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Analysis of an Ion Suppression Device Coupled with an Ion Chromatography System

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Analysis of an Ion Suppression Device Coupled with an Ion Chromatography System

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Abstract:

A priority of chromatographers is to eliminate extraneous and errant noise that can interfere with any experiment. The goal is to focus on what is being analyzed, and in the case of detectors, modifying the instrument being utilized is the method of choice. One way to do that in ion chromatography (IC) is through the use of a suppressor.

Though not all instruments utilized in IC are equipped with suppressors, it is ideal to help reduce the ions already known to be present in the eluent [1]. The premise of this study is simple in nature, but important in its application. It is to ensure the efficacy of a new suppressor prior to its use in everyday workflow. Without the suppressor in use, the instrument would provide data convoluted with extra noise and peaks, which would make analysis of samples unnecessarily tedious. With a suppressor installed in the ion chromatography system (ICS), the baseline can be more stable, the signal-to-noise ratio would be at its optimal level, and sensitivity of the ICS would be enhanced as well.

Introduction:

In 1903, ion chromatography was introduced and pigments were first able to be separated; an experiment that can now be replicated by fledgling scientists through thin-layer chromatography, termed TLC [2]. From the beginning of their educational journey in science, students will learn the periodic table of elements, and will understand that one of the ways that elements differ is in molecular size. This is the same for the ions of every element, and is the basis of IC. Therefore, ionic species will separate differently based on what kind they are, as well as the size of the ion. Chromatography is a method where two phases are used to separate a mixture of ions into its components;

however, this also means that chromatography cannot solely establish the molecular structure of a compound. It wasn't until 1975 that scientists at Dow introduced IC as an analytical technique to distinguish between ions of individual elements [3]. The types of phases used determine what type of chromatography is being used such as gas, liquid, or solid; and the instrument may be in conjunction with another instrument (i.e. a mass spectrometer). Between the differing stationary phases, mobile phases, and detectors, the result is a plethora of combinations of chromatography being used in divers industrial settings.

Liquid chromatography (LC) utilizes an ion exchange method to separate different ions present within a sample. IC is a variant of high-performance liquid chromatography (HPLC), and was introduced to help detect inorganic ions, since typically they do not contain chromophores [4]. IC has been a technique used in conjunction with methods such as normal phase HPLC, reverse-phase HPLC, or even atomic absorption spectrometry (AAS) in the pharmaceutical industry [4]. At its inception, when IC was used in conjunction with spectrophotometric ultraviolet (UV) detection, the samples had to be processed so that organic derivatives and ion-pairs would be detected [5]. An IC system generally consists of a liquid eluent, high-pressure pump, sample injector, guard column, separator column, chemical suppressor, conductivity cell, and a data collection system. From a general standpoint, IC refers to the combination of the separation of analytes via their displacement along with analyte detection. IC utilizes the process from LC to separate the ions present in a sample, and then as those ions pass through a detector, the change is evaluated. In both LC and IC,

there is an ionic solution or mobile phase, otherwise termed the eluent. The eluent is what carries the sample solution through the pathway of the system.

IC was not generally accepted until eluent suppressor columns were introduced, because scientists knew that the background noise of the eluent would be an issue in analyzing data [6]. If a physical suppressor is not usable, acids, bases, or buffers can be added into the mobile phase depending on the sample being analyzed [7]. With one of the appropriate components present in the mobile phase, the suppression of the dissociation of weakly ionizable solutes will occur, and that will mean refined peak shape and better retention [7]. An example of chemical suppression would be the use of a cation exchanger. The metal cations that would travel alongside the analyte would be replaced with hydronium ions that are more conductive while also reducing highly conductive eluents into weakly conductive liquids [5]. If the suppressor being used contained an anion exchange material in the hydroxide form, the hydronium ions would convert to water while the exchange material would convert and leave behind corresponding bases of the analyte that are now highly conductive [3]. The eluent volume is adjusted during the standard calibration, and then should be maintained for the duration of the sample run. The three ways that eluent can be delivered are by using a device that keeps the flow constant, a pump that will keep the pressure constant, or a reciprocating piston pump, which is useful if a gradient elution is being done [8]. To clarify, a gradient elution can be either a change in the composition of the eluent, or a change in the volume of eluent that is passing through.

