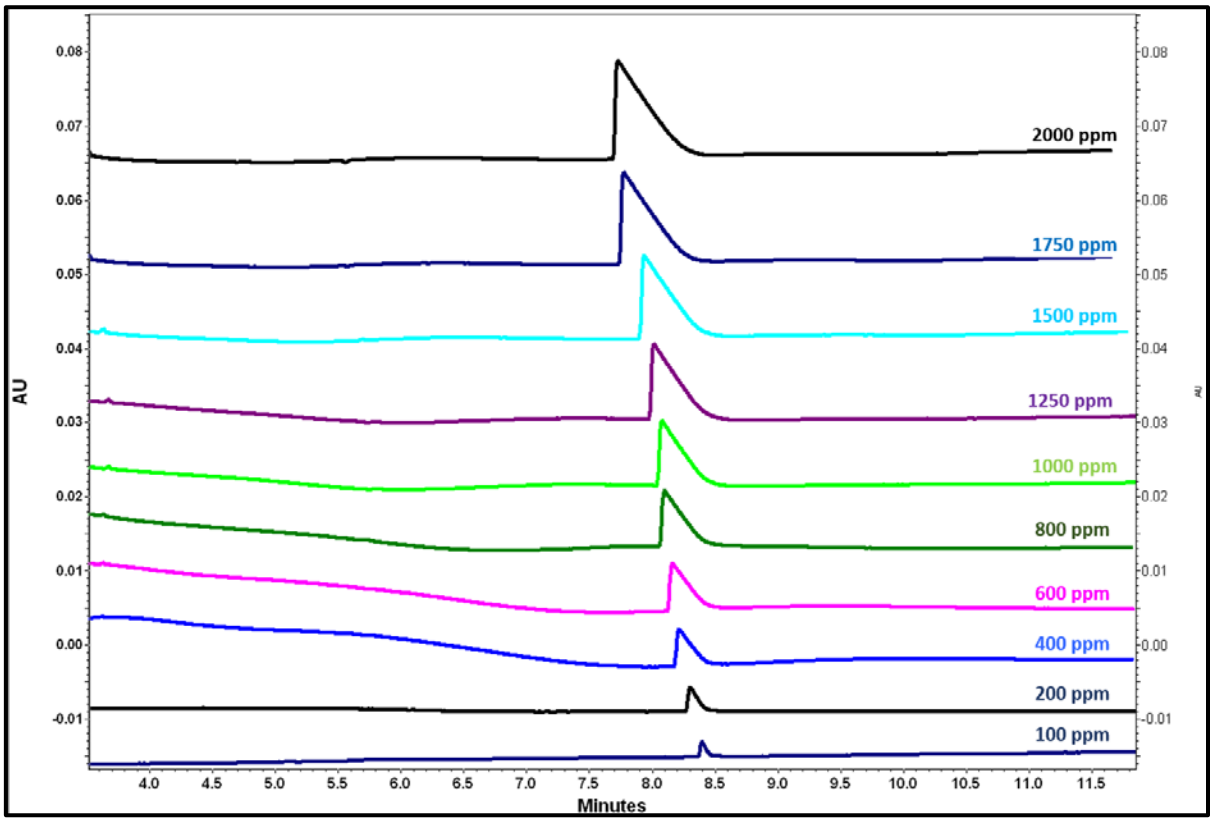

Figure 4.13 - Electropherograms of choline ion in different choline chloride concentrations

External Calibration Method

This is the most common quantitative analysis approach because it is quite simple to be applied for a large number of samples. This method performs the comparison of the signal response for the desired compound in standard solutions. From the calibration curve, the concentration of choline chloride in oilfield water samples were determined by the peak area of choline ion as shown in Figure 4.14.

The external calibration approach might ignore the effects of the matrix because standard solutions and samples are seldom matrix-matched. The peak areas of choline ions and calculated choline chloride concentrations (ppm) of these two sets of samples are shown in Table 4.5 and Table 4.6. The percent choline chloride in the original samples ((% CC) is also calculated and summarized in Table 4.7.

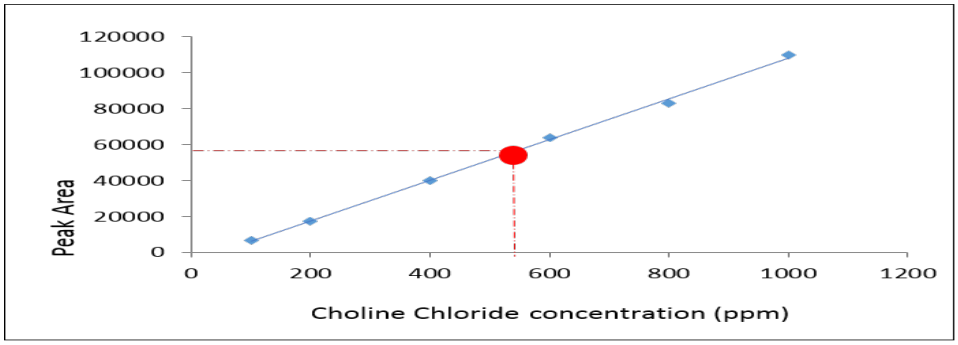

Figure 4.14 - Choline chloride concentration versus the peak area of choline ion based on external calibration method

First set of samples:

Table 4.5 - The concentrations of choline chloride in five samples of the first set based on peak area of choline ion

The calibration equation: $y = 117.14x - 6282.6$ $R^2 = 0.9941$

Sample	Peak area of choline ion			Choline chloride concentration in analyzed samples (ppm)		
	1st	2nd	3rd	1st	2nd	3rd
11241	82734	65633	85678	759.9	613.9	785.0
11242	65717	68273	73730	614.6	636.5	683.1
11243	73143	77821	76920	678.0	718.0	718.2
11244	76541	76310	80626	707.0	705.1	741.9
11245	32669	37020	39960	332.5	369.7	394.8

Second set of samples:

Table 4.6 - The concentrations of choline chloride in six samples of the second set based on peak area of choline ion

The calibration equation: $y = 48.81x - 2477.01$ $R^2 = 0.9874$

Sample	Peak area of choline ion			Choline chloride concentration in analyzed samples (ppm)		
	1st	2nd	3rd	1st	2nd	3rd
11411	10617	10259	12455	298.8	290.7	340.8
11412	23021	20951	21096	581.9	534.7	538.0
11413	25733	25782	24120	643.8	644.9	607.0
11414	27840	24531	31290	691.9	616.4	770.6
11415	33727	32940	33470	826.3	808.3	820.4
11421	32771	33441	30204	804.4	819.7	745.9

Table 4.7 - Summary of choline chloride concentration (% CC) in eleven original samples based on external calibration method

Sample	Concentration of choline chloride (% CC) in original samples based on external calibration method (STD curves)				
	1st	2nd	3rd	Mean (% CC)	% RSD
11241	75.99	61.39	78.50	71.96 ± 9.24	12.84 %
11242	61.46	63.65	68.31	64.47 ± 3.49	5.42 %
11243	67.80	71.80	71.03	70.21 ± 2.31	3.02 %
11244	70.70	70.51	74.19	71.80 ± 2.07	2.89 %
11245	33.25	36.97	39.48	36.57 ± 3.13	8.56 %
11411	29.88	29.07	34.08	31.01 ± 2.69	8.67 %
11412	58.19	53.47	53.80	55.15 ± 2.64	4.78 %
11413	64.38	64.49	60.70	63.19 ± 2.16	3.42%
11414	69.19	61.64	77.06	69.30 ± 7.71	11.13 %
11415	82.63	80.83	82.04	81.83 ± 0.92	1.12 %
11421	80.44	81.97	74.59	79.00 ± 3.90	4.94 %

Standard Addition Method

The standard addition method (also known as spiking method) is another approach to control the matrix effects of the analyte signal. Known amounts of standard solutions are “spiked” into the samples to enhance the signal of the desired analyte. Although this method is extremely effective to minimize the matrix effects, it consumes a huge amount of time because it requires too many measurements per sample. In this work, for each sample, five vials containing 20 µL of sample were “spiked” with 0, 5, 15, 25, 40 µL of 10,000 ppm choline chloride solutions. These vials were diluted with 18 MΩ water to 200 µL. A plot was obtained by plotting the concentration of spiked choline chloride on the x-axis and the corresponding peak areas on the y-axis. By extrapolation to x-axis, the concentration of unknown was determined at the point of the x-intercept with y = 0, as demonstrated in Figure 4.15.

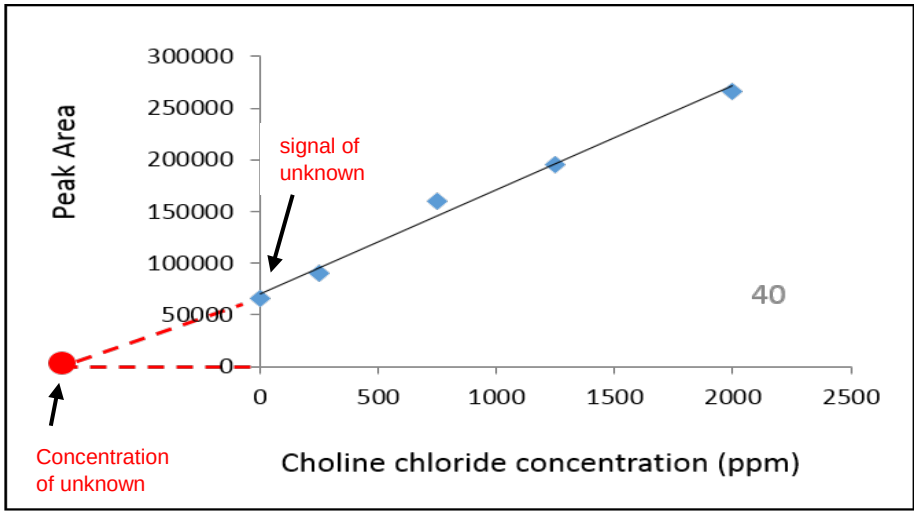

Figure 4.15 - Choline chloride concentration versus the peak area of choline ion based on standard addition method

The results obtained from the standard addition analysis are shown in Table 4.8 and 4.9. An example of the actual electropherogram obtained is displayed in Figure 4.16 for one of the samples. The rest of the electropherograms are shown in the Appendix B.

Table 4.8 - Choline chloride concentrations in diluted samples obtained by standard addition

	Sample	Equation	R^2	Choline chloride concentration in analyzed samples (ppm)
1st	11241	y=100.4x + 88106.1	0.998	877.54
	11242	y=103.2x + 65556.9	0.980	635.04
	11243	y=106.3x + 75014.6	0.994	705.57
	11244	y=107.2x + 75772.6	0.996	706.55
	11245	y=89.3x + 32102.7	0.998	359.47
	11411	y=70.8x + 24187.0	0.987	341.46
	11412	y=75.9x + 35241.0	0.989	464.60
	11413	y=39.3x + 25376.3	0.997	646.49
	11414	y=38.0x +28773.8	0.999	757.22
	11415	y=54.2x + 33891.7	0.999	625.07
	11421	y=38.9x + 24282.9	0.998	625.06
2nd	Sample	Equation	R^2	Choline chloride concentration in analyzed samples (ppm)
	11241	y=84.1x + 72360.6	0.993	859.81
	11242	y=110.6x + 68548.4	0.991	619.58
	11243	y=113.4x +70040.0	0.988	617.64
	11244	y=115.2x + 81356.1	0.993	706.04
	11245	y=123.8x + 42662.9	0.998	344.65
	11411	y=68.5x + 24719.0	0.987	360.46
	11412	y=77.8x + 35712.2	0.996	459.02
	11413	y=38.5x + 24967.3	0.999	648.86
	11414	y=36.4x + 22790.9	0.998	626.76
	11415	y=53.3x + 32875.6	0.999	617.24
	11421	y=48.5x + 29633.4	0.999	610.57
3rd	Sample	Equation	R^2	Choline chloride concentration in analyzed samples (ppm)
	11241	y=102.2x + 83787.6	0.986	820.11
	11242	y=112.8x + 70679.5	0.987	626.57
	11243	y=108.5x + 75157.5	0.999	692.93
	11244	y=112.0x + 83683.4	0.992	747.40
	11245	y=120.5x + 41038.6	0.991	340.68
	11411	y=70.8x + 24001.8	0.991	339.12
	11412	y=77.9x + 36364.3	0.995	467.02
	11413	y=40.2x + 27046.8	0.973	673.14
	11414	y=35.77x + 23849.5	0.999	666.74
	11415	y=48.4x + 32870.7	0.995	679.68
	11421	y=46.6x + 29734.5	0.992	638.11

Table 4.9 - Summary of choline chloride concentration (% CC) in eleven original samples based on standard addition method

SAMPLE	Concentration of choline chloride (% CC) in original samples based on Standard Addition Method				
	1st	2nd	3rd	Mean (% CC)	% RSD
11241	87.76	85.98	82.01	85.25 ± 2.94	3.45%
11242	63.51	61.96	62.66	62.71 ± 0.78	1.24%
11243	70.56	61.76	69.30	67.21 ± 4.75	7.07%
11244	70.66	70.60	74.74	72.00 ± 2.37	3.29%
11245	35.95	34.46	34.07	34.83 ± 0.99	2.84%
11411	34.15	36.05	33.91	34.70 ± 1.17	3.37%
11412	46.46	45.90	46.70	46.35 ± 0.41	0.89%
11413	64.65	64.89	67.31	65.62 ± 1.47	2.25%
11414	75.72	62.68	66.67	68.36 ± 6.68	9.78%
11415	62.51	61.72	67.97	64.07 ± 3.40	5.31%
11421	62.51	61.06	63.81	62.46 ± 1.38	2.21%

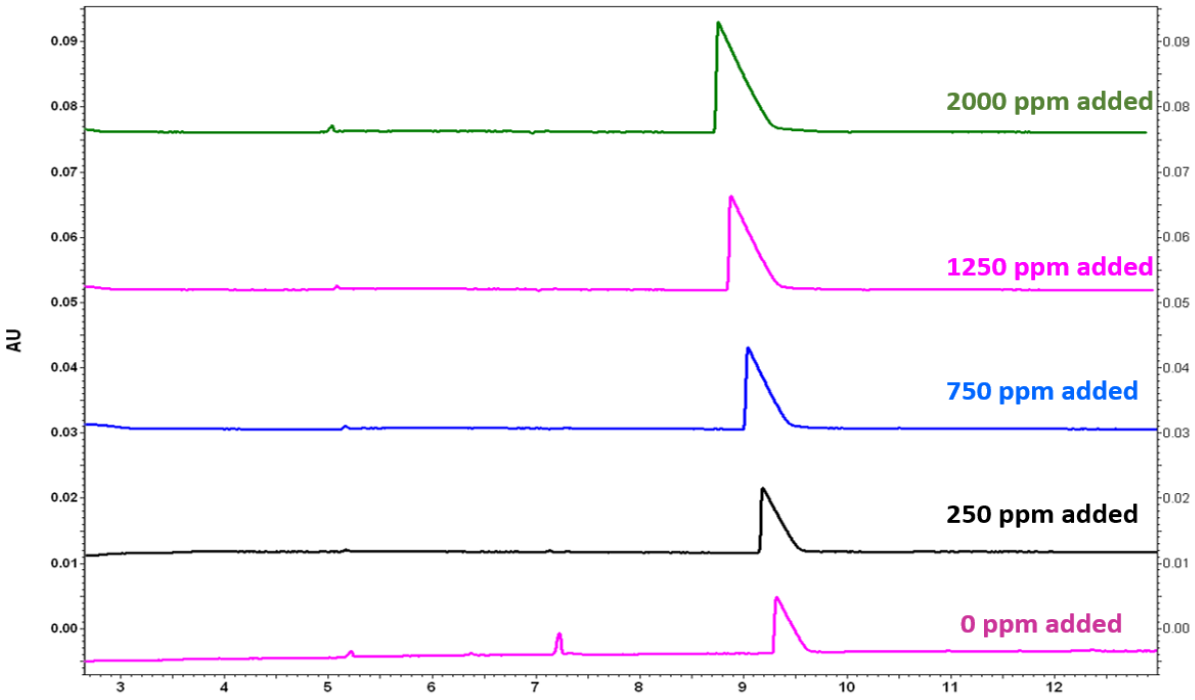

Figure 4.16 - Electropherogram of sample 11241 by standard addition; choline chloride spikes added (0 ppm, 250 ppm, 750 ppm, 1250 ppm, 2000 ppm)

Comparison of the CE Results from the Two Calibration Techniques

A comparison of the CE external calibration and standard addition techniques is also shown in Figure 4.27 and Table 4.10. As can be seen, the two techniques compared favourably well except for the few samples that had large differences of choline chloride levels. But even for these, the differences were less than 20%. The possible reason for the differences could be that the external calibration approach could not eliminate the effect of the matrices on the analyte. Therefore, there may be some interferences in the matrices that have a positive or negative effect on choline ion peak at the same migration time. Nevertheless, because the differences were less than 20%, the two approaches were considered acceptable for choline analysis. Moreover, although the standard addition approach could minimize the effect of matrices, it consumed a huge amount of time and effort to analyze many samples. Therefore, depending on time constraints and purpose, either approach could be chosen for the choline analysis by capillary electrophoresis. It can also be seen from Table 4.10 that for each of the two calibration techniques, the reproducibility is reasonably good with % RSD less than 15%.

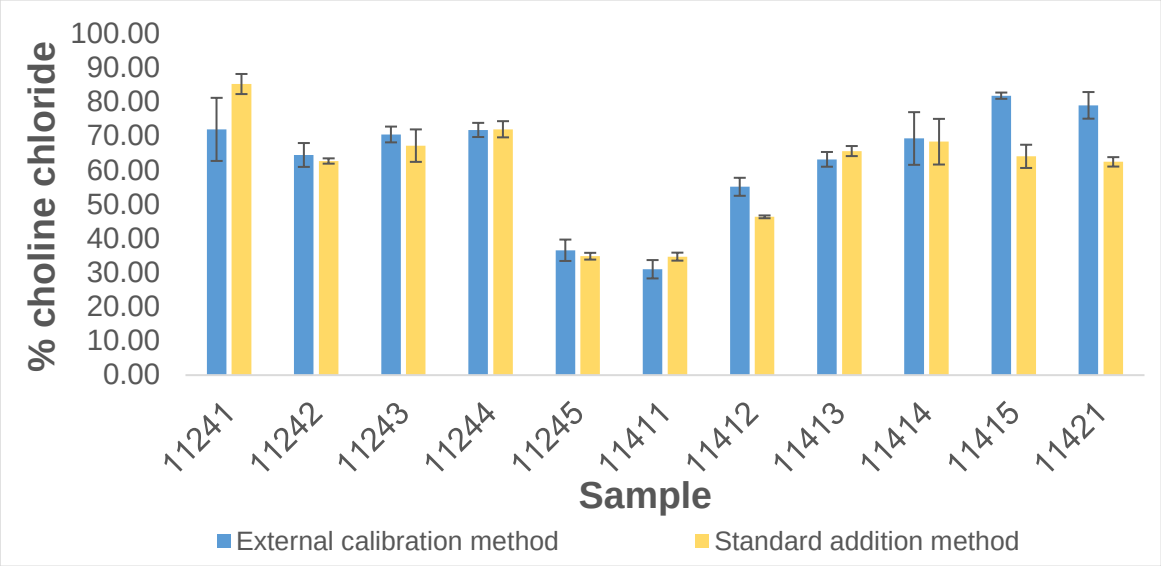

Figure 4.17 - Choline chloride concentrations in oilfield process waters samples determined by capillary electrophoresis with external calibration method and standard addition method.

Table 4.10 - Percent choline chloride in eleven samples by CE with two approaches (n=3)

Sample	External Calibration by CE		STD Addition by CE	
	% CC	% RSD	% CC	% RSD
11241	71.96 ± 9.24	12.84%	85.25 ± 2.94	3.45%
11242	64.47 ± 3.49	5.42%	62.71 ± 0.78	1.24%
11243	70.21 ± 2.31	3.28%	67.21 ± 4.75	7.07%
11244	71.80 ± 2.07	2.89%	72.00 ± 2.37	3.29%
11245	36.57 ± 3.13	8.56%	34.83 ± 0.99	2.84%
11411	31.01 ± 2.69	8.67%	34.70 ± 1.17	3.37%
11412	55.15 ± 2.64	4.78%	46.35 ± 0.41	0.89%
11413	63.19 ± 2.16	3.42%	65.62 ± 1.47	2.25%
11414	69.30 ± 7.71	11.13%	68.36 ± 6.68	9.78%
11415	81.83 ± 0.92	1.12%	64.07 ± 3.40	5.31%
11421	79.00 ± 3.90	4.94%	62.46 ± 1.38	2.21%

Method Validation Results

Intraday and Interday Precision Studies

To investigate the precision of the developed method, intraday and interday analysis were carried out at three different concentrations (200 ppm, 600 ppm and 1000 ppm) of choline chloride. These standard solutions were analyzed at three different times within a day (intraday). These analyses were also repeated on three consecutive days (interday). The data of intraday and interday studies demonstrating the reproducibility of the work are in Table 4.11. As can be seen in the table, the peak area and migration time studies resulted in a % RSD <10% for the intraday and interday analysis. Therefore, the developed method by capillary electrophoresis can be considered highly reproducible.

