

Novel Uses of Ion Chromatography Suppressors Prior to ESI-MS Detection

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ABSTRACT

Ion chromatography is a mode of liquid chromatography wherein ionized analytes, anionic or cationic, are separated using ion-exchange-based analytical columns and (usually) non-volatile strong base or strong acid eluents. The basic or acidic eluents are neutralized using a suppressor in H⁺ or OH⁻ form. The suppressor replaces the cations or anions that are present in the column effluent from both the eluent components and the sample. For example, a sodium chloride ion can be separated on an anion-exchange column using KOH eluent. The K⁺ and Na⁺ are replaced by H⁺ in the suppressor, so the chloride is detected as HCl rather than NaCl and the background is H₂O. This is especially useful if the detector is based on electrical conductivity.

A suppressor could also be used to supply other ions to change the salt form of analytes. For example, we can place a standard acid-form cation-exchange membrane suppressor in series with a salt-form cation-exchange membrane suppressor. As the anions exit the analytical column they are converted to the acid form in the first suppressor. The effluent can either enter the mass spectrometer for detection of the anions or enter the salt-form suppressor so that the anions are converted to the salt form. Second-suppressor counterions that have been investigated include sodium, ammonium, and tetrabutyl-ammonium.

For example, the response in negative electrospray ionization (-ESI) can be enhanced for certain analytes such as propionate. Propionate sees improved electrospray response depending which counterion is present. The -ESI response at *m/z* 73 for propionate is increased three-fold when the counterion is sodium, relative to the response when the counterion is mostly H⁺. If the counterion is NH₄⁺ the signal response is increased six-fold, with a similar increase in s/n ratio. The increased response is due to at least two factors: improved electrospray formation and increased ionization of the weak acid due to the higher pH. The signal of certain anions can also be decreased using this technique by using a counterion that has the ability to form ion pairs. For example, the signal from acetate is decreased more than 90% when the second suppressor supplies TBA⁺.

In this presentation we will discuss some possible uses of post-column ion exchangers to modify analytes and mobile phases in order to improve detection by ESI-MS.

BULK-FLOW ADDITION VS. ION EXCHANGE FOR POST-COLUMN MODIFICATIONS

The ionic content of the effluent from the chromatography system can be modified by the bulk addition of reagents through a mixing tee or via ion exchange of the analyte (and eluent) counterions across an ion-exchange membrane. If such reagents are used with ion-exchange membranes then only the counterions are exchanged. In addition, there is no increase in flow rate due to a bulk addition of reagent. Salts can also be added using ion exchange, without the addition of the involatile counterions.

Analyte	Area counts AMMS (NH ₄ ⁺)/mixing T	S/N AMMS (NH ₄ ⁺)/mixing T
Chloride	1.8	1.5
Formate	0.85	1.4
Acetate	1.8	0.5
Propionate	1.9	0.95
α -Hydroxybutyrate	1.6	0.98
Chloroacetate	1.7	1.5

Table 1: Comparison of peak area counts and s/n ratio for systems using an AMMS[®] III or a mixing tee to add ammonium ion.

In the experiment summarized in Table 1, peak area counts and signal-to-noise ratios were compared for systems utilizing bulk addition of ammonium hydroxide thru a mixing tee and systems using a cation-exchange AMMS III suppressor. Ammonium hydroxide was added to the post-column methanol. Using this technique, the flow rates for the two experiments were identical. The use of the mixing tee required the use of a pump which can also add noise to the overall system. These results show the peak area counts to be generally higher using an ion-chromatography suppressor compared to the mixing tee technique. This is probably due to less signal suppression in the electrospray from a lower ionic strength. Using SIM detection for this work, background and noise vary at each channel. Using the AMMS III, for example, the background at *m/z* 59 is higher than when the mixing tee is used, as reflected in the lower s/n for acetate determination. All other s/n ratios were equal or better using the AMMS III to change ionic form.

Use of a Single Membrane-Based Ion-Exchange Suppressor Prior to ESI-MS Detection

- Standard IC-MS or IC-MS/MS using ion-exchange suppressors
 - Neutralize and desalt column effluent prior to electrospray ionization
 - Change counterion of analytes to H^+ or OH^-

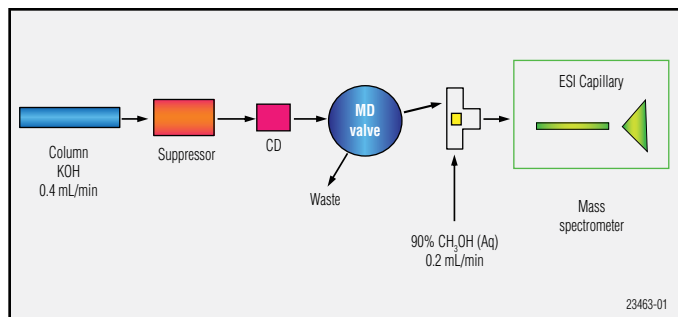


Figure 1. Schematic of standard IC-MS or IC-MS/MS system.