An automated sampler provides assistance in sending the sample through by injecting the sample into already-flowing eluent. The sample is then pushed through the

guard and separator columns. The guard column works to ensure that the sample is ready for action by eliminating contaminants in the sample that could harm the separator column. As the sample traverses through the separator column, which is where the stationary phase resides, the various ions that are present in the eluent begin to separate based on their affinity for the stationary phase.

The greater the number of theoretical plates in a column, the more beneficial it is for chromatographic separations [4]. The separation section of an instrument is the most important component. The higher the resolution that can be obtained, the better the results should be. If a column length cannot be increased, smaller particles can be used to pack the column in order to increase the theoretical plate number [8]. The stationary phase will have a thin layer of an ion exchange resin, which will contain locations on it to interact with ions. The column itself can be replaced depending upon the purpose of the study, varying from positive sites to attract anions or negative sites to have an affinity for cations. Whatever ions are being studied, the affinity of those ions is what is measured, and that is the rate at which the ions attach to the stationary phase and subsequently detach. “The major factors that affect exchange columns are the diameter of the particles, flow rate, column length, and fluid properties such as density and viscosity” [8]. The ions that have a greater attraction for the stationary phase, they will be detained the longest in the column, while those with less affinity will elute more rapidly. The length of time it takes for each ionic species to elute is referred to as retention time, and this is what is measured by the detector. One of two problems can also result at this juncture: 1) if the sample has no interaction with the stationary phase, then it will not bind and will therefore elute at the same rate as the effluent, and 2) if the

sample has no affinity for the mobile phase, it will affix itself to the stationary phase and therefore never elute. This demonstrates that it is important to know what components are being used as well as what is being analyzed. The combination of the solute and mobile phase exiting the column is termed as the eluate, which is comprised of the effluent (the mobile phase exiting the column) and the eluite, the solute that exits the column. IC is sufficiently sensitive to determine eluite even if it is as low as a few parts per billion.

Eluite has been studied, determined, and measured for a variety of ions and therefore, when ions elute from a column at a particular time or rate, it is possible to identify the specific ion that has exited the column. The reason the detector measures regularly is to ensure that all ions, be they from the effluent or the eluite, are quantified as a function of time [6]. A chromatogram is subsequently produced from this data, and will show peaks corresponding to the various substances being analyzed.

In the past, IC consisted of separating with a column that contained an ion exchange stationary phase, with the detection being done by a variety of means such as amperometry, coulometry and so forth [9]. IC has been used in laboratories throughout the world to measure inorganic anions, cations, sugar alcohols, aminoglycosides and more. Anion separation by IC combined with ultraviolet (UV) detection has been designed and used to identify impurities that can be found in heparin; impurities that have resulted in adverse effects [1]. IC can be used to analyze raw materials, culture broths, diluents, waste solutions, and other products that have non-ionic components, but also have ionic analytes which are not responsive to UV absorbance [1]. In regards to anions and cations, IC is typically used to analyze anions

such as fluoride, chloride, nitrite, and cations such as lithium, sodium, potassium, and so forth.

After separation in the ion-exchange column, the flow continues to the suppressor. The suppressor debuted in 1975 and, at that time, needed to be regularly chemically regenerated [10]. To extend the time of a study, the first suppressors were large so that they could be used for a greater period of time before it was necessary to replenish them [10]; however, having a very large suppressor can also be counter effective. If a massive suppressor column is used, band broadening in the void space would severely diminish the efficacy of the separation [11]. On the other hand, if a scientist was patient enough and had sufficient resources, small suppressor beds could be used that required frequent regenerations to minimize the large packed-bed suppressors [12]. The columns used for separation were not robust, and therefore the eluents used were typically of a small ionic strength, which also meant the suppressor had to be regenerated less frequently. The disadvantage of this scheme was that the volume of sample used had to be small [10]. Another issue with suppressors is that the neutralization reaction that occurs is exothermic and that heat results in background noise. However, even though that background noise will be present regardless of the suppressor, it is vital to have the proper suppressor and the optimal mode of operation [13]. The most significant advancement made towards a more robust suppressor was when the evolution of the suppressor turned into a continuously regenerating chemical suppressor, which meant that they would not need to be taken off-line for regeneration, the analytical column could be of a much higher capacity, and large samples could now be put into the system [10]. The use of electrodialytic membrane suppressors are

another way to combat the need for constant regeneration. As an example, with an alkaline eluent on one side of a cation-exchange membrane and pure water on the other side, a positive potential being applied on the membrane would cause eluent cations to traverse the membrane, giving off eluent suppression [14].