Table 4.11 - Intraday and interday precision (% RSD) of choline chloride analysis on CE

Concentration of choline chloride (ppm)	**Peak area (n = 3) % RSD**			**Migration time (n = 3) % RSD**		
	Day 1	**Day 2**	**Day 3**	**Day 1**	**Day 2**	**Day 3**
200	6.10%	5.23%	5.43%	3.39%	0.42%	1.36%
600	1.23%	0.56%	2.33%	0.09%	0.52%	0.38%
1000	2.30%	5.01%	0.81%	0.03%	0.27%	0.06%

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD is the concentration that will give a response with a signal-to-noise (S/N) ratio of 3, while the LOQ is the concentration that will give a response with S/N ratio of 10. By the calculation shown in Appendix A.4, the LOD and LOQ of choline chloride by the CE method were 14.7 ppm and 48.9 ppm, respectively.

Percent Recovery Results

The recoveries of choline chloride in the eleven samples were determined at low (750 ppm) and high concentration (2000 ppm). Good percent recoveries for the selected samples were obtained ranging from 80% - 120% as shown in Table 4.12 and 4.13. Therefore, the developed method by CE can be considered accurate.

- **First set of samples:**

Table 4.12 - Percent recovery results for five samples of the first set by CE

The calibration equation: $y = 117.14x - 6282.6$ $R^2 = 0.9941$

Sample Name	Spiked Concentration (ppm)	Recovered Concentration (ppm)	% Recovery
11241	750	675.4	**90.1%**
	1250	992.2	**79.4%**
	2000	1866.2	**93.3%**
11242	750	751.6	**100.2%**
	1250	1085.6	**86.8%**
	2000	2040.0	**102.0%**
11243	750	715.4	**95.4%**
	1250	1174.7	**94.0%**
	2000	1896.3	**94.8%**
11244	750	789.0	**105.2%**
	1250	1221.1	**97.7%**
	2000	1846.3	**92.3%**
11245	750	624.6	**83.3%**
	1250	1041.5	**83.3%**
	2000	1599.2	**80.0%**

Second set of samples:

Table 4.13 - Percent recovery results for six samples of the second set by CE

The calibration equation: $y = 48.81x - 2477.01$ $R^2 = 0.9874$

Sample Name	Spiked Concentration (ppm)	Recovered Concentration (ppm)	% Recovery
11411	750	820.6	**109.4%**
	1250	1281.4	**102.5%**
	2000	2128.6	**106.4%**
11412	750	650.3	**86.7%**
	1250	1222.3	**97.8%**
	2000	1960.2	**98.0%**
11413	750	668.4	**89.1%**
	1250	1145.0	**91.6%**
	2000	1801.5	**90.1%**
11414	750	756.3	**100.8%**
	1250	1157.2	**92.6%**
	2000	1803.9	**90.2%**
11415	750	790.3	**105.4%**
	1250	1353.9	**108.3%**
	2000	2303.5	**115.2%**
11421	750	724.8	**96.6%**
	1250	1215.2	**97.2%**
	2000	2188.8	**109.4%**

Liquid Chromatography - Mass Spectrometry (LC/MS)

Standard Solutions Calibration

Standard choline chloride solutions with varying concentrations from 50 ppm to 1000 ppm were analyzed by LC/MS. The retention time of choline ion in the extracted-ion chromatogram (EIC) was around 1.5 min as shown in Figure 4.28. The mass spectrum in Figure 4.29 indicated choline identity at a mass-to-charge ratio (*m/z*) of approximately 104.

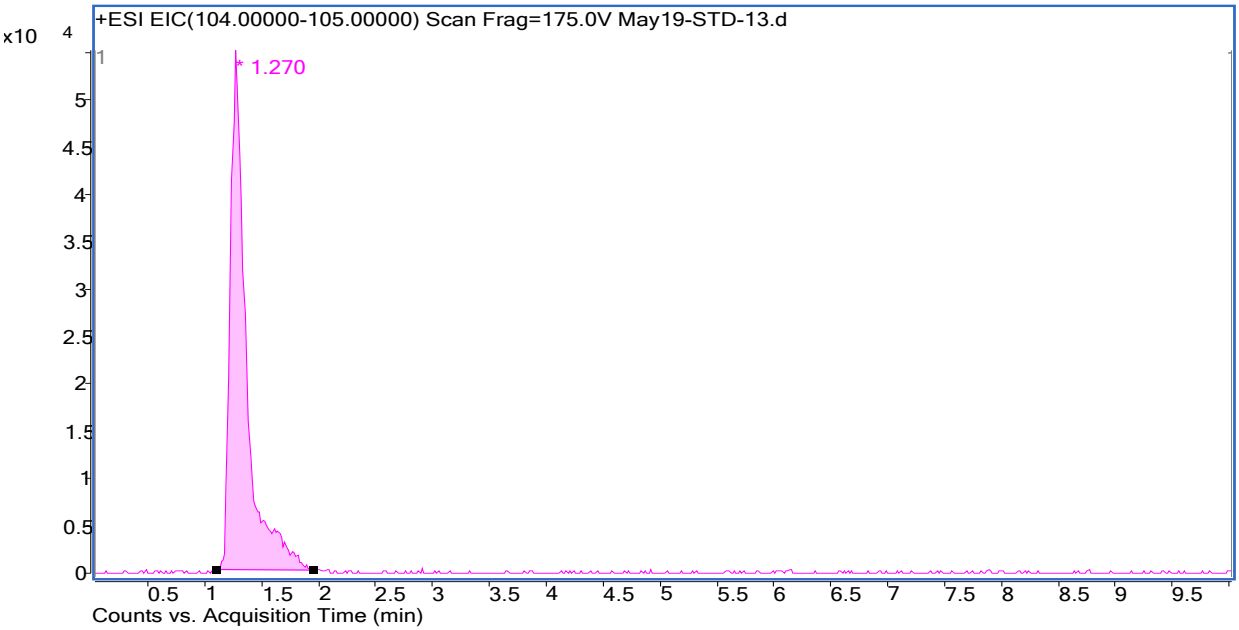

Figure 4.18 - Chromatogram showing choline peak in 750 ppm choline chloride standard solution (y-axis is counts and x-axis is time in min)

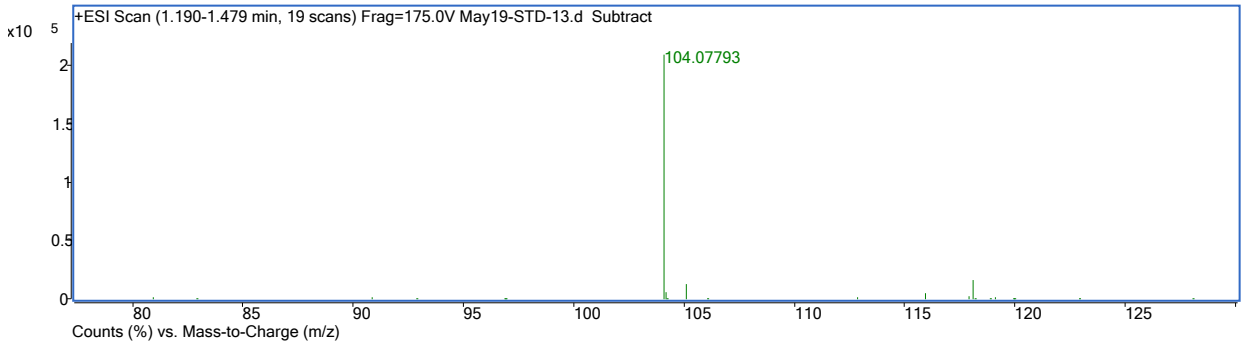

Figure 4.19 - Mass spectrum for choline present in 750 ppm choline chloride standard solution (y-axis is signal intensity and x-axis is *m/z*)

The peak areas of choline ion increased with increasing choline chloride concentrations as shown in Table 4.14., and produced a good calibration curve shown in Figure 4.30.

Table 4.14 - The peak area of choline ion versus concentration of standard choline chloride solutions from 50 ppm to 1000 ppm by LC/MS

Standard choline chloride solutions	
Concentration (ppm)	**Peak Area**
50	99581
100	114680
250	205643
500	408919
750	536049
1000	649328

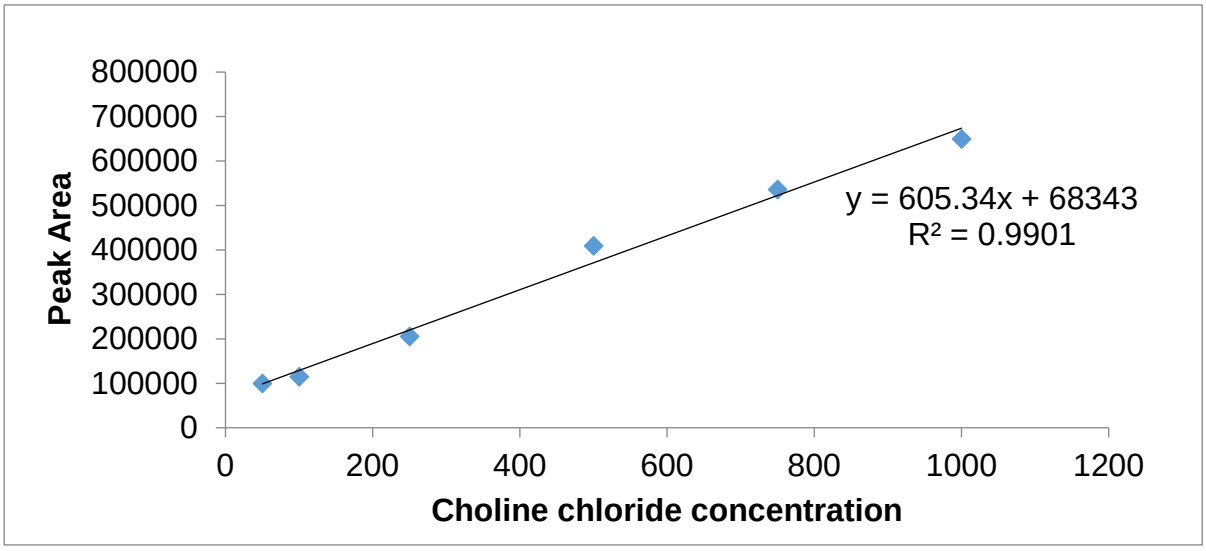

Figure 4.20 - Calibration curve of peak area of choline ion versus choline chloride concentration by LC/MS

Analysis of Choline Samples

The purpose of the LC/MS analysis of choline is to validate the CE results obtained for the oilfield water samples with the aim of establishing that the results obtained by CE analysis were accurate. In the preparation, all eleven oilfield waters were diluted 1,000 times for the analysis by the external calibration method. The analysis of choline chloride by LC/MS was carried out by the external calibration curve

approach because of limited time. Therefore, the obtained results might be affected by the matrices. Moreover, the analyzed conditions could not provide a simultaneous separation of choline ion and other cations as seen in the CE method. By LC/MS, choline ion was detected at a retention time of 1-2 min. The identity of the peak was confirmed by the mass spectrum. The chromatograms and the mass spectrum of choline ion in all samples were obtained. The peak areas of choline ion were applied for quantitation of choline chloride concentration (ppm) using the calibration equation shown in Table 4.15. Then, the percentage choline chloride in the original water samples was calculated by multiplying the choline chloride concentrations (ppm) by 1,000 before dividing by 10,000 as shown in Table 4.16.

The calibration equation: $y = 605.34x + 68343$ $R^2 = 0.9901$

Sample	Peak area of choline ion			Choline chloride concentration in analyzed samples (ppm)		
	1st	2nd	3rd	1st	2nd	3rd
11241	545578	547219	531950	788.38	791.09	765.87
11242	552196	550834	549869	799.31	797.06	795.47
11243	564237	551765	549014	819.20	798.60	794.06
11244	538253	544380	537835	776.28	786.40	775.59
11245	327395	325319	310492	427.95	424.52	400.02
11411	279333	281465	278464	348.55	352.07	347.11
11412	451878	455306	452422	633.59	639.25	634.49
11413	578185	569329	566010	842.25	827.62	822.13
11414	576492	583192	582897	839.45	850.52	850.03
11415	515572	519899	511384	738.81	745.96	731.89
11421	540143	550108	530678	779.40	795.86	763.76

Table 4.15 - The concentrations of choline chloride in samples based on external calibration

Table 4.16 - Summary of choline chloride concentration (% CC) in eleven samples based on external calibration method by LC/MS

Sample	Concentration of choline chloride (% CC) in original samples based on external calibration method				
	1st	2nd	3rd	Mean (% CC)	% RSD
11241	78.84	79.11	76.59	78.18 ± 1.38	1.77%
11242	79.93	79.71	79.55	79.73 ± 0.19	0.24%
11243	81.92	79.86	79.41	80.40 ± 1.34	1.67%
11244	77.63	78.64	77.56	77.94 ± 0.61	0.78%
11245	42.79	42.45	40.00	41.75 ± 1.52	3.65%
11411	34.85	35.21	34.71	34.92 ± 0.26	0.73%
11412	63.36	63.93	63.45	63.58 ± 0.30	0.48%
11413	84.22	82.76	82.21	83.07 ± 1.04	1.25%
11414	83.94	85.05	85.00	84.67 ± 0.63	0.74%
11415	73.88	74.60	73.19	73.89 ± 0.70	0.95%
11421	77.94	79.59	76.38	77.97 ± 1.61	2.06%

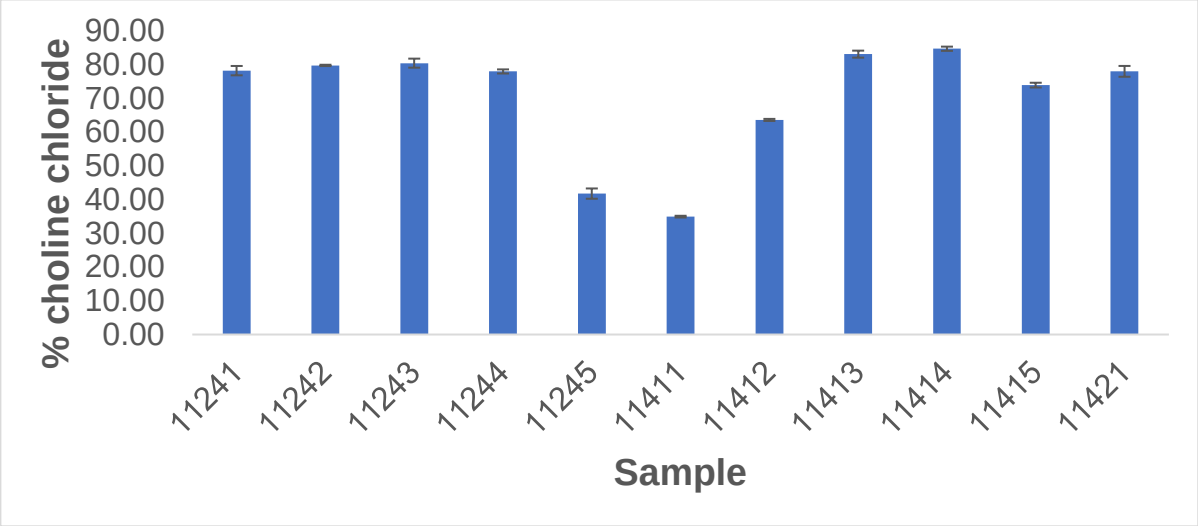

Figure 4.21 - Choline chloride concentrations in oilfield process water samples determined by LC/MS with external calibration method

The percentage choline chloride in the eleven oilfield water samples with standard deviation error bars are shown in Figure 3.31. The retention times of choline peaks in all samples were similar with those in standard solutions. The extracted ion

chromatograms and mass spectra for two samples 11241 and 11421 are illustrated in Figures 4.32-4.35. The chromatograms and mass spectra of the other remaining nine (9) samples are shown in the Appendix C.

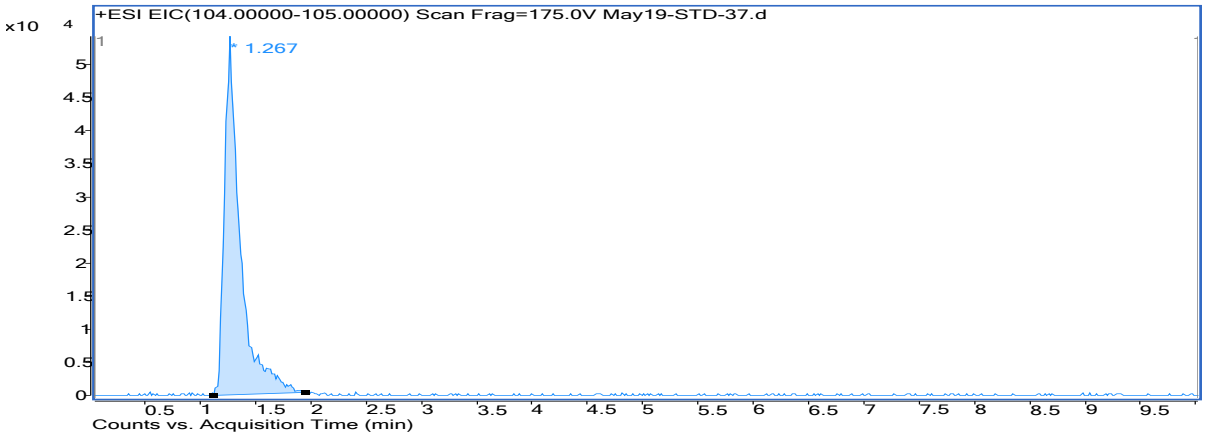

Figure 4.22 – Extracted Ion Chromatogram showing choline peak in sample 11241 (y-axis is counts and x-axis is time in min)

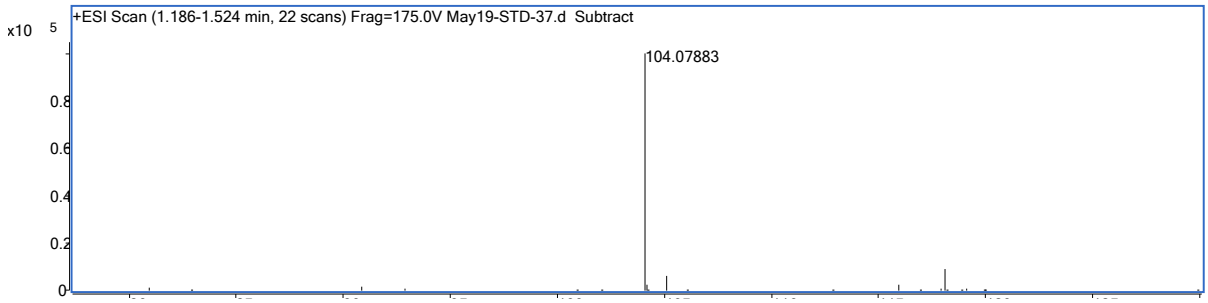

Figure 4.24 - Mass spectrum for choline present in sample 11241 (y-axis is signal intensity and x-axis is *m/z*)

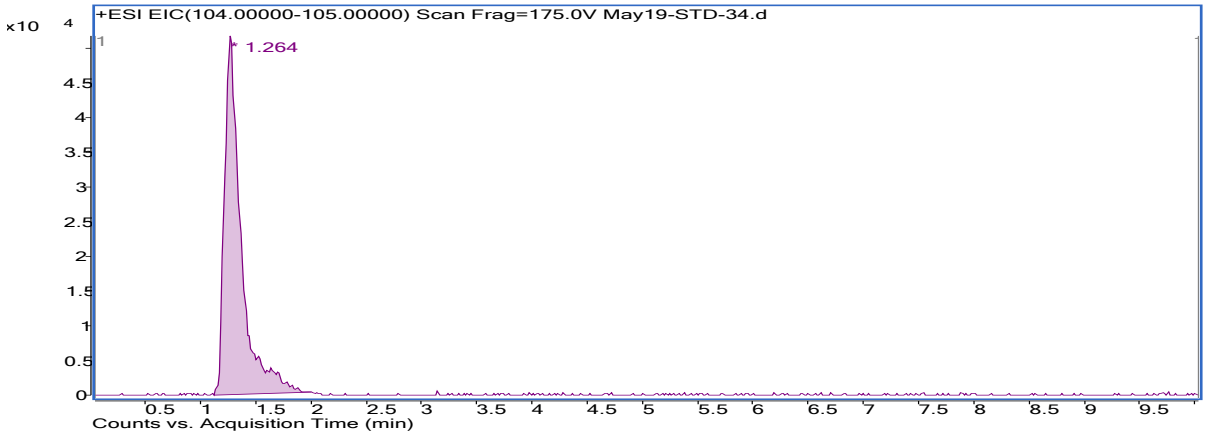

Figure 4.23 – Extracted Ion Chromatogram showing choline peak in sample 11421 (y-axis is counts and x-axis is time in min)

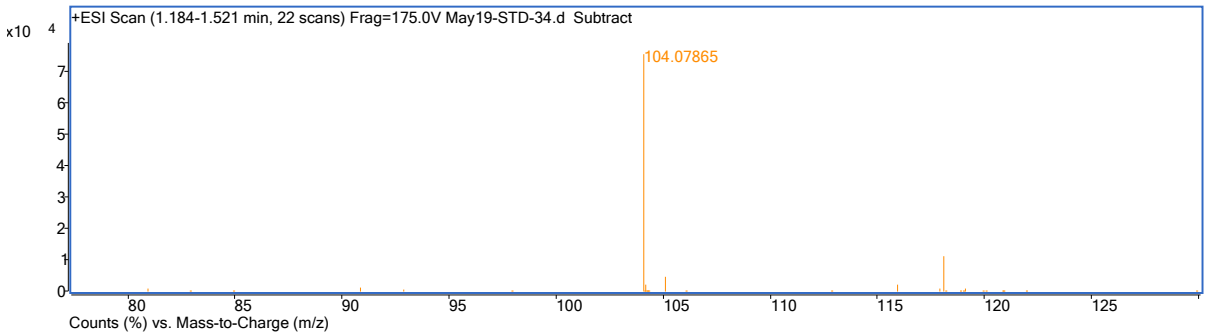

Figure 4.25 - Mass spectrum for choline present in sample 11421 (y-axis is signal intensity and x-axis is *m/z*)

Interday and Intraday Precision

Intraday and interday precision studies were carried out to evaluate the precision of the LC/MS method. Six different choline chloride concentrations from 50 ppm to 1000 ppm were analyzed three times in a day for the intraday study. The interday precision was investigated by measuring the same choline chloride concentrations in triplicate during three consecutive days as shown in Tables 4.17 - 4.20. The results showed a good reproducibility for choline analysis by LC/MS indicated by the excellent repeatability which was achieved for the peak area (RSD < 10%) and migration times (RSD < 10%).

Table 4.17 - Intraday and interday precision (% RSD) of choline chloride analysis on LC/MS

Concentration of choline chloride (ppm)	**Peak area (n = 3) % RSD**			**Retention time (n = 3) % RSD**		
	Day 1	**Day 2**	**Day 3**	**Day 1**	**Day 2**	**Day 3**
50	5.17%	9.34%	4.47%	1.27%	1.14%	2.55%
100	3.55%	6.02%	2.24%	0.32%	0.91%	0.40%
250	4.72%	4.11%	1.63%	0.12%	0.80%	0.81%
500	1.13%	0.65%	3.17%	0.72%	0.64%	0.64%
750	1.10%	1.43%	2.65%	0.16%	0.34%	0.27%
1000	3.01%	1.99%	1.01%	0.31%	0.08%	0.08%

Table 4.18 - First day interday precision studies for choline ion on LC/MS

	Concentration (ppm)	Peak area (n = 3)			Mean	SD	% RSD
Day 1	50	99581	100545	91407	97178	5020.7	5.17%
	100	114680	111502	106842	111008	3942.3	3.55%
	250	205643	217907	225939	216496	10221.3	4.72%
	500	408919	410615	401902	407145	4619.4	1.13%
	750	536049	547769	543773	542530	5958.0	1.10%
	1000	649328	641049	612948	634442	19068.8	3.01%
	Concentration (ppm)	**Migration time (n =3)**			**Mean**	**SD**	**% RSD**
	50	1.262	1.294	1.283	1.280	0.016	1.27%
	100	1.279	1.282	1.287	1.283	0.004	0.32%
	250	1.29	1.287	1.289	1.289	0.002	0.12%
	500	1.274	1.268	1.256	1.266	0.009	0.72%
	750	1.27	1.269	1.273	1.271	0.002	0.16%
	1000	1.267	1.271	1.275	1.271	0.004	0.31%

Table 4.19 - Second day interday precision studies for choline ion on LC/MS

	Concentration (ppm)	Peak area (n = 3)			Mean	SD	% RSD
Day 2	50	112083	101222	93072	102126	9537.7	9.34%
	100	137850	122727	127102	129226	7782.1	6.02%
	250	257443	278117	274859	270140	11115.6	4.11%
	500	444694	440260	439299	441418	2877.8	0.65%
	750	527074	541960	537546	535527	7645.7	1.43%
	1000	626659	602499	618375	615844	12277.2	1.99%
	Concentration (ppm)	**Migration time (n = 3)**			**Mean**	**SD**	**% RSD**
	50	1.299	1.329	1.313	1.314	0.015	1.14%
	100	1.261	1.284	1.274	1.273	0.012	0.91%
	250	1.275	1.255	1.267	1.266	0.010	0.80%
	500	1.276	1.261	1.263	1.267	0.008	0.64%
	750	1.267	1.275	1.274	1.272	0.004	0.34%
	1000	1.271	1.272	1.273	1.272	0.001	0.08%

Table 4.20 - Third day interday precision studies for choline ion on LC/MS

	Concentration (ppm)	Peak area (n = 3)			Mean	SD	% RSD
Day 3	50	83396	88190	80767	84118	3763.8	4.47%
	100	142897	141113	147367	143792	3221.7	2.24%
	250	304597	313397	304868	307621	5004.3	1.63%
	500	424112	438049	451892	438018	13890.0	3.17%
	750	554913	527306	534631	538950	14301.3	2.65%
	1000	588337	599349	589720	592469	5998.5	1.01%
	Concentration (ppm)	**Migration time (n = 3)**			**Mean**	**SD**	**% RSD**
	50	1.262	1.326	1.309	1.299	0.033	2.55%
	100	1.292	1.285	1.295	1.291	0.005	0.40%
	250	1.305	1.288	1.286	1.293	0.010	0.81%
	500	1.286	1.273	1.288	1.282	0.008	0.64%
	750	1.29	1.287	1.283	1.287	0.004	0.27%
	1000	1.289	1.288	1.29	1.289	0.001	0.08%

Reinecke Salt Gravimetric Method

Standard Solutions Calibration

A stock solution of choline chloride was prepared with a 100 mL total volume containing 250 mg of choline chloride. The increasing red color of choline reinecke precipitates are displayed in Figure 4.36. The precipitates of choline reinecke increased corresponding to the volume of stock solution used in the experiment. The linear relationship between the mass of choline precipitates and choline chloride content in the used volume is demonstrated in Figure 4.37. The recovered choline chloride contents were calculated, providing a good percent recovery in the range 92% - 98% as shown in Table 4.21.

Figure 4.26 - Collected choline reinecke precipitates

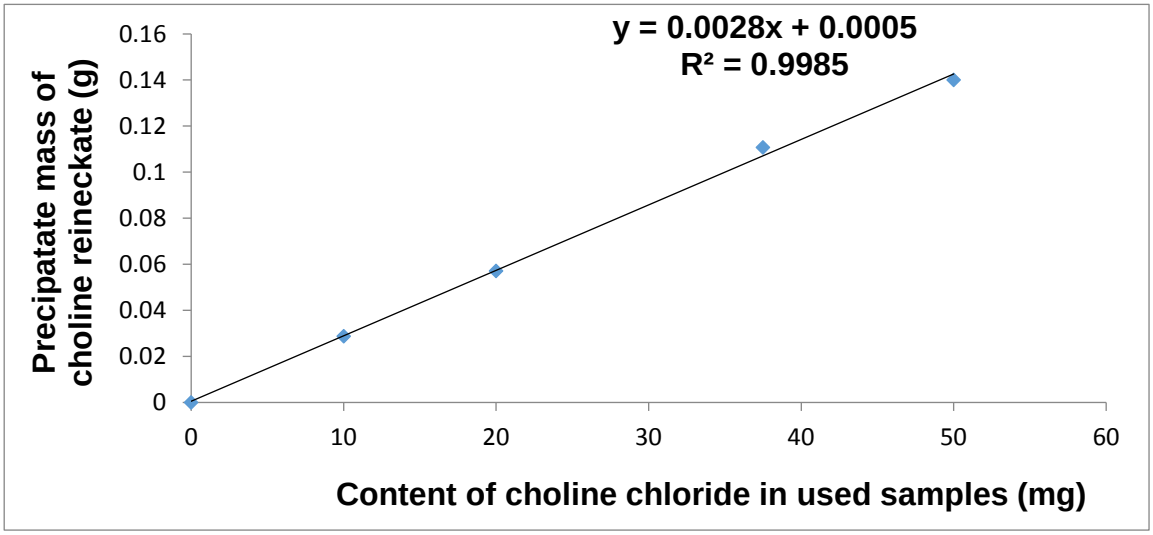

Figure 4.27- Calibration curve of the mass of choline precipitates versus choline chloride content

Table 4.21 - The percent recovery of choline chloride in stock solution

Vol. Used (mL)	Content of CC (mg)	Precipitate mass (g)	Recovered choline chloride= 0.3304 x m (mg)	% Recovery
20	50	0.1401	46.2890	92.58
15	37.5	0.1107	36.5753	97.53
8	20	0.0571	18.8658	94.33
4	10	0.0287	9.4825	94.82

Analysis of Oilfield Choline Water Samples

Original samples were diluted 100 times for the analysis. Each sample was analyzed four times with % RSD less than 10%. Concentrations of choline chloride in the eleven samples were calculated and the results displayed in Figure 4.38 and Table 4.22.

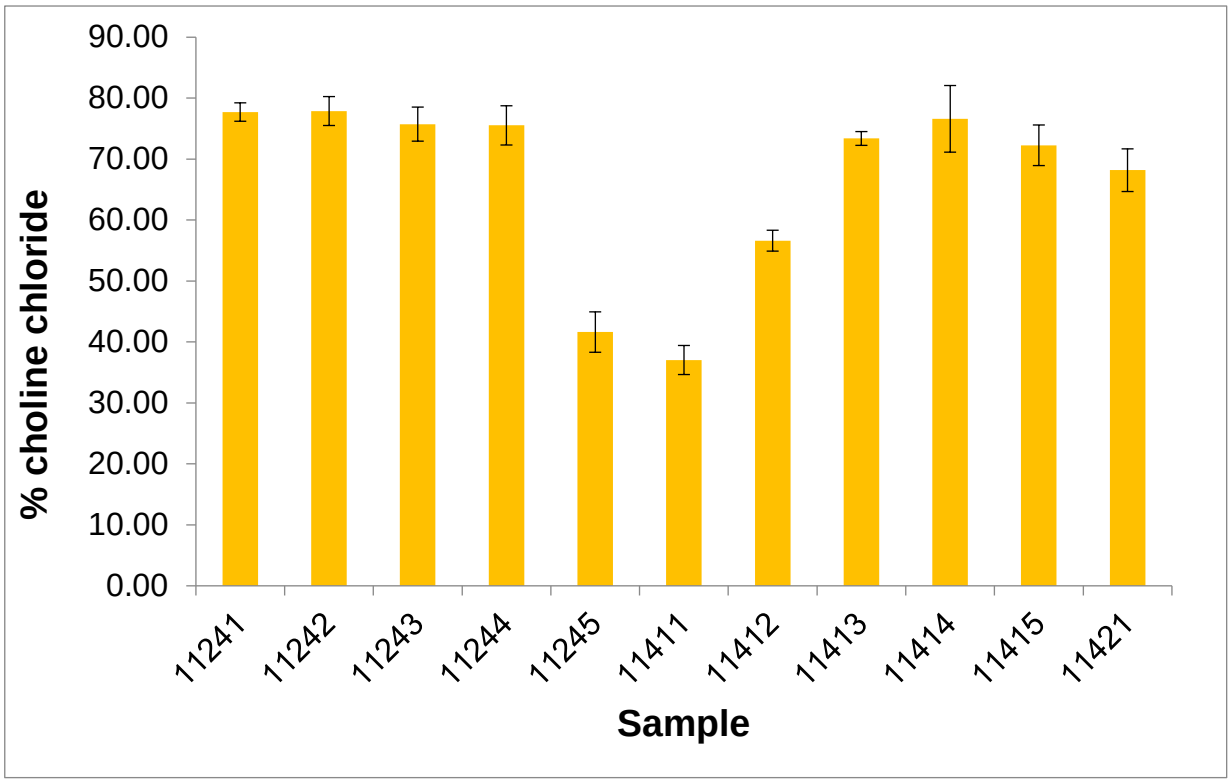

Figure 4.28 - Choline chloride concentrations in oilfield process water samples determined by Reinecke salt gravimetric method

Table 4.22 - Choline chloride concentration in oilfield water samples by Reinecke salt gravimetric method

Diluted Sample	**Vol. Used (mL)**	**Precipitate mass** m(g)	**CC content** = 0.3304 x m (mg)	**C(ppm) in original sample** = CC content (mg)/used vol. (L) * 100	**% CC**	**Mean (% CC) (n = 4)**	**% RSD**
11241	1	0.024	7.930	792960	79.30	77.13 ± 1.51	1.9%
	1	0.0238	7.864	786352	78.64		
	1	0.0230	7.599	759920	75.99		
	2	0.0466	15.397	769832	76.98		
11242	1	0.024	7.930	792960	79.30	77.89 ± 2.37	3.0%
	1	0.0225	7.434	743400	74.34		
	1	0.0239	7.897	789656	78.97		
	2	0.0478	15.793	789656	78.97		
11243	1	0.0226	7.467	746704	74.67	75.74 ± 2.80	3.7%
	1	0.0231	7.632	763224	76.32		
	1	0.022	7.269	726880	72.69		
	2	0.048	15.859	792960	79.30		
11244	1	0.0225	7.434	743400	74.34	75.54 ± 3.22	4.3%
	1	0.0242	7.996	799568	79.96		
	1	0.0219	7.236	723576	72.36		
	2	0.0457	15.099	754964	75.50		
11245	1	0.0122	4.031	403088	40.31	41.63 ± 3.31	8.0%
	1	0.0121	3.998	399784	39.98		
	1	0.0141	4.659	465864	46.59		
	2	0.0240	7.930	396480	39.65		

Table 4.22 (cont.)

11411	1	0.0115	3.800	379960	38.00	37.05 ± 2.38	6.4%
	1	0.0121	3.998	399784	39.98		
	1	0.0106	3.502	350224	35.02		
	2	0.0213	7.038	351876	35.19		
11412	1	0.0169	5.584	558376	55.84	56.62 ± 1.71	3.0%
	1	0.0179	5.914	591416	59.14		
	1	0.017	5.617	561680	56.17		
	2	0.0335	11.068	553420	55.34		
11413	1	0.0219	7.236	723576	72.36	73.39 ± 1.13	1.5%
	1	0.0226	7.467	746704	74.67		
	1	0.0224	7.401	740096	74.01		
	2	0.0439	14.505	725228	72.52		
11414	1	0.0212	7.004	700448	70.04	76.61 ± 5.47	7.1%
	1	0.0252	8.326	832608	83.26		
	1	0.0235	7.764	776440	77.64		
	2	0.0457	15.099	754964	75.50		
11415	1	0.0231	7.632	763224	76.32	72.28 ± 3.33	4.6%
	1	0.021	6.938	693840	69.38		
	1	0.0223	7.368	736792	73.68		
	2	0.0422	13.943	697144	69.71		
11421	1	0.0206	6.806	680624	68.06	68.19 ± 3.5	5.1%
	1	0.0192	6.344	634368	63.44		
	1	0.0217	7.170	716968	71.70		
	2	0.0421	13.910	695492	69.55		

Summary of Results

CE vs LC/MS

For the LC/MS, the choline chloride concentrations in samples were mostly higher than the results obtained by CE as shown in Table 4.23 and Figure 4.39 with the differences being less than 15%. This observed small difference may probably be due to the differences in the sensitivity between the two methods. Compared to CE, the LC/MS method might not be as selective and sensitive enough to separate choline ion from other species that might have the same response in the matrices. Overall, the two methods can be considered to compare quite favourably.

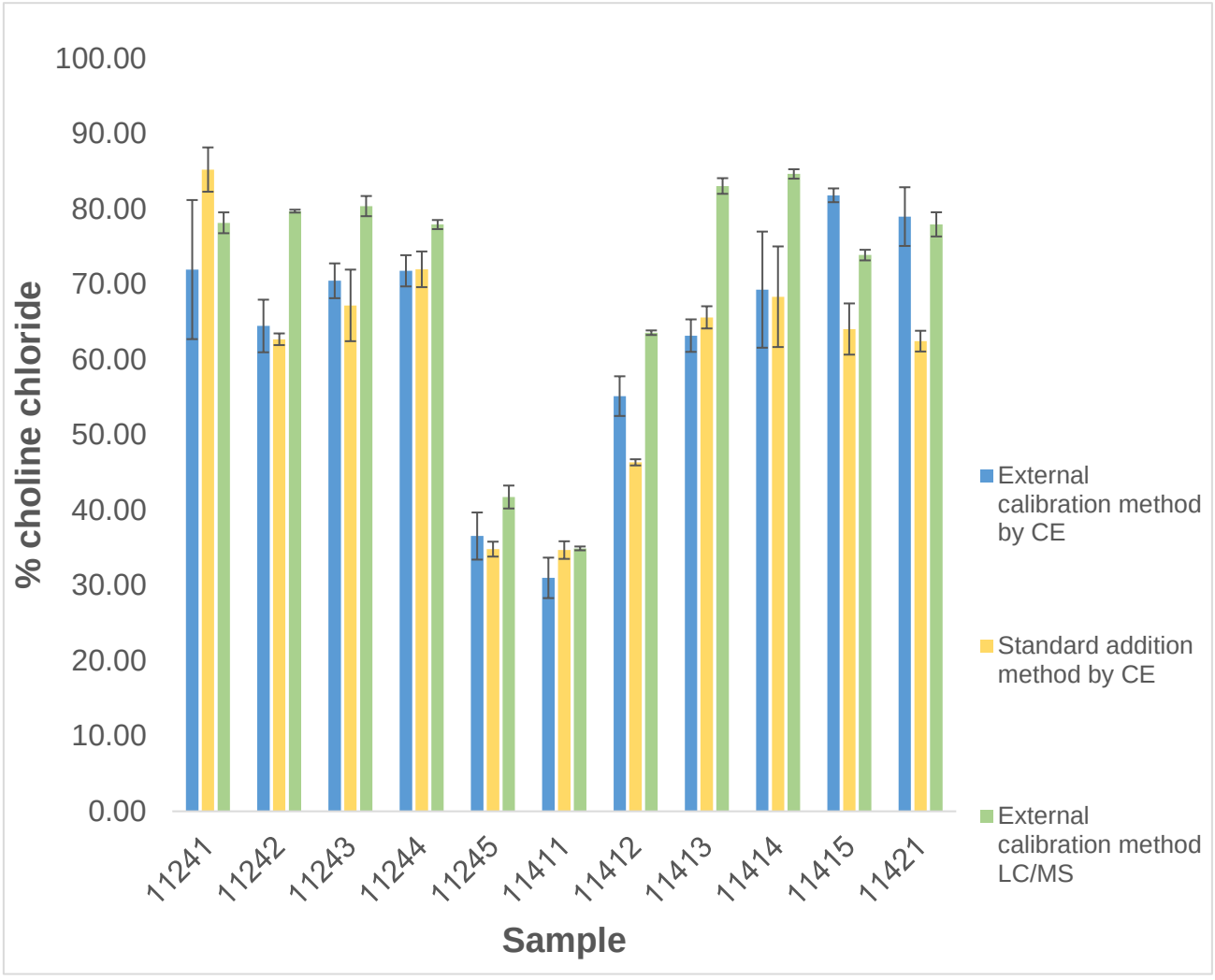

Figure 4.29 - Choline chloride concentrations in samples determined by LC/MS (external calibration) compared to CE (external calibration and standard addition)

To further confirm this, a *t*-test calculation was done and at the 95% confidence level there was no significant difference between the two methods. In addition, results from *F*-test calculations at the 95% confidence level show similar precisions for the two methods.