A suppressor is made to work according to its name by suppressing the detection of the ions in the eluent, which is done by providing suppressor derived ions to take the place of eluent and sample counter ions [15]. By exchanging the ions, several events occur; the background conductivity is lessened, as is the noise that accompanies the sample, while the conductivity response of most analytes is increased [10]. From the perspective of lowering the signal-to-noise ratio, the ratio is higher in a suppressed IC as opposed to an ICS without a suppressor.

The purpose of suppression is to place regenerant ions in the stead of eluent and sample counter ions. By doing this, the eluent will be altered into a weakly dissociated form prior to entering the detector. This will greatly improve the chances of detection of the analyte ions by providing a clearer background. This is how suppression can greatly enhance the signal-to-noise ratio in IC; by creating a low background as well as decreased noise that is associated with the signal itself. To help enhance the signal, the analytes are converted to their conductive acidic or basic form, which yields more fully dissociated species. The subsequent result in the enhancement is an improvement in the signal-to-noise ratio when observing the detection limits.

For the purposes of this experiment, the previous Dionex suppressor used was an Anion Self-Regenerating Suppressor (ASRS) 300 Ultra, 4mm- which, according to Thermo Scientific will no longer be manufactured. The ASRS Ultra has been in

widespread use, owing to its ease of use and ability to handle large sample volumes [13]. The new suppressor to be tested for equivalency was a Thermo Scientific Dionex Electrolytically Regenerated Suppressor (AERS) 500, because the company stated that the ASRS would be discontinued. The suppressor being used in the instrument has two side-regenerant channels, and a third eluent channel, which is centrally located. The eluent in and out ports are independent ports that define the fluidic pathway, which is similar to a column.

The regenerant flow is arranged to be counter-current to the eluent flow, and by having this orientation, this ensures that regeneration will occur for the entire device. The suppressor being used is designed for continuous operation, and does not require switching or off-line regeneration. The suppressor cannot be changed when running the standard or sample solutions, to ensure that the analytical parameters will be set and consistent throughout. If the suppressor is operated without current during installation or startup, it can lose its regenerated form, and that would result in diminishing the peak area response as well as giving an unstable baseline. This would mean that before electrolytic suppression can begin, the suppressor would have to be chemically regenerated in order to restore it to its proper form [10]. The move to the detector from the suppressor is the final step in the process. In the detector, a conductivity cell measures the conductance of the ions as they elute from the suppressor, and signals are created based on the analyte's properties.

Materials & Instrumentation:

The instrument used for this project was an Ion Chromatography System-1000 High Performance Liquid Chromatography (ICS-1000 HPLC) [Figure 1]. The

autosampler is in place for the samples to be placed once they are prepared, and where they can be automatically injected in the correct order without any human interference [Figure 2]. The column used was a Thermo Scientific IONPAC AS14 analytical column, 4 x 250mm, and was kept at ambient temperature. The guard used was a Thermo Scientific IONPAC AG14 guard column, 4 x 50mm. The columns and suppressor were housed in the same unit and are displayed with an outer view [Figure 3], as well as the inner view [Figure 5]. A diagram of the path flow can be seen in Figure 4. Both the flush reservoir, which houses Laboratory Ultrapure (LUP) water, and the bottle containing the eluent were kept pressurized using a nitrogen cylinder, such as N UHP300, with an accompanying regulator such as Hewlett Packard Multi-Stage Regulator, HP Part # 5183-4645. Eluent used was prepared by first making the stock solution. This was done by weighing out 6.7301g. of $NaHCO_3$ {Certified ACS, Fisher Lot# 126930, Expiration: 11-2017, M.W. 84.01}, 29.611g of Na_2CO_3 {Anhydrous, extra pure, 99.95%, Acros Lot: AO294874, Expiration: 12-16-15, M.W. 105.99}, placing in a 1,000mL flask, and then adding in enough LUP water to dissolve the material. Once it was dissolved, the flask was filled to volume with LUP water, and thoroughly mixed. The eluent working solution was prepared by placing 50.0mL of the eluent stock solution in a 4,000mL flask, and then adding sufficient quantity of LUP water to the mark. The eluent working solution did not need to be filtered or degassed before use. The suppressor being used was the AERS 500. An IC Millex- LG filter 0.2µm was utilized. The standard stock solutions used were:

Table 1-Standard Stock Solutions	
SPEX CertiPrep	**SPEX CertiPrep**
Anion Std: 1000ug/mL Chloride	Anion Std: 1000ug/mL Phosphate
C of A: 997 ppm	C of A: 1000 ppm
Lot# 3-57Cl-2Y	Lot# 3-132PO_4-2Y
Cat# AS-Cl9-2Y	Cat# AS-$PO_4$9-2Y
CAS #: H_2O [7732-18-5]	CAS #: H_2O [7732-18-5]
Vol. 125mL	Vol. 125mL

Table 2-Calibration Verification Stock Solutions	
RICCA	**RICCA**
Phosphate Standard 1000ppm PO4{3-} Exp. 10-2016	Chloride Standard 1.00mL = 1.00mg Cl- (1000 ppm Cl-) Exp. 4-2016, Vol. 120mL
C of A: 1000ppm PO_4^-	C of A: 1000 ppm Cl^-
Lot#4504625	Lot# 4410997
Cat# 5839-4	Cat No. 1955-4
Contains: Water [7732-18-5] & Potassium Dihydrogen Phosphate [7778-77-0]	Prepared w/ ACS Reagent Grade Sodium Chloride, CAS No. 7647-14-5, in ACS Reagent Grade Water, CAS No. 7732-18-5. Suitable for use in Ion Chromatography, with Ion Selective Electrodes, and for other techniques where the above matrix is acceptable.
Specifications: Phosphate (PO4): 995-1005ppm	

The chloride and phosphate standards were prepared using the following table.

Table 3-Standard Preparation for Suitability Run					
	1000 ppm Std	**Total volume in LUP (water)**	**Dilution factor**	**Final Cl^- Concentration (mM)**	**Final PO_4^{3-} Concentration (mM)**
Standard 1	1 mL	200 mL	200	0.14062	0.05260

Table 3-Standard Preparation for Suitability Run					
	1000 ppm Std	**Total volume in LUP (water)**	**Dilution factor**	**Final Cl^- Concentration (mM)**	**Final PO_4^{3-} Concentration (mM)**
Standard 2	2 mL	200 mL	100	0.28124	0.10519
Standard 3	3 mL	200 mL	66.6666	0.42186	0.15778
Standard 4	4 mL	200 mL	50	0.56248	0.21038
Check Standard	3 mL	200 mL	66.6666	0.42313	0.15794

An example calculation for Chloride Standard 2 would be:

$[(x)*(997\ ppm\ Cl^-)] / [(200.0mL)*(35.45) = y\ mM\ Cl^-$

with 'x' being 1, 2, 3, or 4 mL of the chloride standard, 200 mL QS of LUP water, and 35.45 being the molecular weight of a chloride ion, and 'y' is the result with units of millimolar.

An example calculation for Phosphate Standard 2 would be:

$[(x)*(1000\ ppm\ PO_4^{3-})] / [(200.0mL)*(94.97) = y\ mM\ PO_4^{3-}$

with 'x' being 2, 3, or 4 mL of the phosphate standard, 200 mL QS of LUP water, and 94.97 being the molecular weight of a phosphate ion, and 'y' is the result with units of millimolar.

All samples are filtered through 3 mL disposable Latex-free syringes, Mfg: BD, Lot: 4153696. They are filtered into WATERS Autosampler Vials and Caps: P/N: 600000668CV, L/N: 0668533100.

The background conductivity was set at 16 μS, with the limit of the conductivity set to be ≦20μS. The samples analyzed in the study will be called Analyte A (due to

confidentiality and proprietary concerns, the name of the sample will be withheld from this paper).

Method & Analysis:

The research that was done was through a method that was based on the determination of anions by IC.