Table 4.23 - Comparison of choline chloride concentrations in samples determined by LC/MS (external calibration) and CE (external calibration and standard addition)

Sample	External calibration by CE		STD addition by CE		External calibration by LC/MS	
	% CC	% RSD	% CC	% RSD	% CC	% RSD
11241	71.96 ± 9.24	12.84%	85.25 ± 2.94	3.45%	78.18 ± 1.38	1.77%
11242	64.47 ± 3.49	5.42%	62.71 ± 0.78	1.24%	79.73 ± 0.19	0.24%
11243	70.21 ± 2.31	3.28%	67.21 ± 4.75	7.07%	80.40 ± 1.34	1.67%
11244	71.80 ± 2.07	2.89%	72.00 ± 2.37	3.29%	77.94 ± 0.61	0.78%
11245	36.57 ± 3.13	8.56%	34.83 ± 0.99	2.84%	41.75 ± 1.52	3.65%
11411	31.01 ± 2.69	8.67%	34.70 ± 1.17	3.37%	34.92 ± 0.26	0.73%
11412	55.15 ± 2.64	4.78%	46.35 ± 0.41	0.89%	63.58 ± 0.30	0.48%
11413	63.19 ± 2.16	3.42%	65.62 ± 1.47	2.25%	83.07 ± 1.04	1.25%
11414	69.30 ± 7.71	11.13%	68.36 ± 6.68	9.78%	84.67 ± 0.63	0.74%
11415	81.83 ± 0.92	1.12%	64.07 ± 3.40	5.31%	73.89 ± 0.70	0.95%
11421	79.00 ± 3.90	4.94%	62.46 ± 1.38	2.21%	77.97 ± 1.61	2.06%

CE vs Reinecke Salt Gravimetric Method

Using the Reinecke salt gravimetric method, the concentrations of choline chloride in samples were less than 15% different from results analyzed by CE as shown in Table 4.24 and Figure 4.40. The Reinecke method might not be as selective and sensitive enough as the CE to separate choline ion from other species in the matrices. With the differences being less than 15% both methods can be considered comparable. Overall, the two methods can be considered to compare quite favourably. A further confirmation by a *t*-test calculation at the 95% confidence level showed that there was no significant difference between the two methods. Results from *F*-test calculations at the 95% confidence level showed similar precisions for the two methods.

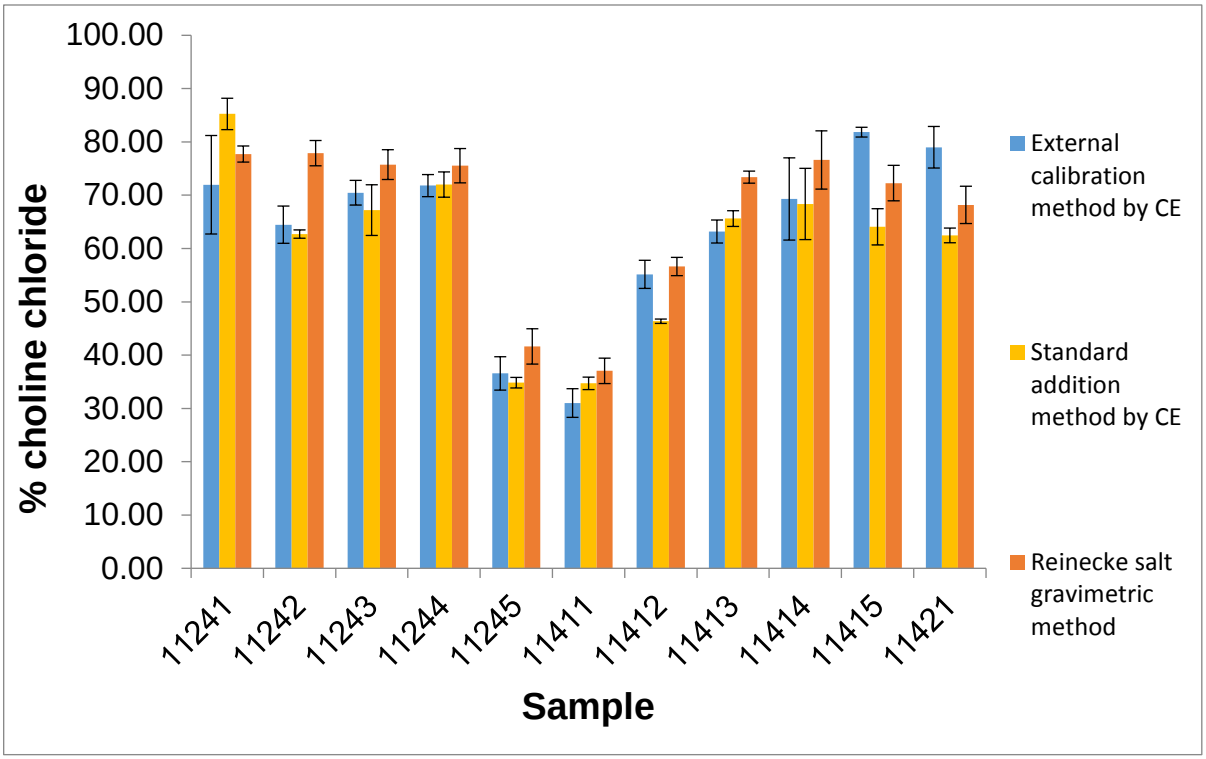

Figure 4.30 - Choline chloride concentrations in samples determined by Reinecke salt gravimetric method compared to CE (external calibration and standard addition)

Table 4.24 - Comparison of choline chloride concentrations in samples determined by Reinecke salt gravimetric method and CE (external calibration and standard addition)

Sample	External calibration by CE		STD addition by CE		Reinecke salt gravimetric method	
	% CC	% RSD	% CC	% RSD	% CC	% RSD
11241	71.96 ± 9.24	12.84%	85.25 ± 2.94	3.45%	77.13 ± 1.51	1.94%
11242	64.47 ± 3.49	5.42%	62.71 ± 0.78	1.24%	77.89 ± 2.37	3.05%
11243	70.21 ± 2.31	3.28%	67.21 ± 4.75	7.07%	75.74 ± 2.80	3.69%
11244	71.80 ± 2.07	2.89%	72.00 ± 2.37	3.29%	75.54 ± 3.22	4.26%
11245	36.57 ± 3.13	8.56%	34.83 ± 0.99	2.84%	41.63 ± 3.31	7.96%
11411	31.01 ± 2.69	8.67%	34.70 ± 1.17	3.37%	37.05 ± 2.38	6.44%
11412	55.15 ± 2.64	4.78%	46.35 ± 0.41	0.89%	56.62 ± 1.71	3.03%
11413	63.19 ± 2.16	3.42%	65.62 ± 1.47	2.25%	73.39 ± 1.13	1.54%
11414	69.30 ± 7.71	11.13%	68.36 ± 6.68	9.78%	76.61 ± 5.47	7.14%
11415	81.83 ± 0.92	1.12%	64.07 ± 3.40	5.31%	72.28 ± 3.33	4.61%
11421	79.00 ± 3.90	4.94%	62.46 ± 1.38	2.21%	68.19 ± 3.5	5.13%

LC/MS vs Reinecke Salt Gravimetric Method

The concentrations of choline chloride in samples obtained by LC/MS and the Reinecke salt gravimetric method were quite similar. The differences in the results by these two methods were less than 11% as shown in Table 4.25 and Figure 4.41. By calculating *t*-test, it was found thattheir means are similar and the two methods are not significantly different at 95% confidence level. *F*-test calculations also showed that their precisions are similar at the 95% confidence level.

Table 4.25 - Comparison of choline chloride concentrations in samples determined by Reinecke salt gravimetric method and LC/MS

Sample	External calibration by LC/MS		Reinecke salt gravimetric method	
	% CC	% RSD	% CC	% RSD
11241	78.18 ± 1.38	1.77%	77.13 ± 1.51	1.94%
11242	79.73 ± 0.19	0.24%	77.89 ± 2.37	3.05%
11243	80.40 ± 1.34	1.67%	75.74 ± 2.80	3.69%
11244	77.94 ± 0.61	0.78%	75.54 ± 3.22	4.26%
11245	41.75 ± 1.52	3.65%	41.63 ± 3.31	7.96%
11411	34.92 ± 0.26	0.73%	37.05 ± 2.38	6.44%
11412	63.58 ± 0.30	0.48%	56.62 ± 1.71	3.03%
11413	83.07 ± 1.04	1.25%	73.39 ± 1.13	1.54%
11414	84.67 ± 0.63	0.74%	76.61 ± 5.47	7.14%
11415	73.89 ± 0.70	0.95%	72.28 ± 3.33	4.61%
11421	77.97 ± 1.61	2.06%	68.19 ± 3.5	5.13%

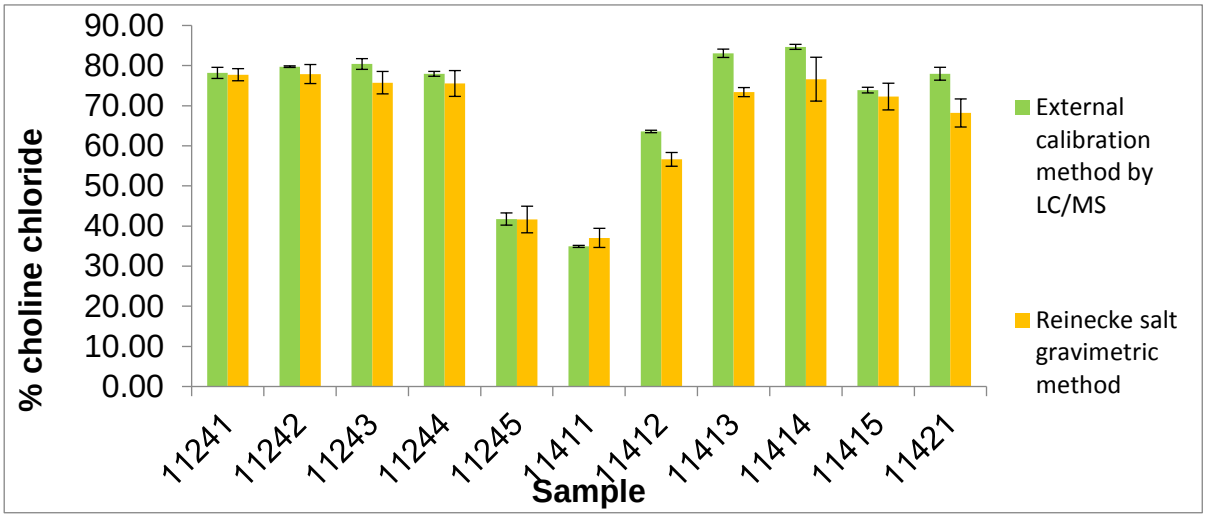

Figure 4.31 - Choline chloride concentrations in samples determined by Reinecke salt gravimetric method compared to LC/MS

Comparison of All Three Methods (CE vs LC/MS vs Reinecke Salt Gravimetric Method)

The concentrations of choline chloride in eleven oilfield process choline water samples that were obtained by CE, LC/MS and Reinecke salt gravimetric methods are summarized in Table 4.26 and Figure 4.42. As can be observed in the table and the figure, the differences of the results among these three methods were quite small and in fact, less than 15%. Therefore, the developed CE analysis can be considered comparable to LC/MS and Reinecke salt gravimetric analyses. Furthermore, the small differences between the CE and the other two methods confirm that the CE results obtained are reasonable and accurate.

Table 4.26 - Comparison of choline chloride concentrations in samples determined by CE, LC/MS and Reinecke salt gravimetric method

Sample	External calibration by CE		STD addition by CE		External calibration by LC/MS		Reinecke salt gravimetric method	
	% CC	% RSD	% CC	% RSD	% CC	% RSD	% CC	% RSD
11241	71.96 ± 9.24	12.84%	85.25 ± 2.94	3.45%	78.18 ± 1.38	1.77%	77.13 ± 1.51	1.94%
11242	64.47 ± 3.49	5.42%	62.71 ± 0.78	1.24%	79.73 ± 0.19	0.24%	77.89 ± 2.37	3.05%
11243	70.21 ± 2.31	3.28%	67.21 ± 4.75	7.07%	80.40 ± 1.34	1.67%	75.74 ± 2.80	3.69%
11244	71.80 ± 2.07	2.89%	72.00 ± 2.37	3.29%	77.94 ± 0.61	0.78%	75.54 ± 3.22	4.26%
11245	36.57 ± 3.13	8.56%	34.83 ± 0.99	2.84%	41.75 ± 1.52	3.65%	41.63 ± 3.31	7.96%
11411	31.01 ± 2.69	8.67%	34.70 ± 1.17	3.37%	34.92 ± 0.26	0.73%	37.05 ± 2.38	6.44%
11412	55.15 ± 2.64	4.78%	46.35 ± 0.41	0.89%	63.58 ± 0.30	0.48%	56.62 ± 1.71	3.03%
11413	63.19 ± 2.16	3.42%	65.62 ± 1.47	2.25%	83.07 ± 1.04	1.25%	73.39 ± 1.13	1.54%
11414	69.30 ± 7.71	11.13%	68.36 ± 6.68	9.78%	84.67 ± 0.63	0.74%	76.61 ± 5.47	7.14%
11415	81.83 ± 0.92	1.12%	64.07 ± 3.40	5.31%	73.89 ± 0.70	0.95%	72.28 ± 3.33	4.61%
11421	79.00 ± 3.90	4.94%	62.46 ± 1.38	2.21%	77.97 ± 1.61	2.06%	68.19 ± 3.5	5.13%

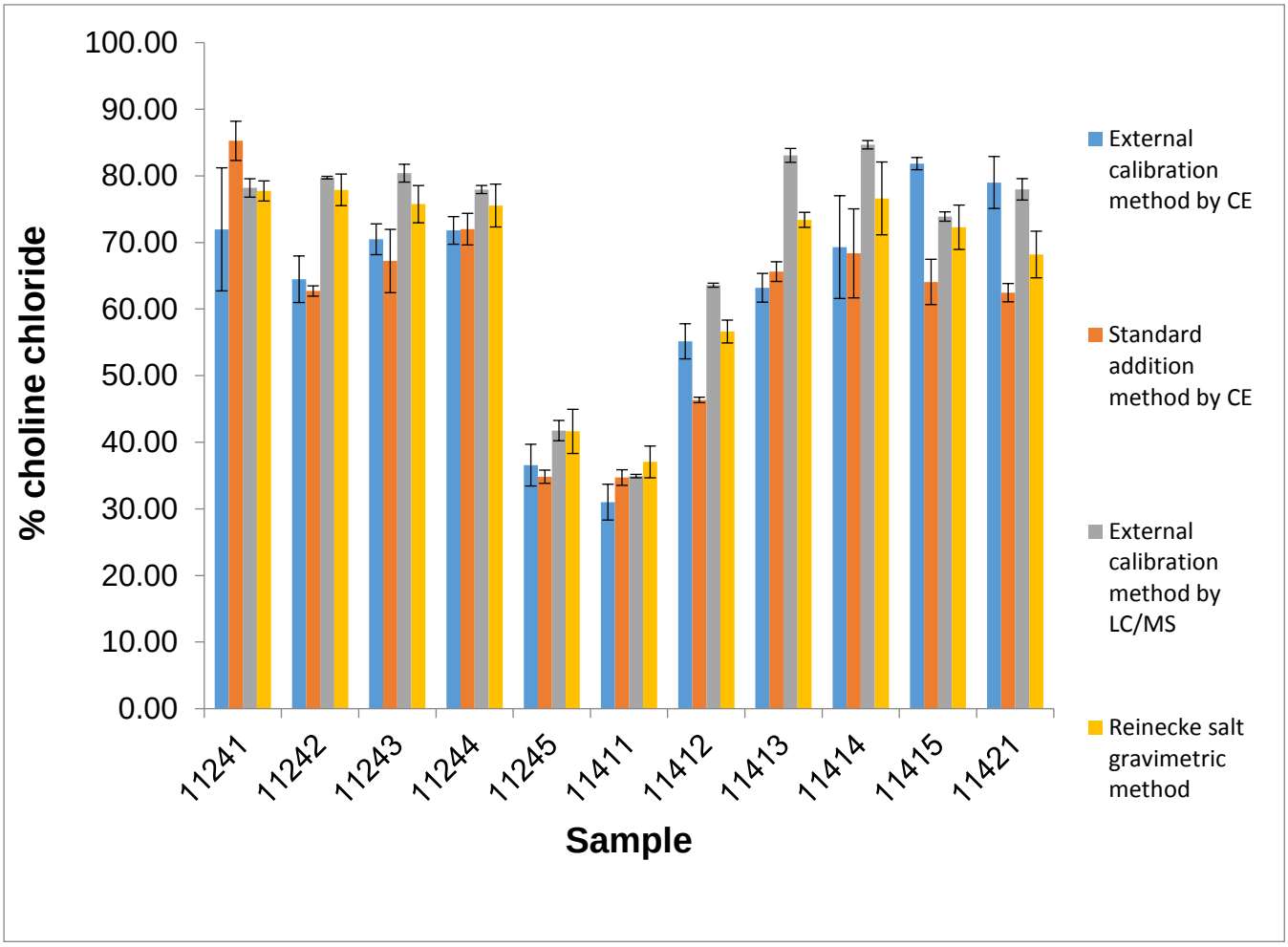

Figure 4.32 - Choline chloride concentrations in samples determined by CE, LC/MS and Reinecke salt gravimetric method methods.

CHAPTER 5

CONCLUSIONS

Conclusions

In this work, an efficient, robust and sensitive analytical method for the qualitative and quantitative analysis of choline chloride in oilfield waters using capillary electrophoresis (CE) was developed successfully. This method offered good selectivity and sensitivity for the detection of choline. The developed CE method using a BGE of imidazole containing 18-crown-6 provided a complete separation between choline ion and other cations commonly found in oilfield process waters. The developed method yielded a good approach for choline analysis in oilfield water samples. The analyzed concentrations of choline chloride in the provided samples by either external calibration or standard addition were mostly similar. For the capillary electrophoresis method, the intraday and interday analysis indicated good reproducibility with % RSD of the peak area and migration time less than 10% confirming that the developed capillary electrophoresis method is reproducible. The percent recoveries for the selected samples obtained ranged from 80% - 120%, indicating that the developed method is accurate. The LOD and LOQ of choline chloride analysis by CE method were determined to be 14.7 ppm and 48.9 ppm, respectively, which shows that the method is reasonably sensitive. In order to validate the CE results, the samples were analyzed by LC/MS and Reinecke salt gravimetric analyses. The differences of choline chloride concentrations obtained by these three methods were less than 15%. Therefore, the CE method is considered accurate and comparable to LC/MS and Reinecke salt gravimetric analyses. The advantage of CE over the other two methods was the ability to separate choline ion from other cations that might be present in oilfield process waters. It has been demonstrated in this work that CE can become a robust tool for many suppliers and oil and gas companies in analyzing choline chloride. This would enable them to evaluate the quality of choline chloride or identify any adulteration in clay stabilizer products on the market. Besides, CE also can replace the current traditional methods used for the routine analysis of choline chloride levels. In addition, the CE method would be available for analysis of a large number of samples automatically in a short time. In addition, the CE method can also help the environmental

specialists or ecologists in their bid to investigate the ecotoxicity of choline and its potential effects on marine life as well as human health.

Future Work

Choline chloride is considered biodegradable but there is no research to characterize this ability. The developed CE method can be a robust tool to access the biodegradability of choline chloride in different water types such as wastewater or a water aquifer around an oilfield.

Choline is also present in many kinds of food, feed and supplements. Nevertheless, the information about the suitable intake or its toxicity has not been completely reported for different species of animals or humans. It is necessary for government agencies and scientists to look for the content of choline added into feed and supplements and understand the way choline behaves in the human or animal body to prevent any side effects.

Because of time constraints, the LC/MS method used in this work was not been evaluated with respect to the percent recovery, the LOD and the LOQ. The choline analysis by LC/MS was only done with the external calibration approach, thus in the future it would be good to do the analysis with the standard addition technique as well. The current project has laid the groundwork for using CE to determine choline chloride in a challenging matrix of oilfield process waters. In the future, the method developed in this work can be applied on other industrial and environmental matrices for the quantification of choline chloride.

The precision obtained in this work is good but it can be improved even further. The capillary could be flushed and coated more frequently to make the EOF more stable. Choline can be also extracted and preconcentrated from the matrix by solid phase extraction technique before the CE analysis.

To improve the LOD and LOQ, the CE technique of large volume sample stacking and dynamic pH junction technique can be explored for this project. Also, a suitable fluorophore can be investigated for choline and a laser induced fluorescence (LIF) detection could be employed. This method can possibly enhance the sensitivity.

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APPENDIX

Appendix A: Method Validation Results for CE

Optimization of CE conditions

Table A.1 – Theoretical plate and asymmetry factor of choline peak with different concentrations of imidazole in BGE at 30 mM, 40 mM, and 50 mM.

Buffer concentration	Theoretical plate (N)	Asymmetry factor
50 mM	79403038.40	6.4
40 mM	80219920.81	6.3
30 mM	85402574.79	23.1

Table A.2 - Theoretical plate and asymmetry factor of choline peak with different pHs of BGE at 4, 5 and 6.

Buffer pH	Theoretical plate (N)	Asymmetry factor
6	90642844.60	9.3
5	80219920.81	6.3
4	71562357.33	6.8

Table A.3 – The resolutions values of various pairs of cations with different BGE: Imidazole; Imidazole with 1-propanol; Imidazole with 1-propanol and 18-crown-6.

Resolutions values			
Cation	**Imidazole**	**Imidazole + IP**	**Imidazole+IP$^+$ 18-crown-6**
Ch$^+$ vs NH$_4^+$	3.40	4.29	4.94
Ch$^+$ vs K$^+$	3.40	4.29	4.73
Ch$^+$ vs Na$^+$	1.89	2.51	2.83
K$^+$ vs NH$_4^+$	0.00	0.00	2.49
Na$^+$ vs NH$_4^+$	4.17	5.63	4.73
Na$^+$ vs K$^+$	4.17	5.63	4.09

Interday and Intraday Studies

Table A.4 - First day interday and intraday precision studies for peak areas and migration times by CE

	Concentration (ppm)	Peak area (n = 3)			Mean	SD	% RSD
Day 1	200	17369	19577	18137	18361	1120.9	6.10%
	600	63710	64718	65272	64567	791.9	1.23%
	1000	109881	106742	111725	109449	2519.4	2.30%
	Concentration (ppm)	**Migration time (n = 3)**			**Mean**	**SD**	**% RSD**
	200	8.387	8.676	8.975	8.68	0.29	3.39%
	600	9.333	9.317	9.325	9.33	0.01	0.09%
	1000	9.229	9.229	9.225	9.23	0.00	0.03%

Table A.5 - Second day interday and intraday precision studies for peak areas and migration times by CE

	Concentration (ppm)	Peak area (n = 3)			Mean	SD	% RSD
Day 2	200	24243	24149	26453	24948	1303.9	5.23%
	600	84778	84036	83905	84240	470.8	0.56%
	1000	140552	144409	154741	146567	7336.6	5.01%
	Concentration (ppm)	**Migration time (n = 3)**			**Mean**	**SD**	**% RSD**
	200	8.342	8.367	8.412	8.37	0.04	0.42%
	600	8.846	8.887	8.938	8.89	0.05	0.52%
	1000	8.942	8.95	8.988	8.96	0.02	0.27%

Table A.6 - Third day interday and intraday precision studies for peak areas and migration times by CE

	Concentration(ppm)	Peak area (n = 3)			Mean	SD	% RSD
Day 3	200 ppm	19366	19911	21484	20254	1099.8	5.43%
	600 ppm	67631	70818	69714	69388	1618.4	2.33%
	1000 ppm	124367	125081	123090	124179	1008.7	0.81%
	Concentration(ppm)	**Migration time (n = 3)**			**Mean**	**SD**	**% RSD**
	200 ppm	7.725	7.819	7.938	7.83	0.11	1.36%
	600 ppm	7.888	7.938	7.942	7.92	0.03	0.38%
	1000 ppm	7.867	7.862	7.858	7.86	0.00	0.06%

Limit of Detection (LOD) and Limit of Quantitation (LOQ) of CE Method

Table A.7 - Calculation of LOD and LOQ of choline chloride by CE method

peak area of 100 ppm choline chloride (n=6) (the calibration curve: y= 117.14 x - 6282.6)						**SD**	**LOD (ppm)**	**LOQ (ppm)**
6056	7162	7081	6634	6956	7775	573	14.7	48.9

Percent recovery calculations for CE method

First set of samples:

Table A.8 - Percent recovery calculations for CE method for 5 samples of the first set

The calibration equation: y = 117.14x - 6282.6 R^2 = 0.9941

Sample Name	**Spiked Concentration (Spiked) true**	**Peak area**	**∆ = Peak area (spiked) -peak area (unspiked)**	**Spike(exp)**	**% Rec. = Spiked(exp)/ Spiked(true)**
11241	0	85678			
	750	158517	72839	675.44	**90.1%**
	1250	195617	109939	992.16	**79.4%**
	2000	298006	212328	1866.23	**93.3%**
11242	0	73730			
	750	155487	81757	751.58	**100.2%**
	1250	194617	120887	1085.62	**86.8%**
	2000	306416	232686	2040.03	**102.0%**
11243	0	77844			
	750	155360	77516	715.37	**95.4%**
	1250	209164	131320	1174.68	**94.0%**
	2000	293694	215850	1896.30	**94.8%**
11244	0	76541			
	750	162680	86139	788.98	**105.2%**
	1250	213301	136760	1221.13	**97.7%**
	2000	286533	209992	1846.29	**92.3%**
11245	0	32669			
	750	99553	66884	624.61	**83.3%**
	1250	138391	105722	956.16	**83.3%**
	2000	213717	181048	1599.20	**80.0%**

Second set of samples:

Table A.9 - Percent recovery calculations for CE method for 6 samples of the second set

The calibration equation: $y = 48.81x - 2477.01$ $R^2 = 0.9874$

Sample Name	Spiked Concentration (Spiked) true	Peak area	Δ = Peak area (spiked) -peak area (unspiked)	Spike(exp)	% Rec. = Spiked(exp)/ Spiked(true)
11411	0	10617			
	750	44095	33478	820.57	**109.4%**
	1250	64288	53671	1281.42	**102.5%**
	2000	101407	90790	2128.55	**106.4%**
11412	0	23021			
	750	49037	26016	650.27	**86.7%**
	1250	74100	51079	1222.26	**97.8%**
	2000	106435	83414	1960.22	**98.0%**
11413	0	25733			
	750	52542	26809	668.37	**89.1%**
	1250	73428	47695	1145.03	**91.6%**
	2000	102194	76461	1801.53	**90.1%**
11414	0	27840			
	750	58502	30662	756.30	**100.8%**
	1250	76068	48228	1157.20	**92.6%**
	2000	104405	76565	1803.91	**90.2%**
11415	0	33727			
	750	65877	32150	790.26	**105.4%**
	1250	90575	56848	1353.92	**108.3%**
	2000	132183	98456	2303.51	**115.2%**
11421	0	32771			
	750	62053	29282	724.81	**96.6%**
	1250	83541	50770	1215.21	**97.2%**
	2000	126200	93429	2188.78	**109.4%**

Appendix B: Electropherograms of Ten Choline Samples

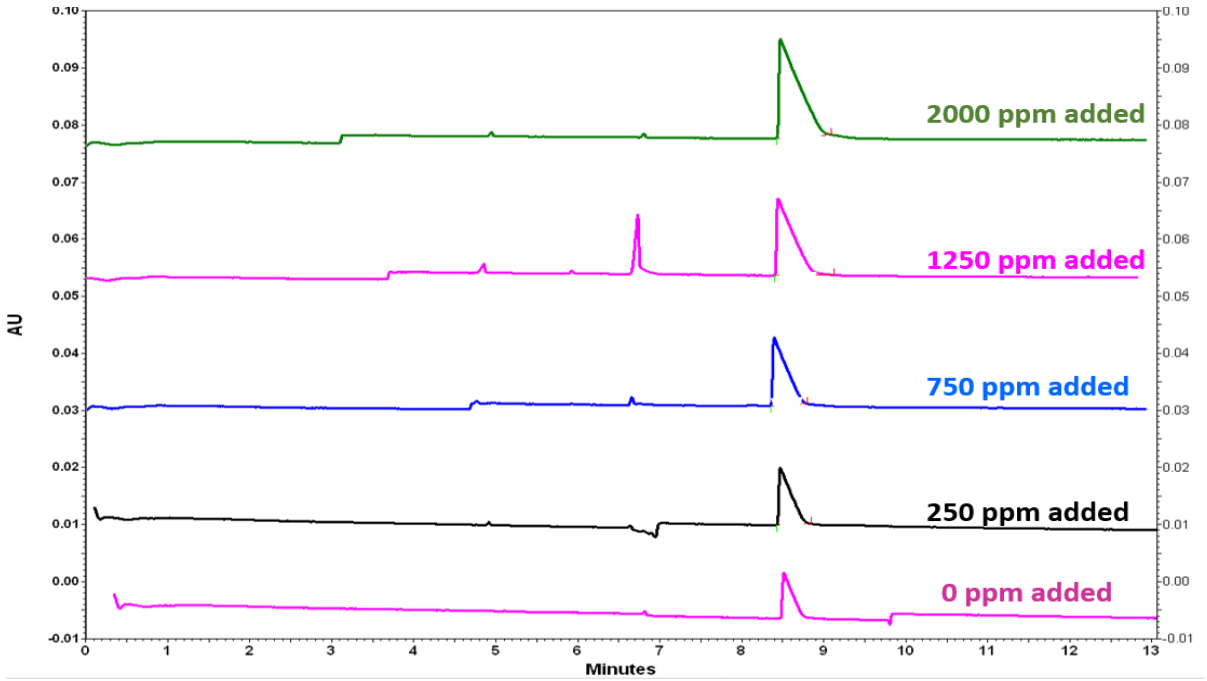

Figure B.1 - Electropherogram of sample 11242 by standard addition; choline chloride spikes added (0 ppm, 250 ppm, 750 ppm, 1250 ppm, 2000 ppm)

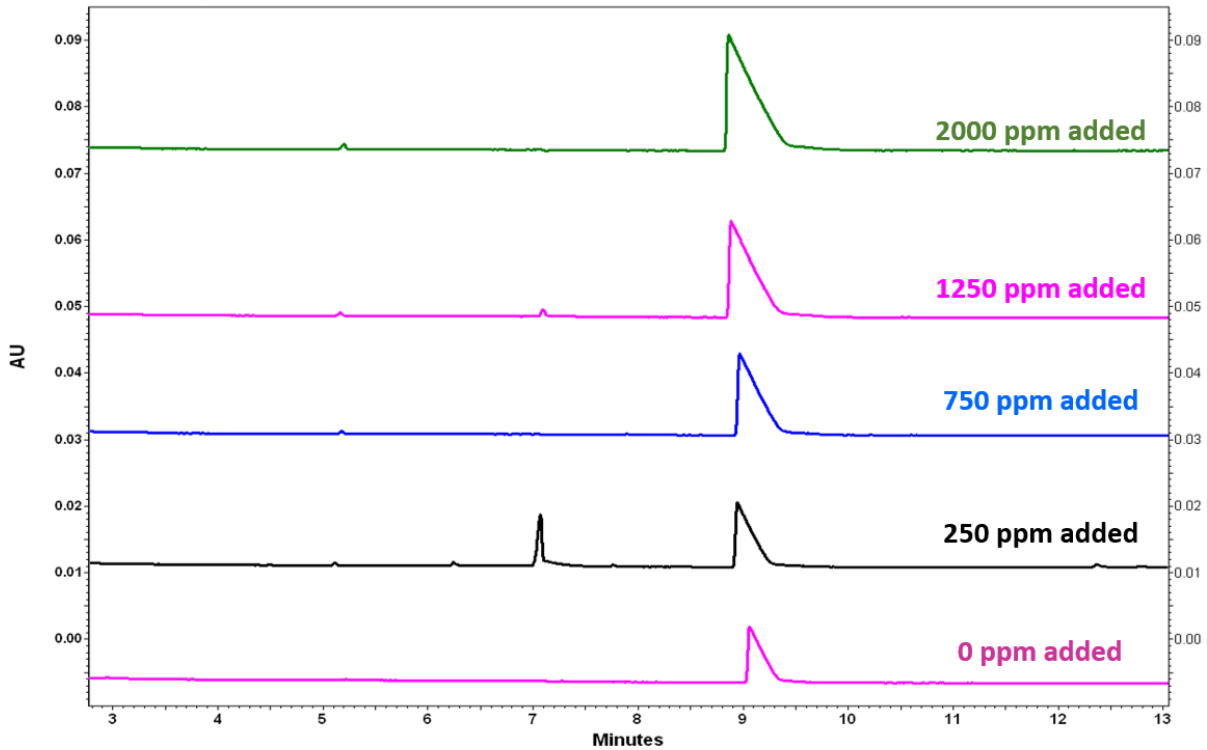

Figure B.2 - Electropherogram of sample 11243 by standard addition; choline chloride spikes added (0 ppm, 250 ppm, 750 ppm, 1250 ppm, 2000 ppm)

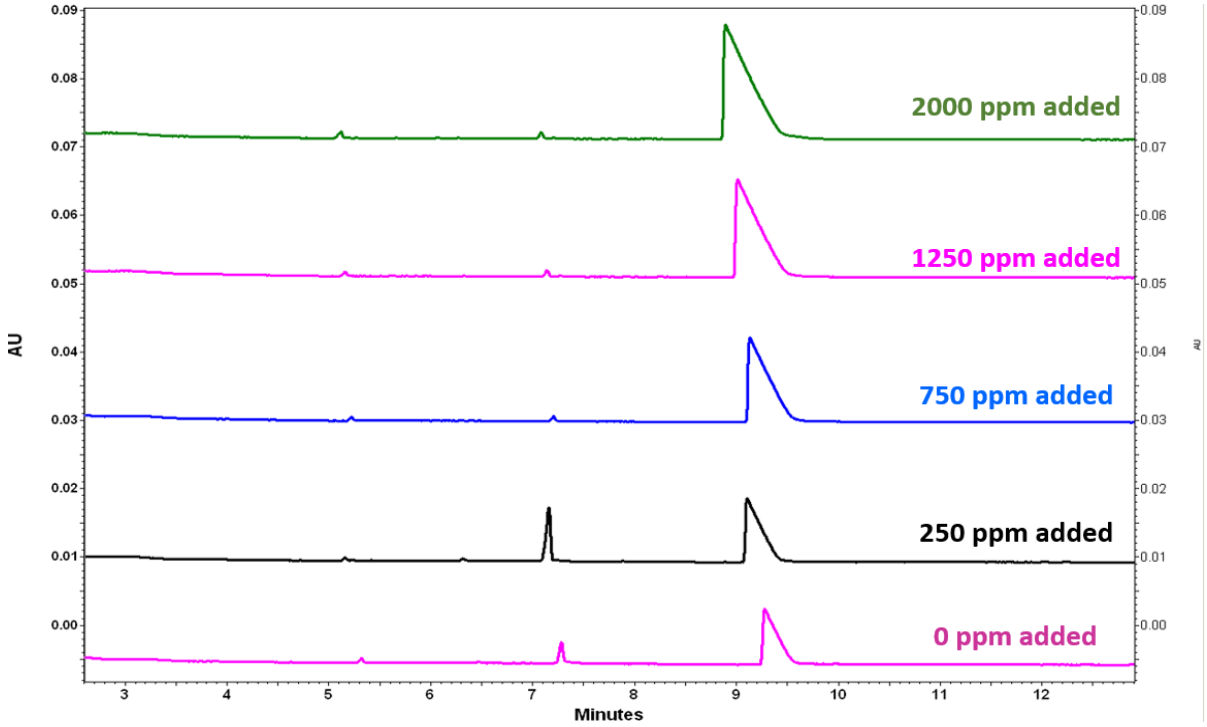

Figure B.3 - Electropherogram of sample 11244 by standard addition; choline chloride spikes added (0 ppm, 250 ppm, 750 ppm, 1250 ppm, 2000 ppm)

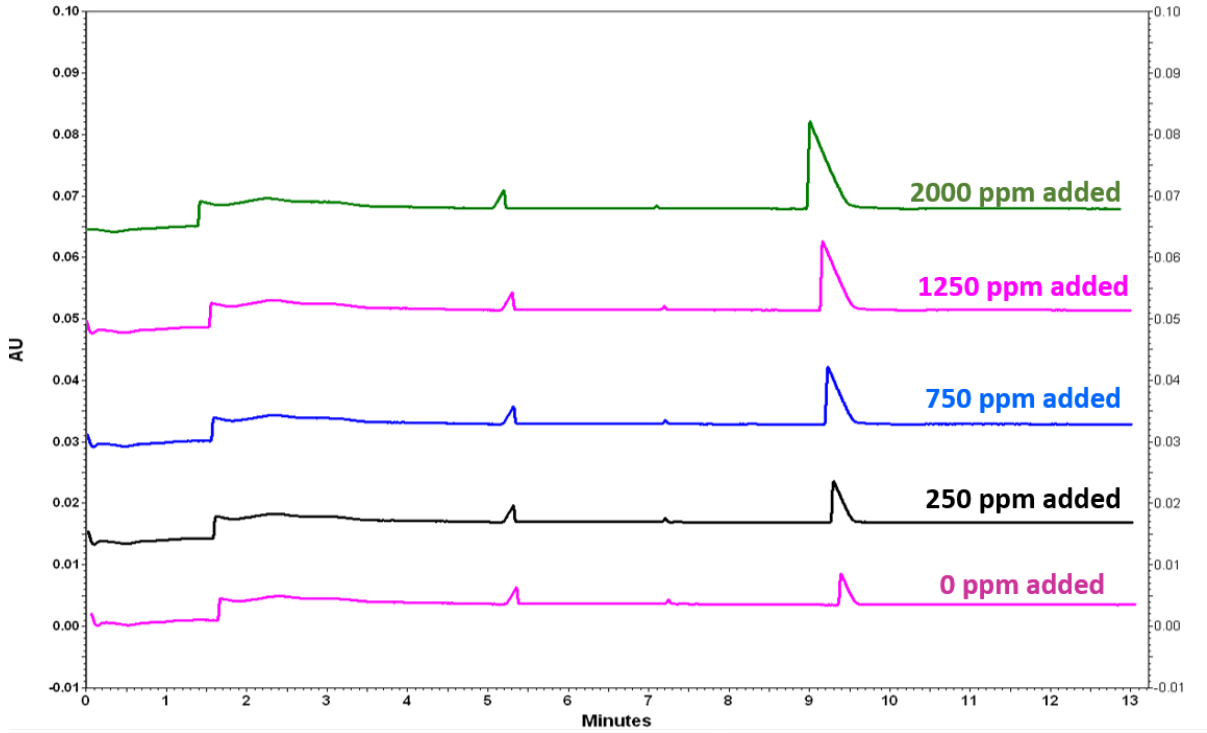

Figure B.4 - Electropherogram of sample 11245 by standard addition; choline chloride spikes added (0 ppm, 250 ppm, 750 ppm, 1250 ppm, 2000 ppm)

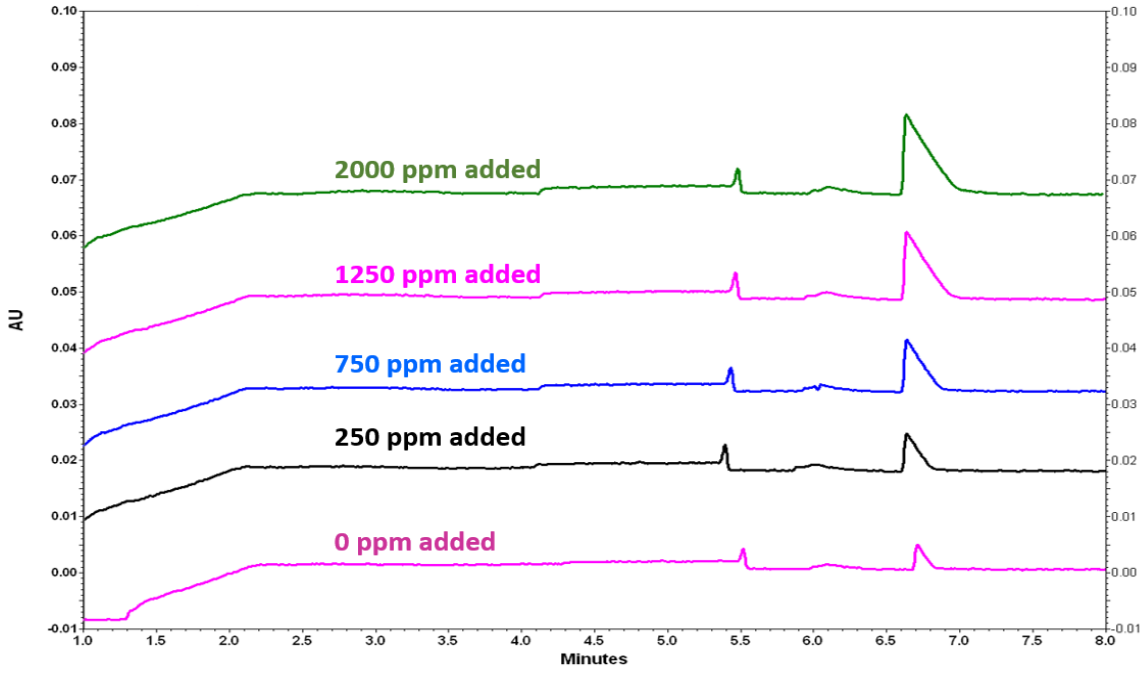

Figure B.5 - Electropherogram of sample 11411 by standard addition; choline chloride spikes added (0 ppm, 250 ppm, 750 ppm, 1250 ppm, 2000 ppm)