Table 4-ICS Operating Parameters	
Autosampler	
	Column Temperature
50 µL loop	Ambient
Sample syringe 250 µL	Detector Settings
Settings at Autosampler	SRS.....24mA
Sample Mode: Normal	DS3 Setpoint.....35^{o}C
Injection type: Full Loop	Eluent & Needle Wash Pressure Setting
Settings at Chromeleon	Between 6 to 10 psi
Syringe Speed: 4	System Suitability Parameters
Flush volume: 500 µL	Minimum peak resolution: 8
Injection volume: 50 µL	Number of expected components: 2
Injections per vial: 1	Aysmmetry/Tailing: Min. 0.8 Max 1.3

The procedure to be followed is to analyze three samples of Analyte A each containing different masses following a set method with the ASRS 300. The next step is to replace the suppressor with the AERS 500, and perform a Laboratory Performance Qualification (LPQ). The LPQ itself has qualifications that must be met before it is approved, but that procedure will not be discussed in this paper because it is done merely to assess that the new suppressor has been installed correctly and is ready for testing. After a

passing LPQ is completed, the same analyses that were performed with the ASRS 300 will be done again with the AERS 500. All samples run were done in duplicate.

Before running any sample, the system must be calibrated using a standard solution. The solution is prepared using known concentrations of ions, so that once a chromatogram is produced of the standard solution, it can be determined whether the system is running adequately, or if further maintenance is required before continuing. Standard 2 for the ions will be run for a suitability check and will be injected six times. To be considered passing, the Relative Standard Deviation (RSD) of all six injections must be less than or equal to 3%. Once the suitability has been established, the retention times of each ion must be within plus or minus 3% of the average retention time of that ion's respective six Standard 2 injections.

Results & Discussion:

Table 5--CHLORIDE ION (mM)

	Current Suppressor (ASRS 300)			Replacement Suppressor (AERS 500)			Absolute Diff. (AERS-ASRS)
Sample ID	ASRS Results	Ave	% Diff	AERS Results	Ave	% Diff	
1g, Lot a	35.9944	36.09	0.50	35.9401	35.87	0.37	-0.22
	36.1760			35.8091			
1g, Lot b	36.1073	36.13	0.10	36.3999	36.24	0.88	0.11
	36.1438			36.0818			
1g, Lot c	35.9831	36.24	1.40	35.8332	35.84	0.04	-0.40
	36.4900			35.8492			
4g, Lot a	38.9045	38.88	0.14	38.4445	38.47	0.13	-0.41

Table 5--CHLORIDE ION (mM)							
	38.8495			38.4929			
4g, Lot b	37.7181	38.16	2.31	38.3883	38.27	0.60	0.11
	38.6012			38.1576			
4g, Lot c	39.4249	39.35	0.40	38.4437	38.72	1.44	-0.63
	39.2674			38.9999			
5g, Lot a	38.7404	38.67	0.38	38.4846	38.51	0.12	-0.16
	38.5945			38.5306			
5g, Lot b	38.5206	38.75	1.18	38.4236	38.51	0.47	-0.24
	38.9764			38.6048			

Table 6--PHOSPHATE ION (mM)							
	Current Suppressor (ASRS 300)			Replacement Suppressor (AERS 500)			Absolute Diff. (AERS-ASRS)
Sample ID	ASRS Results	Ave	% Diff	AERS Results	Ave	% Diff	
1g, Lot a	16.9686	16.92	0.56	16.9310	16.82	1.34	-0.10
	16.8744			16.7050			
1g, Lot b	17.0912	17.07	0.24	17.2602	17.15	1.23	0.08
	17.0505			17.0496			
1g, Lot c	16.9974	17.04	0.55	16.8954	16.90	0.03	-0.14
	17.0915			16.9011			
4g, Lot a	18.0346	18.00	0.40	17.5825	17.59	0.08	-0.41
	17.9619			17.5966			
4g, Lot b	17.5536	17.72	1.90	17.6775	17.55	1.47	-0.17

Table 6--PHOSPHATE ION (mM)							
	17.8911			17.4200			
4g, Lot c	18.1479	18.09	0.63	17.5716	17.73	1.82	-0.36
	18.0347			17.8948			
5g, Lot a	17.8672	17.80	0.78	17.6333	17.62	0.12	-0.18
	17.7276			17.6117			
5g, Lot b	17.6248	17.72	1.08	17.6112	17.60	0.12	-0.12
	17.8156			17.5905			