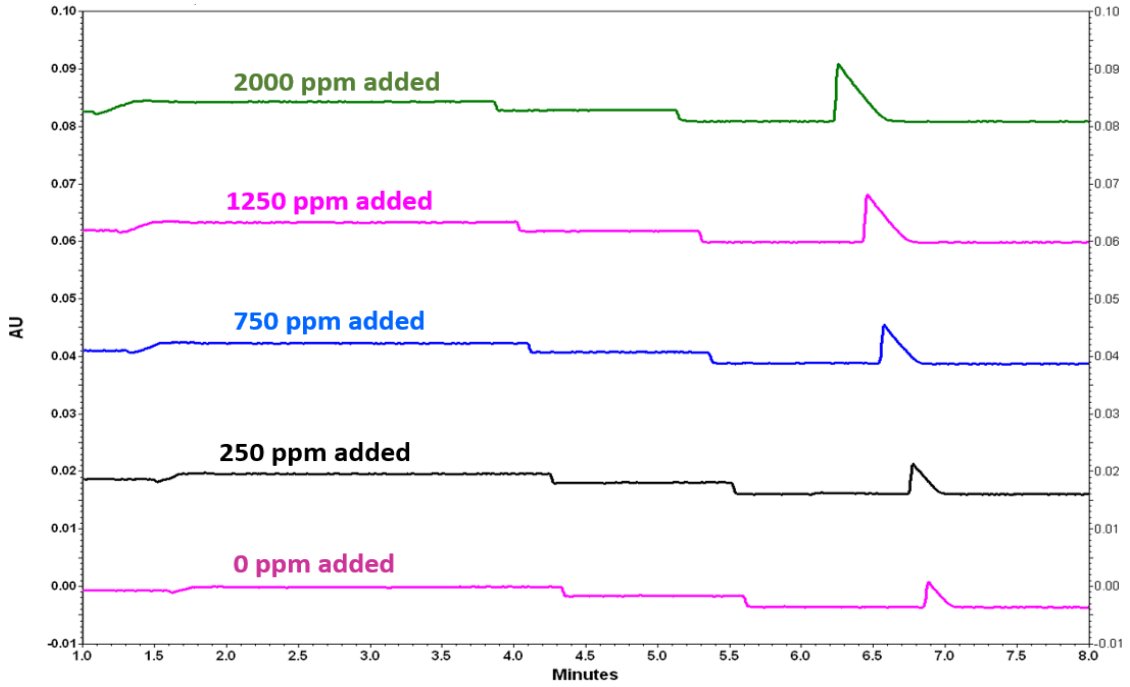

Figure B.6 - - Electropherogram of sample 11413 by standard addition; choline chloride spikes added (0 ppm, 250 ppm, 750 ppm, 1250 ppm, 2000 ppm)

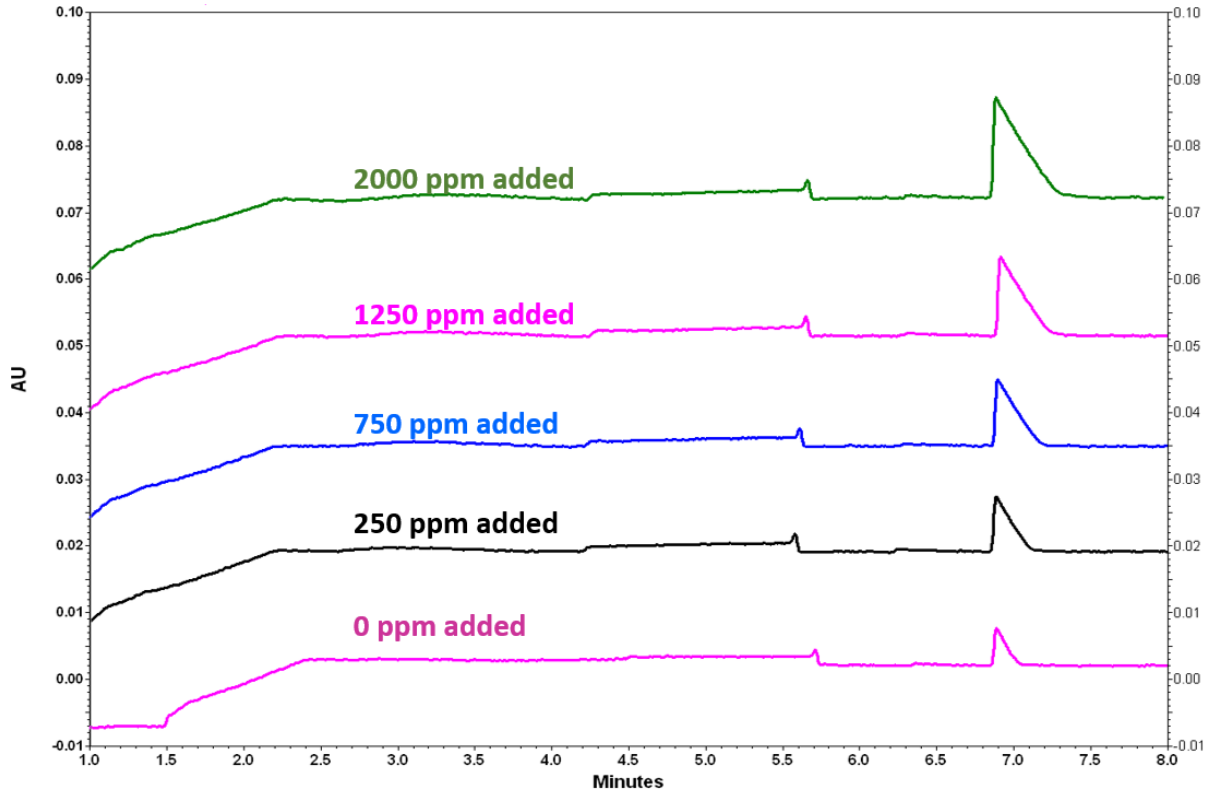

Figure B.7 - Electropherogram of sample 11412 by standard addition; choline chloride spikes added (0 ppm, 250 ppm, 750 ppm, 1250 ppm, 2000 ppm)

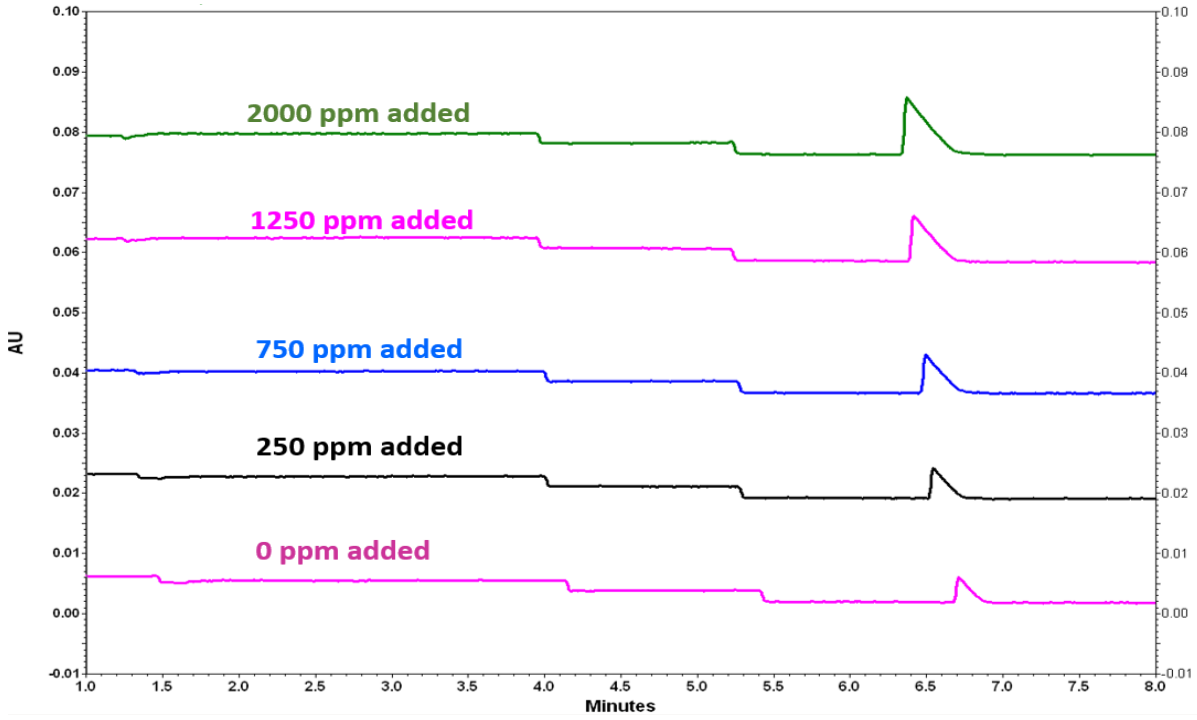

Figure B.8 - Electropherogram of sample 11414 by standard addition; choline chloride spikes added (0 ppm, 250 ppm, 750 ppm, 1250 ppm, 2000 ppm)

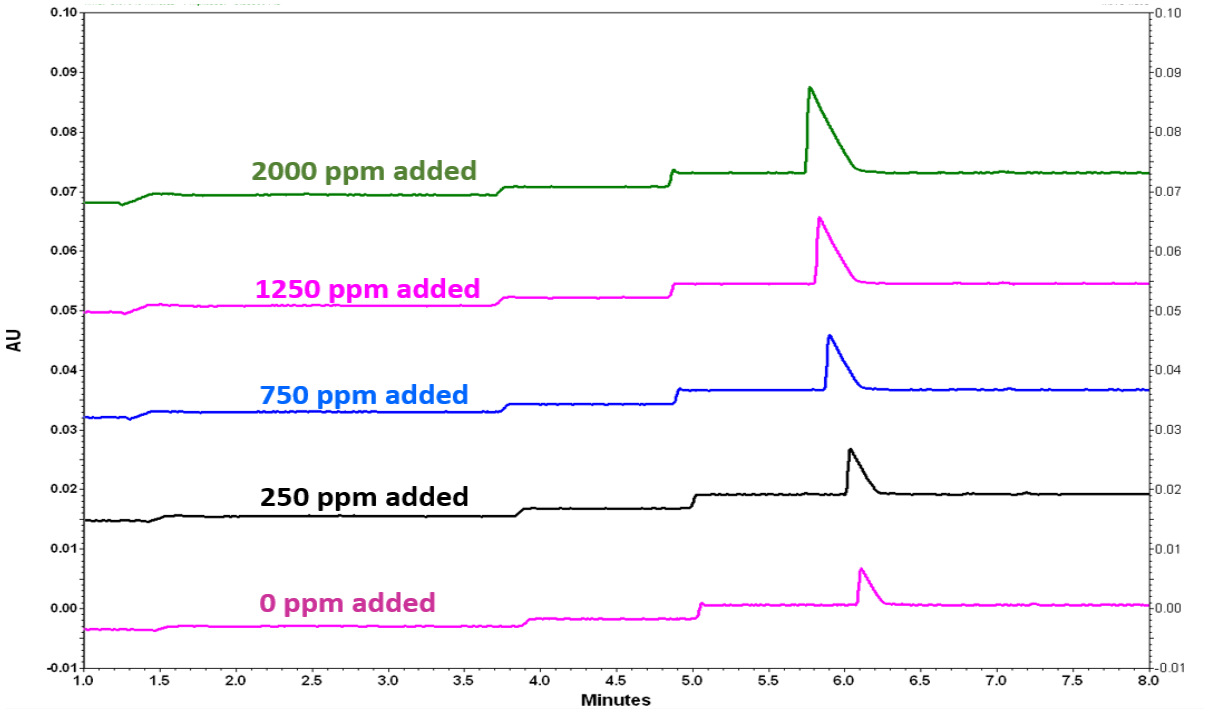

Figure B.10 - Electropherogram of sample 11415 by standard addition; choline chloride spikes added (0 ppm, 250 ppm, 750 ppm, 1250 ppm, 2000 ppm)

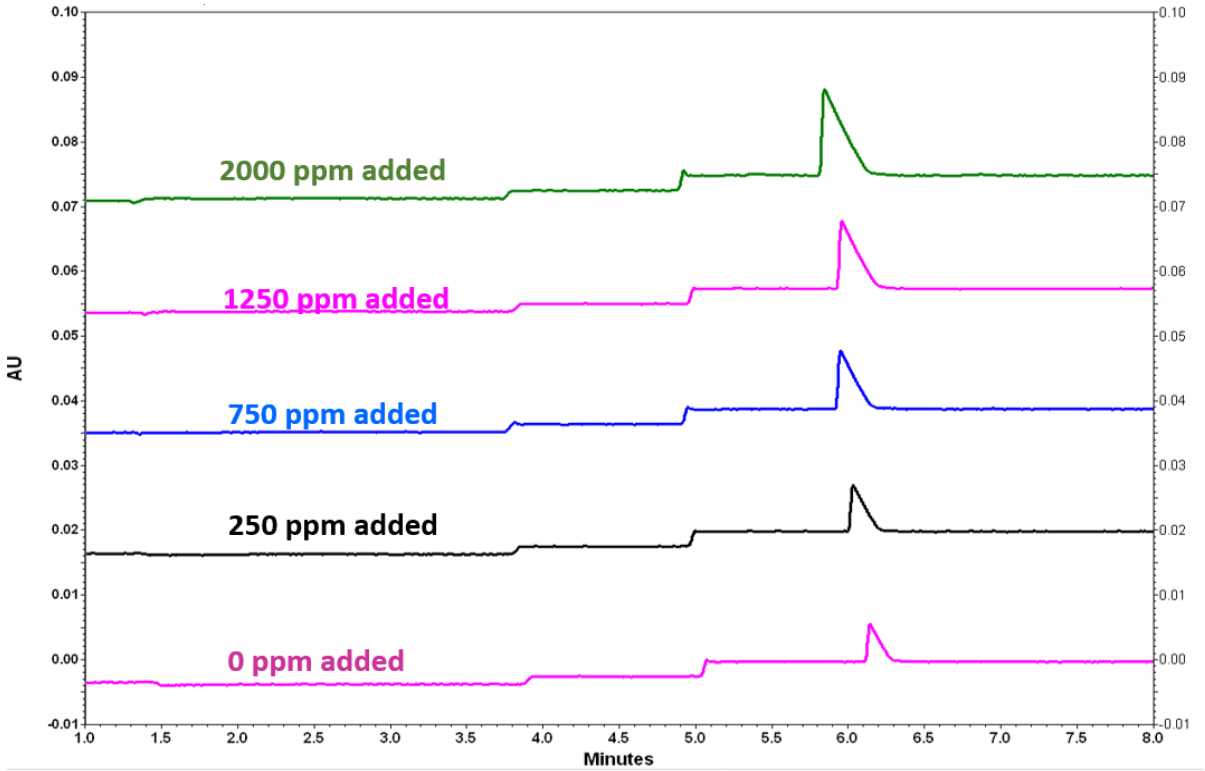

Figure B.9 - Electropherogram of sample 11421 by standard addition; choline chloride spikes added (0 ppm, 250 ppm, 750 ppm, 1250 ppm, 2000 ppm)

Appendix C: LC/MS Chromatograms of Nine Choline Samples

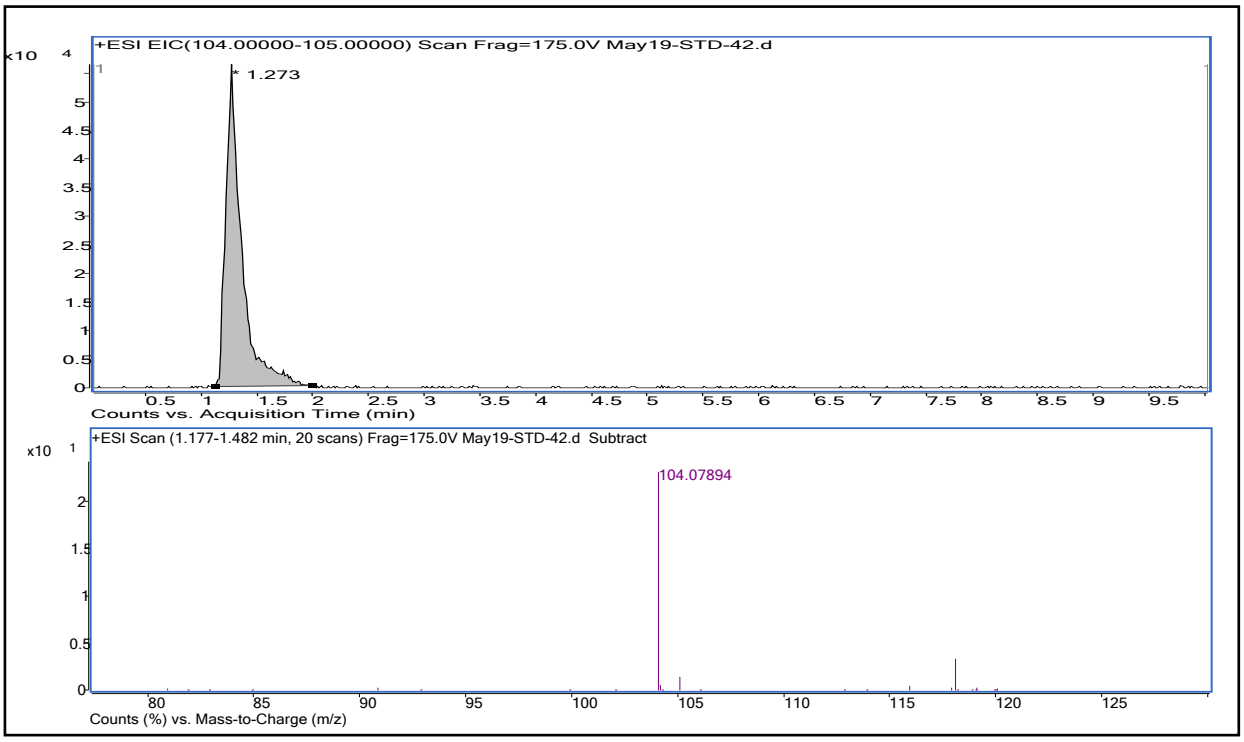

Figure C.1 - Extracted ion chromatogram and mass spectrum for choline ion present in sample 11242

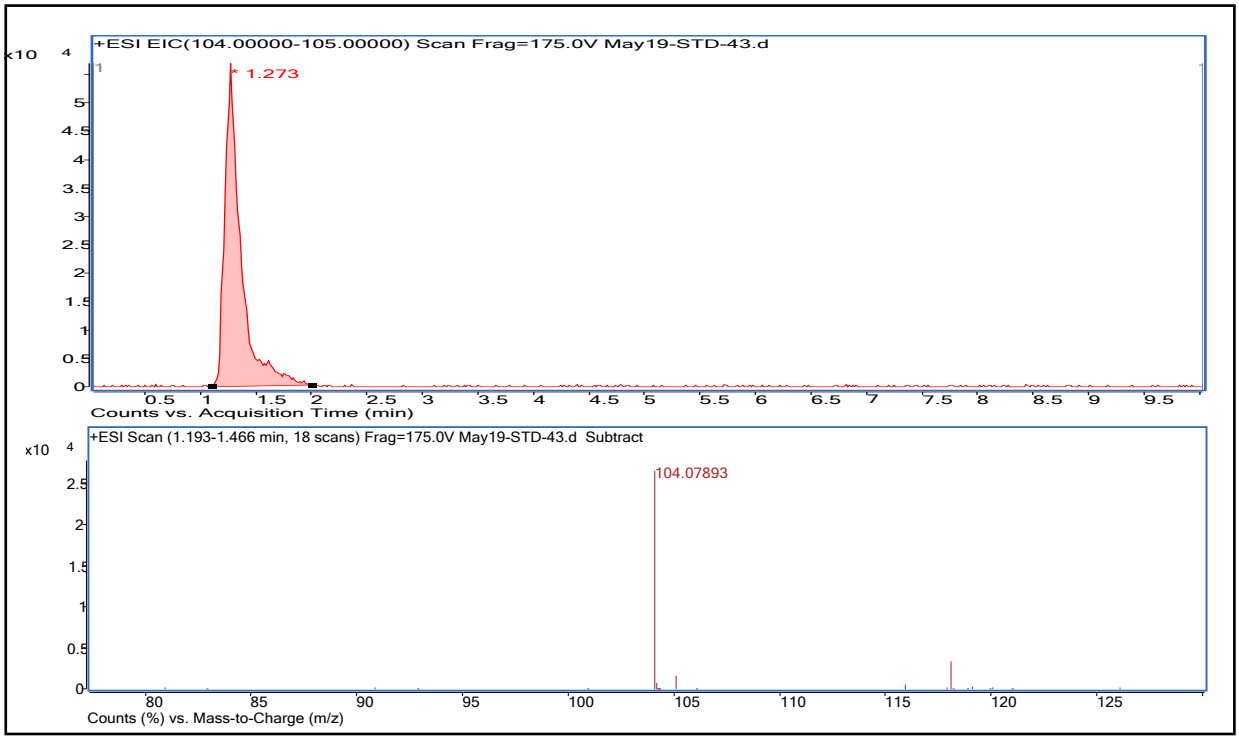

Figure C.2 - Extracted ion chromatogram and mass spectrum for choline ion present in sample 11243

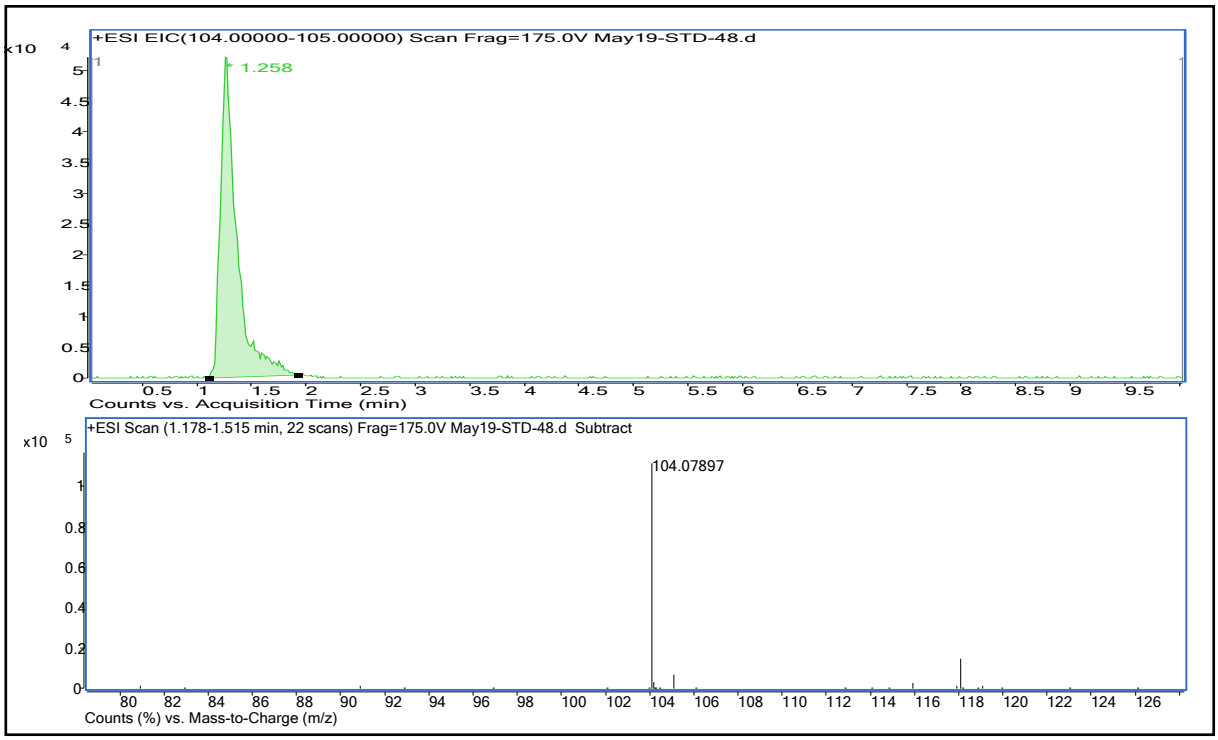

Figure C.3 - Extracted ion chromatogram and mass spectrum for choline ion present in sample 11244

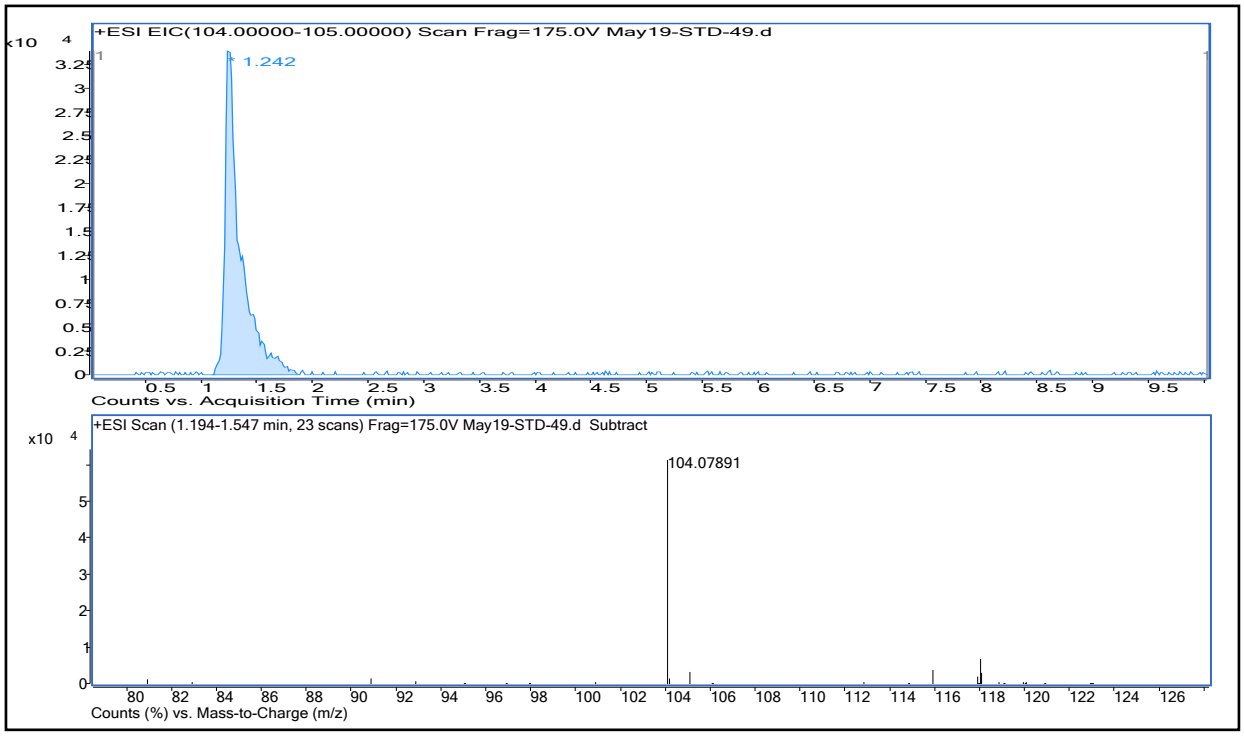

Figure C.4 - Extracted ion chromatogram and mass spectrum for choline ion present in sample 11245

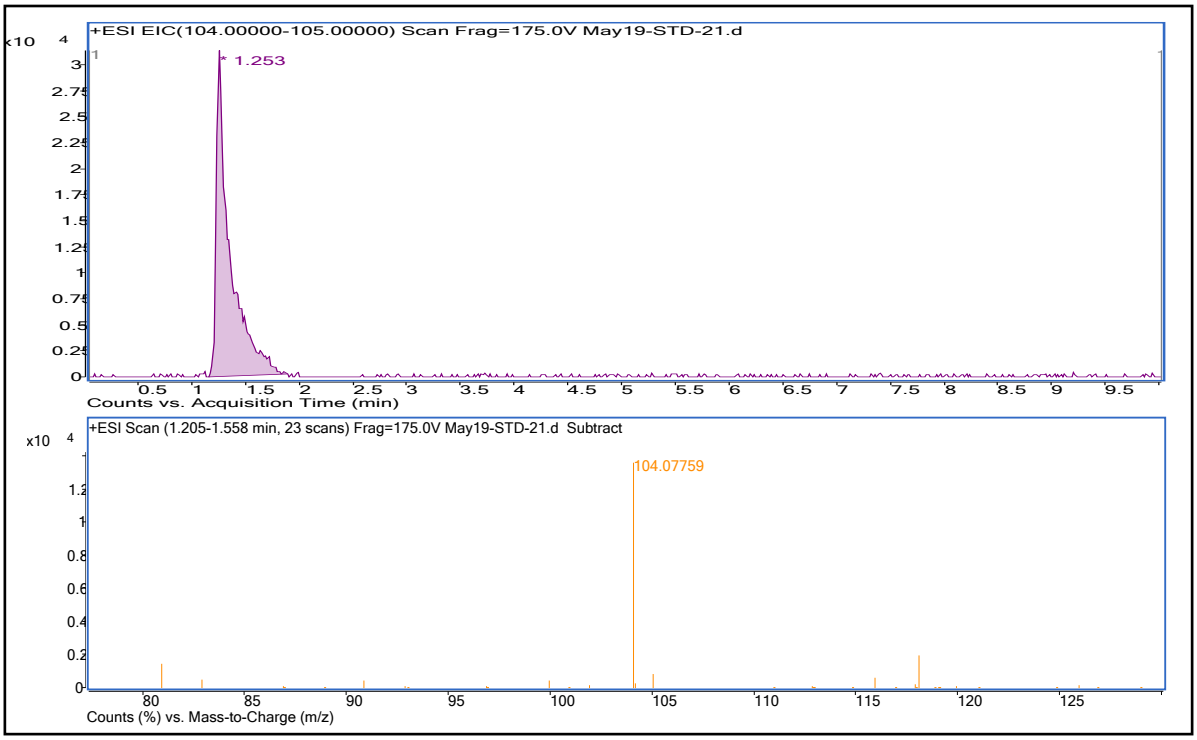

Figure C.5 - Extracted ion chromatogram and mass spectrum for choline ion present in sample 11411

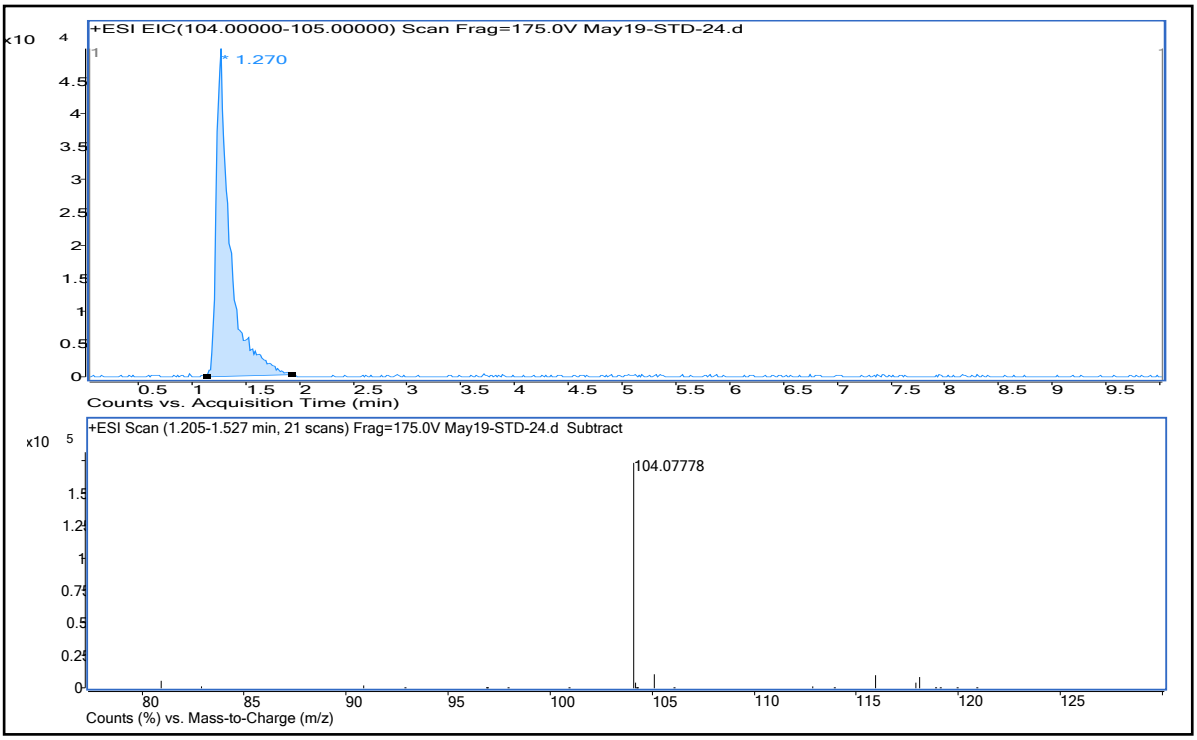

Figure C 6 - - Extracted ion chromatogram and mass spectrum for choline ion present in sample 11412

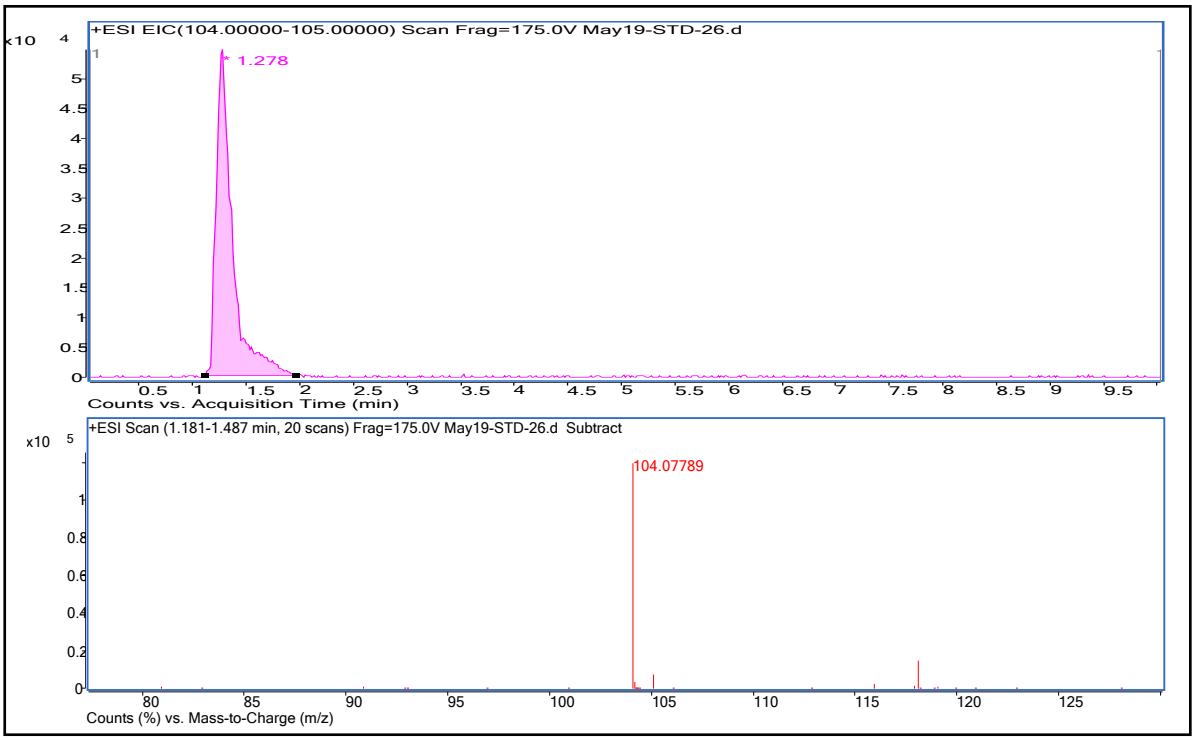

Figure C.7 - Extracted ion chromatogram and mass spectrum for choline ion present in sample 11413

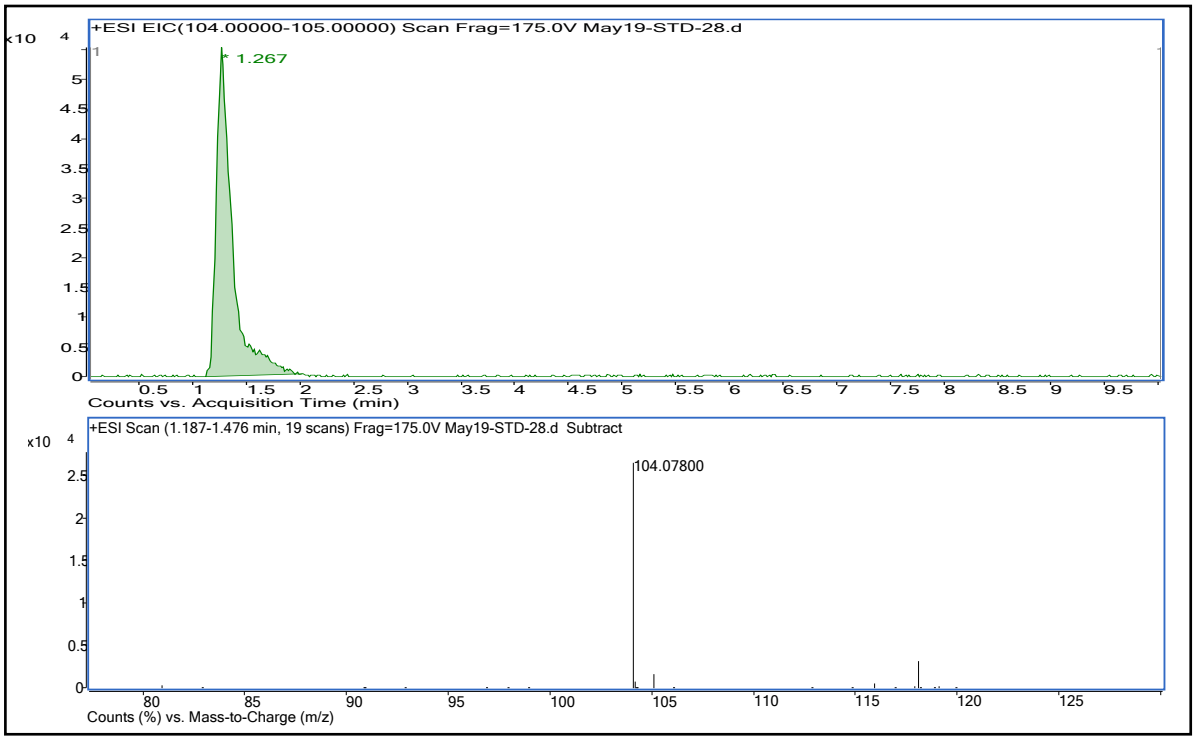

Figure C.8 - Extracted ion chromatogram and mass spectrum for choline ion present in sample 11414

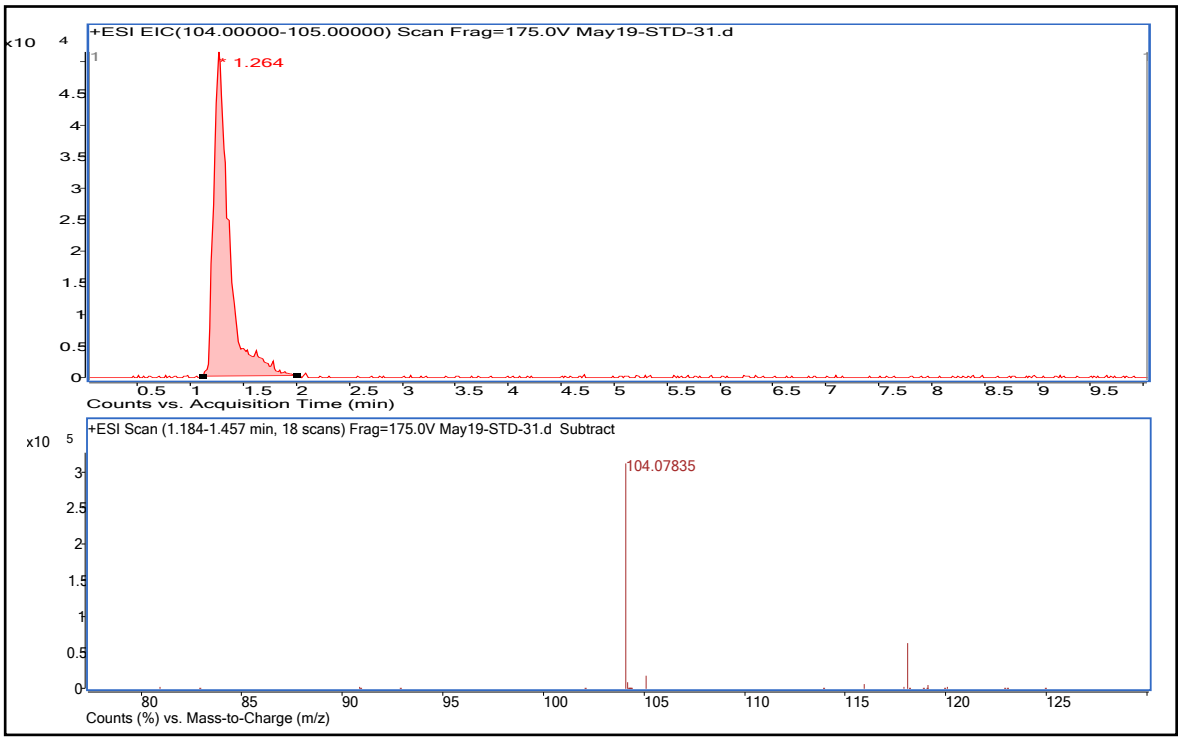

Figure C.9 - Extracted ion chromatogram and mass spectrum for choline ion present in sample 11415