Before the run is started, the background conductivity of the system is checked and recorded. The conductivity cannot be equal or greater than 20µS, if so, then the eluent would have to be remade and reran until that parameter was obtained. The background conductivity was recorded at 16 µS for the study. Injection of a LUP water blank begins the run. The sole requirement for the blank injection is that no other peaks other than the negative peak at the system void time should be detected from the water. For a peak to be considered detectable, it must be three times the baseline noise. Therefore, if peaks are detected, then another water blank must be injected to see if the peaks appear again. If so, then an investigation must be done, ranging from glassware cleanliness, laboratory technique, or even possible contamination of the sample vial. If no peaks are detected, the run may continue.

Table 3 listed the standard preparation for the suitability run. Parameters for the suitability are listed in Table 4. From Table 3, Standard 2 is injected 6 times after the blank. The 6 injections must fall within the parameters to be considered a valid suitability. The resolution for the peaks must be greater than or equal to 8. For each ion, chloride and phosphate, the tailing factor will be based from the chromatogram of

the Standard 2 injection. The tailing factor has to be greater than or equal to 0.8, but less than or equal to 1.3. If the resolution or the tailing factor does not meet the parameters stated, the same steps would be taken for each to determine if the problem can be rectified. That is, the column and the suppressor would be evaluated to see if either or both of them require replacement, and the preparation of the mobile phase and standards would have to be scrutinized to ensure that they were correctly prepared.

With a passing suitability, the run is now ready for further injections. Each sample that is to be injected must be assayed in duplicate. Between those two injections of a single sample, the variability must be less than or equal to 3%. With each set of injections, be they for chloride or phosphate, the check standard listed in Table 3 will also be run. That check standard recovery must be 100 plus or minus 3.5%. If that criteria is not met, that run is invalid. The recovery is calculated by dividing the peak amount of the anion of the check standard by the prepared concentration of the anion of the check standard based on the nominal concentration, multiplied by 100.

Tables 5 and 6 list the data compiled from the runs for the chloride ion and the phosphate ion respectively. The layout of the tables are the same. The first column is the lot identification for each sample run. The next 3 columns are for the ASRS 300 suppressor; the retention time of each set of duplicate sample injections, the average of each set, and the percent difference between the duplicate sample injections. This is calculated by taking the absolute value of the difference of the duplicate injections, dividing by the average of the duplicate injections, and then multiplying by 100. The next 3 columns are for the AERS 500 and follow the exact same format as listed

previously for the ASRS 300. The 8th and final column in both Tables 5 & 6 is the difference between the results from the averages of the duplicate injections from each suppressor. That is, the average from the AERS 500 is subtracted from the average of the ASRS 300 for each set of samples.

The first two sets of samples on the ASRS 300 had to be reassayed because when they were run, there were passing peaks as well as peaks that didn't pass the requirements set forth for the samples. Unfortunately, the samples were mislabeled, which resulted in the entire set of samples having to be remade instead of simply reassaying the ones that had failed the peak requirements. One possible source of error for the ICS-1000 is that it is an isocratic delivery system, which means that during the entire run, the concentration of the eluent as well as it's composition must remain the same. Any deviation on this part can cause errant results.

Conclusion:

The final determination of this study is that the AERS 500 will be up to the task of replacing the ASRS 300. Referring back to Tables 5 and 6, the 8th column is the column that will bear the final scrutiny. By comparing the averages and the differences of the ions run under each suppressor, it was clear to see that the transition to the new suppressor would not negatively affect any future experiments using the ICS. The next step would be to determine how to go forward with this study. One route of study would be to investigate other suppressors. For instance, the suppressor in the study was an electrolytically regenerated suppressor whereas a chemically regenerated suppressor could be utilized to determine if there is a variation in the resultant chromatograms.

Another avenue would be to couple the ICS with a RFIC-ER system, which is a Reagent-Free Ion Chromatography-Eluent Regeneration system, and would help to recover more analyte from the suppressor since the suppressor waste is eluent [10].