Appendix D: Statistical Analysis of Methods Used

Table D.1 - The uncertainty in the choline chloride concentrations of eleven samples determined by external calibration curve by CE

Samples	CC concentration in original samples (ppm)	$S_{x(o)}$	%RSD	CC (ppm) in original samples	% CC in original samples
11241	719631.2	93513.3	13.0%	719631.21 ± 93513.33	71.96 ± 9.35
11242	644720.8	85461.9	13.3%	644720.85 ± 85461.88	71.96 ± 8.55
11243	702099.5	91609.3	13.0%	702099.48 ± 91609.28	70.21 ± 9.16
11244	718014.9	93337.3	13.0%	718014.91 ± 93337.33	77.80 ± 9.33
11245	365650.2	58577.7	16.0%	365650.22 ± 58577.71	36.57 ± 5.86
11411	310093.0	49871.0	16.1%	310093.05 ± 49871.05	31.01 ± 4.99
11412	551529.0	73152.2	13.3%	551529.02 ± 73152.20	55.15 ± 7.32
11413	631916.4	82217.2	13.0%	631916.39 ± 82217.16	63.19 ± 8.22
11414	692973.4	89337.7	12.9%	692973.36 ± 89337.69	69.30 ± 8.93
11415	818312.8	104401.1	12.8%	818312.84 ± 104401.10	81.83 ± 10.44
11421	790005.7	100956.5	12.8%	790005.71 ± 100956.5	79.00 ± 10.10

Table D.2 - The uncertainty in the choline chloride concentrations of eleven samples determined by standard addition technique by CE

Samples	CC concentration in original samples (ppm)	$S_{x(o)}$	%RSD	CC (ppm) in original samples	% CC in original samples
11241	852500	136729	16.0%	852500 ± 136729.17	85.25 ± 13.67
11242	627100	134286	21.4%	627100 ± 134285.83	62.71 ± 13.43
11243	672100	105419	15.7%	672100 ± 105419.05	67.21 ± 10.54
11244	720000	117094	16.3%	720000 ± 117094.5	72.00 ± 11.71
11245	348300	59573	17.1%	348300 ± 59572.94	34.83 ± 5.96
11411	347000	96068	27.7%	347000 ± 96068.06	34.70 ± 9.61
11412	463500	89234	19.3%	463500 ± 89233.97	46.35 ± 8.92
11413	656200	112990	17.2%	656200 ± 112990.98	65.62 ± 11.30
11414	683600	89129	13.0%	683600 ± 89128.52	68.36 ± 8.91
11415	640700	87953	13.7%	640700 ± 87952.54	64.07 ± 8.80
11421	624600	91252	14.6%	624600 ± 91251.72	62.46 ± 9.13

Table D.3 - The uncertainty in the choline chloride concentrations of eleven samples determined by external calibration curve by LC/MS

Samples	**CC concentration in original samples (ppm)**	**$S_{X(o)}$**	**%RSD**	**CC (ppm) in original samples**	**% CC in original samples**
11241	781778.6	97887.1	12.5%	781778.61 ± 97887.07	78.18 ± 9.79
11242	797280.7	99781.8	12.5%	797280.72 ± 99781.76	79.73 ± 9.98
11243	803953.0	100597.8	12.5%	803953.04 ± 100597.8	80.40 ± 10.06
11244	779422.3	97599.2	12.5%	779422.35 ± 97599.24	77.94 ± 9.76
11245	417496.1	54274.7	13.0%	417596.11 ± 54174.66	41.76 ± 5.42
11411	349245.4	46522.1	13.3%	349245.42 ± 46522.14	34.92 ± 4.65
11242	635776.7	80146.8	12.6%	635776.73 ± 80146.84	63.58 ± 8.01
11413	830664.3	103867.5	12.5%	830664.33 ± 103867.5	80.07 ± 10.39
11414	846664.8	105828.3	12.5%	846664.79 ± 105828.3	84.67 ± 10.58
11415	738886.8	92654.4	12.5%	738886.80 ± 92654.36	78.39 ± 9.27
11421	779676.2	97630.3	12.5%	779676.20 ± 97630.25	78.00 ± 9.76

Table D.4 – *t*-test results between external calibration method and standard addition method by CE (Note: “Accepted” means “Not significantly different”)

	CE (Calibration curve) (n = 3)		CE (STD addition) (n =3)					
Samples	% CC (Mean)	S_X	% CC (Mean)	S_X	Spooled	*t* calculated	*t* value at 99% CI	Results
11241	71.96	9.35	85.25	13.67	11.713	1.389	4.604	Accepted
11242	64.47	8.55	62.71	13.43	11.255	0.192	4.604	Accepted
11243	70.47	9.16	67.21	10.54	9.876	0.405	4.604	Accepted
11244	71.80	9.33	72.00	11.71	10.588	0.023	4.604	Accepted
11245	36.57	5.86	34.83	5.96	5.908	0.360	4.604	Accepted
11411	31.01	4.99	34.70	9.61	7.654	0.591	4.604	Accepted
11412	55.15	7.32	46.35	8.92	8.159	1.321	4.604	Accepted
11413	63.19	8.22	65.62	11.30	9.881	0.301	4.604	Accepted
11414	69.30	8.93	68.36	8.91	8.923	0.129	4.604	Accepted
11415	81.83	10.44	64.07	8.80	9.653	2.254	4.604	Accepted
11421	79.00	10.10	62.46	9.13	9.623	2.105	4.604	Accepted

Table D.5 - *t*-test results between external calibration method by CE and external calibration method by LC/MS (Note: “Accepted” means “Not significantly different”)

Samples	CE (Calibration curve) (n = 3)		LC/MS (Calibration curve) (n = 3)		Spooled	*t* calculated	*t* value at 99% CI	Results
	% CC (Mean)	Sx	% CC (Mean)	Sx				
11241	71.96	9.35	78.18	9.79	9.573	0.7951	4.604	Accepted
11242	64.47	8.55	79.73	9.98	9.290	2.0113	4.604	Accepted
11243	70.47	9.16	80.40	10.06	9.621	1.2631	4.604	Accepted
11244	71.80	9.33	77.94	9.76	9.549	0.7876	4.604	Accepted
11245	36.57	5.86	41.75	5.43	5.647	1.1245	4.604	Accepted
11411	31.01	4.99	34.92	4.65	4.823	0.9943	4.604	Accepted
11412	55.15	7.32	63.58	8.01	7.673	1.3448	4.604	Accepted
11413	63.19	8.22	83.07	10.39	9.367	2.5987	4.604	Accepted
11414	69.30	8.93	84.67	10.58	9.793	1.9221	4.604	Accepted
11415	81.83	10.44	73.89	9.27	9.870	0.9855	4.604	Accepted
11421	79.00	10.10	77.97	9.76	9.931	0.1274	4.604	Accepted

Table D.6 - *t*-test results between standard addition method by CE and external calibration method by LC/MS (Note: “Accepted” means “Not significantly different”)

Samples	CE (STD Addition) (n = 3)		LC/MS (Calibration curve) (n = 3)		Spooled	*t* calculated	*t* value at 99% CI	Results
	% CC (Mean)	Sx	% CC (Mean)	Sx				
11241	85.25	13.67	78.18	9.79	11.890	0.7282	4.604	Accepted
11242	62.71	13.43	79.73	9.98	11.830	1.7622	4.604	Accepted
11243	67.21	10.54	80.40	10.06	10.304	1.5679	4.604	Accepted
11244	72.00	11.71	77.94	9.76	10.779	0.6753	4.604	Accepted
11245	34.83	5.96	41.75	5.43	5.699	1.4879	4.604	Accepted
11411	34.70	9.61	34.92	4.65	7.548	0.0362	4.604	Accepted
11412	46.35	8.92	63.58	8.01	8.481	2.4871	4.604	Accepted
11413	65.62	11.30	83.07	10.39	10.852	1.9693	4.604	Accepted
11414	68.36	8.91	84.67	10.58	9.784	2.0416	4.604	Accepted
11415	64.07	8.80	73.89	9.27	9.033	1.3317	4.604	Accepted
11421	62.46	9.13	77.97	9.76	9.449	2.0102	4.604	Accepted

Table D.7 - *t*-test results between external calibration method by CE and Reinecke salt gravimetric method (Note: “Accepted” means “Not significantly different”)

Samples	CE (Calibration curve) (n = 3)		Gravimetric Reinecke (n = 4)		Spooled	*t* calculated	*t* value at 99% CI	Results
	% CC (Mean)	S_x	% CC (Mean)	SD				
11241	71.96	9.35	77.73	1.51	6.029	1.252	4.032	Accepted
11242	64.47	8.55	77.89	2.37	5.709	3.078	4.032	Accepted
11243	70.47	9.16	75.74	2.80	6.185	1.116	4.032	Accepted
11244	71.80	9.33	75.54	3.22	6.408	0.763	4.032	Accepted
11245	36.57	5.86	41.63	3.31	4.508	1.471	4.032	Accepted
11411	31.01	4.99	37.05	2.38	3.655	2.163	4.032	Accepted
11412	55.15	7.32	56.62	1.71	4.813	0.400	4.032	Accepted
11413	63.19	8.22	73.39	1.13	5.273	2.532	4.032	Accepted
11414	69.30	8.93	76.61	5.47	7.061	1.356	4.032	Accepted
11415	81.83	10.44	72.28	3.33	7.089	1.765	4.032	Accepted
11421	79.00	10.10	68.19	3.50	6.937	2.041	4.032	Accepted

Table D.8 - *t*-test results between standard addition method by CE and Reinecke salt gravimetric method (Note: “Accepted” means “Not significantly different”)

Samples	CE (STD Addition) (n = 3)		Gravimetric Reinecke (n = 4)		Spooled	*t* calculated	*t* value at 99% CI	Results
	% CC (Mean)	S_x	% CC (Mean)	SD				
11241	85.25	13.67	77.73	1.51	8.726	1.129	4.032	Accepted
11242	62.71	13.43	77.89	2.37	8.690	2.288	4.032	Accepted
11243	67.21	10.54	75.74	2.80	7.010	1.595	4.032	Accepted
11244	72.00	11.71	75.54	3.22	7.814	0.593	4.032	Accepted
11245	34.83	5.96	41.63	3.31	4.560	1.954	4.032	Accepted
11411	34.70	9.61	37.05	2.38	6.350	0.483	4.032	Accepted
11412	46.35	8.92	56.62	1.71	5.798	2.319	4.032	Accepted
11413	65.62	11.30	73.39	1.13	7.200	1.414	4.032	Accepted
11414	68.36	8.91	76.61	5.47	7.050	1.533	4.032	Accepted
11415	64.07	8.80	72.28	3.33	6.132	1.753	4.032	Accepted
11421	62.46	9.13	68.19	3.50	6.376	1.176	4.032	Accepted

Table D.9 - *t*-test results between external calibration method by LC/MS and Reinecke salt gravimetric method (Note: “Accepted” means “Not significantly different”)

Samples	LC/MS (Calibration curve) (n = 3)		Gravimetric Reinecke (n = 4)		Spooled	*t* calculated	*t* value at 99% CI	Results
	% CC (Mean)	S_x	% CC (Mean)	SD				
11241	78.18	9.79	77.73	1.51	6.301	0.094	4.032	Accepted
11242	79.73	9.98	77.89	2.37	6.573	0.366	4.032	Accepted
11243	80.40	10.06	75.74	2.80	6.721	0.906	4.032	Accepted
11244	77.94	9.76	75.54	3.22	6.657	0.473	4.032	Accepted
11245	41.75	5.43	41.63	3.31	4.287	0.036	4.032	Accepted
11411	34.92	4.65	37.05	2.38	3.474	0.800	4.032	Accepted
11412	63.58	8.01	56.62	1.71	5.240	1.738	4.032	Accepted
11413	83.07	10.39	73.39	1.13	6.627	1.912	4.032	Accepted
11414	84.67	10.58	76.61	5.47	7.920	1.332	4.032	Accepted
11415	73.89	9.27	72.28	3.33	6.403	0.330	4.032	Accepted
11421	77.97	9.76	68.19	3.50	6.744	1.899	4.032	Accepted

Table D.10 – *F*-test results between external calibration method and standard addition method by CE (Note: “Accepted” means “Precision are similar”)

Samples	CE (Calibration curve) (n= 3)		CE (STD addition) (n=3)		*F* calculated	*F* at 95% CI	Results
	% CC (Mean)	S_x	% CC (Mean)	S_x			
11241	71.96	9.35	85.25	13.67	2.138	19.000	Accepted
11242	64.47	8.55	62.71	13.43	2.469	19.000	Accepted
11243	70.47	9.16	67.21	10.54	1.324	19.000	Accepted
11244	71.80	9.33	72.00	11.71	1.574	19.000	Accepted
11245	36.57	5.86	34.83	5.96	1.034	19.000	Accepted
11411	31.01	4.99	34.70	9.61	3.711	19.000	Accepted
11412	55.15	7.32	46.35	8.92	1.488	19.000	Accepted
11413	63.19	8.22	65.62	11.30	1.889	19.000	Accepted
11414	69.30	8.93	68.36	8.91	1.005	19.000	Accepted
11415	81.83	10.44	64.07	8.80	1.409	19.000	Accepted
11421	79.00	10.10	62.46	9.13	1.224	19.000	Accepted

Table D.11 - *F*-test results between external calibration method by CE and external calibration method by LC/MS (Note: “Accepted” means “Precisions are similar”)

Samples	CE (Calibration curve) (n = 3)		LC/MS (Calibration curve) (n = 3)		*F* calculated	*F* at 95% CI	Results
	% CC (Mean)	S_X	% CC (Mean)	S_X			
11241	71.96	9.35	78.18	9.79	1.096	19.000	Accepted
11242	64.47	8.55	79.73	9.98	1.363	19.000	Accepted
11243	70.47	9.16	80.40	10.06	1.206	19.000	Accepted
11244	71.80	9.33	77.94	9.76	1.093	19.000	Accepted
11245	36.57	5.86	41.75	5.43	1.165	19.000	Accepted
11411	31.01	4.99	34.92	4.65	1.149	19.000	Accepted
11412	55.15	7.32	63.58	8.01	1.200	19.000	Accepted
11413	63.19	8.22	83.07	10.39	1.596	19.000	Accepted
11414	69.30	8.93	84.67	10.58	1.403	19.000	Accepted
11415	81.83	10.44	73.89	9.27	1.270	19.000	Accepted
11421	79.00	10.10	77.97	9.76	1.069	19.000	Accepted

Table D.12 - *F*-test results between standard addition method by CE and external calibration method by LC/MS (Note: “Accepted” means “Precisions are similar”)

Samples	CE (STD Addition) (n = 3)		LC/MS (Calibration curve) (n = 3)		*F* calculated	*F* at 95% CI	Results
	% CC (Mean)	S_X	% CC (Mean)	S_X			
11241	85.25	13.67	78.18	9.79	1.951	19.000	Accepted
11242	62.71	13.43	79.73	9.98	1.811	19.000	Accepted
11243	67.21	10.54	80.40	10.06	1.098	19.000	Accepted
11244	72.00	11.71	77.94	9.76	1.439	19.000	Accepted
11245	34.83	5.96	41.75	5.43	1.205	19.000	Accepted
11411	34.70	9.61	34.92	4.65	4.264	19.000	Accepted
11412	46.35	8.92	63.58	8.01	1.240	19.000	Accepted
11413	65.62	11.30	83.07	10.39	1.183	19.000	Accepted
11414	68.36	8.91	84.67	10.58	1.410	19.000	Accepted
11415	64.07	8.80	73.89	9.27	1.110	19.000	Accepted
11421	62.46	9.13	77.97	9.76	1.145	19.000	Accepted

Table D.13 - *F*-test results between external calibration method by CE and Reinecke salt gravimetric method (Note: “Accepted” means “Precisions are similar”; “Rejected” means “Precisions not similar”)

Samples	CE (Calibration curve) (n = 3)		Gravimetric Reinecke (n = 4)		*F* calculated	*F* at 95% CI	Results
	% CC (Mean)	SD	% CC (Mean)	SD			
11241	71.96	9.24	77.73	1.51	37.391	19.200	Rejected
11242	64.47	3.49	77.89	2.37	2.168	19.200	Accepted
11243	70.47	2.31	75.74	2.80	1.463	9.550	Accepted
11244	71.80	2.07	75.54	3.22	2.411	9.550	Accepted
11245	36.57	3.13	41.63	3.31	1.121	9.550	Accepted
11411	31.01	2.69	37.05	2.38	1.272	19.200	Accepted
11412	55.15	2.64	56.62	1.71	2.369	19.200	Accepted
11413	63.19	2.16	73.39	1.13	3.638	19.200	Accepted
11414	69.30	7.71	76.61	5.47	1.991	19.200	Accepted
11415	81.83	0.92	72.28	3.33	13.220	9.550	Rejected
11421	79.00	3.90	68.19	3.50	1.241	19.200	Accepted

Table D.14 - *F*-test results between standard addition method by CE and Reinecke salt gravimetric method (Note: “Accepted” means “Precisions are similar”; “Rejected” means “Precisions not similar”)

Samples	CE (STD Addition) (n = 3)		Gravimetric Reinecke (n = 4)		*F* calculated	*F* at 95% CI	Results
	% CC (Mean)	SD	% CC (Mean)	SD			
11241	85.25	2.94	77.73	1.51	3.793	19.200	Accepted
11242	62.71	0.78	77.89	2.37	9.348	19.200	Accepted
11243	67.21	4.75	75.74	2.80	2.892	9.550	Accepted
11244	72.00	2.37	75.54	3.22	1.842	9.550	Accepted
11245	34.83	0.99	41.63	3.31	11.209	9.550	Rejected
11411	34.70	1.17	37.05	2.38	4.149	19.200	Accepted
11412	46.35	0.41	56.62	1.71	17.443	19.200	Accepted
11413	65.62	1.47	73.39	1.13	1.699	19.200	Accepted
11414	68.36	6.68	76.61	5.47	1.495	19.200	Accepted
11415	64.07	3.40	72.28	3.33	1.043	9.550	Accepted
11421	62.46	1.38	68.19	3.50	6.455	19.200	Accepted

Table D.15 - *F*-test results between external calibration method by LC/MS and Reinecke salt gravimetric method (Note: “Accepted” means “Precisions are similar”; “Rejected” means “Precisions not similar”)

Samples	LC/MS (Calibration curve) (n = 3)		Gravimetric Reinecke (n = 4)		*F* calculated	*F* at 95% CI	Results
	% CC (Mean)	SD	% CC (Mean)	SD			
11241	78.18	1.38	77.73	1.51	1.191	19.200	Accepted
11242	79.73	0.19	77.89	2.37	150.959	19.200	Rejected
11243	80.40	1.34	75.74	2.80	4.351	9.550	Accepted
11244	77.94	0.61	75.54	3.22	28.275	9.550	Rejected
11245	41.75	1.52	41.63	3.31	4.739	9.550	Accepted
11411	34.92	0.26	37.05	2.38	87.340	19.200	Rejected
11412	63.58	0.30	56.62	1.71	31.698	19.200	Rejected
11413	83.07	1.04	73.39	1.13	1.184	19.200	Accepted
11414	84.67	0.63	76.61	5.47	76.391	19.200	Rejected
11415	73.89	0.70	72.28	3.33	22.416	9.550	Rejected
11421	77.97	1.61	68.19	3.50	4.756	19.200	Accepted