The advancement of suppressors once focused on continuity, capacity, and making the suppressor easier to use. Going forward, there should hopefully be improvement in backpressure tolerance, peak efficiency, and full electrolytic regeneration [10].

Figure 1--Ion Chromatography System-1000 High Performance Liquid Chromatography (ICS-1000 HPLC)

Figure 2--Automated sampler

Figure 3--Column housing (exterior)

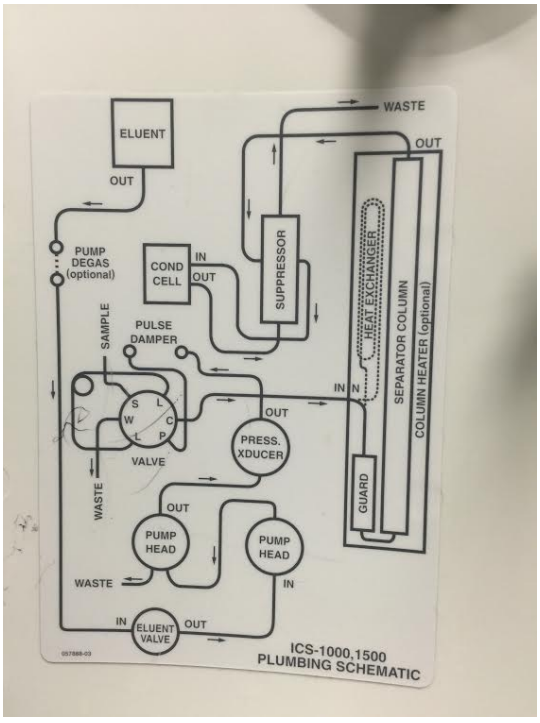

Figure 4--Plumbing schematic

Figure 5--Column housing (interior)

References:

1. Jenke, D. (2011). Application of ion chromatography in pharmaceutical and drug analysis. Journal of chromatographic science, 49(7), 524-539.

2. Joachim, W. (1986). Handbook of ion chromatography. Dionex Corporation. Sunnyvale, California.

3. Tarter, J. G., & Worsfold, P. J. (1988). Ion chromatography (chromatographic science series, vol. 37). Analytica Chimica Acta, 207, 381.

4. Majors, R. E. (2007). Column Pressure Considerations in Analytical HPLC.

5. De Vera, G. A. D., & Espino, M. P. B. (2011). Anions Analysis in Ground and Tap Waters by Sequential Chemical and CO2-Suppressed Ion Chromatography. Science Diliman, 23(1).

6. Maugh 2nd, T. H. (1983). A survey of separative techniques. Science (New York, NY), 222(4621), 259-266.

7. Han, S. Y., Ming, X., Qi, Z. C., Sheng, D., & Lian, H. Z. (2010). Retention prediction and hydrophobicity estimation of weak acidic compounds by reversed-phase liquid chromatography using acetic and perchloric acids as ion suppressors. Analytical and bioanalytical chemistry, 398(6), 2731-2743.

8. Rantakokko, P., Mustonen, S., Yritys, M., & Vartiainen, T. (2004). Ion chromatographic method for the determination of selected inorganic anions and organic acids from raw and drinking waters using suppressor current switching to reduce the background noise. Journal of liquid chromatography & related technologies, 27(5), 829-842.

9. Swadesh, J. K. (Ed.). (2000). HPLC: practical and industrial applications. CRC Press.

10. Madden, J., Verma, M. (2013). What is the Role of a Next Generation Suppressor? Sunnyvale: Thermo Scientific.

11. Scott, C. D. "High-pressure ion exchange chromatography." Science 186.4160 (1974): 226-233.

12. Small, H. (1989). Ion Chromatography. New York: Plenum Press.

13. Poole, C. F., & Schuette, S. A. (1984). Contemporary practice of chromatography (Vol. 5). Elsevier.

14. Small, H., Liu, Y., & Avdalovic, N. (1998). Electrically polarized ion-exchange beds in ion chromatography: eluent generation and recycling. Analytical chemistry, 70(17), 3629-3635.

15. Strong, D. L., & Dasgupta, P. K. (1989). Electrodialytic membrane suppressor for ion chromatography. Analytical Chemistry, 61(9), 939-945.