The membrane suppressor, shown in Figure 2, contains two cation-exchange membranes that remove the cations from the eluent and sample matrix. The analyte counterion is also removed and replaced by H^+ , generated by the electrolysis of externally-supplied water. The schematic shows the device configured for analysis of anions, X^- . A device configured for the analysis of cations and amines uses anion-exchange membranes and the regenerating ion is OH^- produced by the electrolysis of water.

Methanol or acetonitrile is added through a mixing tee, shown in Figure 1, to improve volatility and sensitivity for some IC-MS and IC-MS/MS methods. Detection limits for small aliphatic carboxylic acids, perchlorate, and haloacetic acids are improved five to ten times by the addition of post-column solvent.



Figure 2. Schematic of ASRS® electrolytic suppressor.

Figures 3 and 4 show two important applications of IC-MS/MS and IC-MS using standard electrolytic suppressors for eluent neutralization and replacement of X^- ions with H^+ . The separation and detection of haloacetic acids along with the matrix diversion of chloride, sulfate, and carbonate is shown in Figure 3. The detection limits using internal standards are less than 400 ppb for chloroacetate, bromoacetate, dichloroacetate, dibromoacetate, and trichloroacetate. The detection limits are less than 1 ppb for bromochloroacetate, bromodichloroacetate, chlorodibromoacetate, and tribromoacetate. The linearities are greater than 90% for all of these anions in a salty matrix of 250 ppm each of chloride and sulfate, 100 ppm ammonium chloride, 20 ppm nitrate, and 150 ppm carbonate.

The determination of perchlorate in lettuce shown in Figure 4 was run using U.S. EPA Method 332.0.

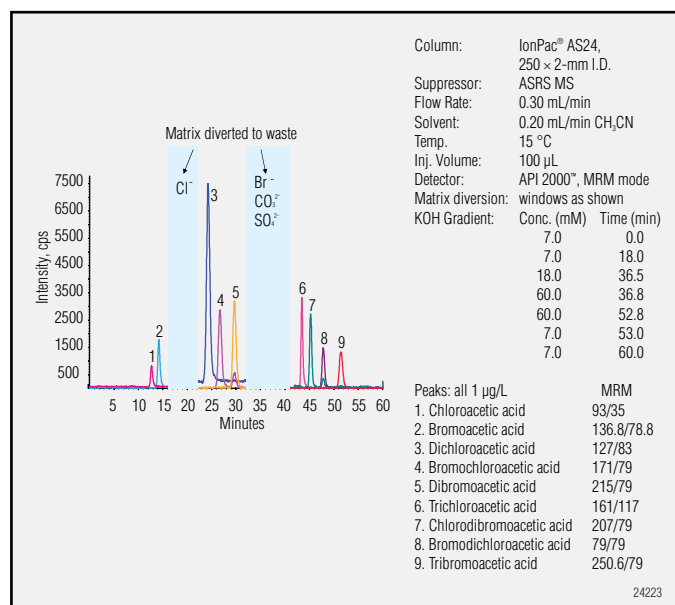


Figure 3. Determination of haloacetic acids by direct injection using IC-MS/MS.

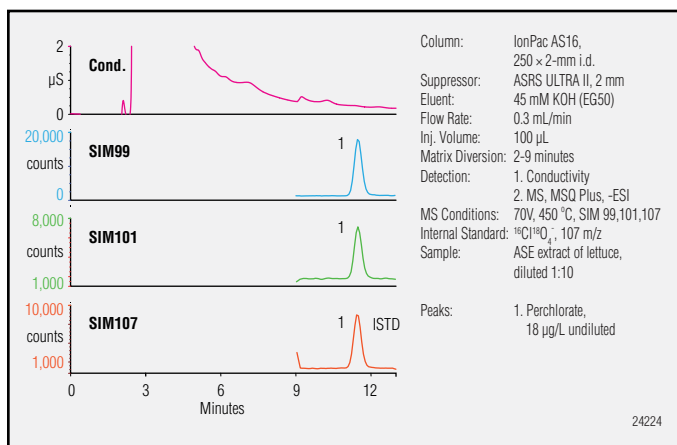


Figure 4. Determination of perchlorate in lettuce using a standard IC-MS system.

ION EXCHANGE DESALTER TECHNOLOGY

Ion-exchange suppressor technology may also be used as a desalter for oligosaccharide separations. A high capacity version of the device is used to replace the Na^+ from a sodium acetate eluent with H^+ prior to detection using amperometry with fraction collection and MALDI-TOF mass spectrometry. A schematic of the system layout is shown in Figure 5.

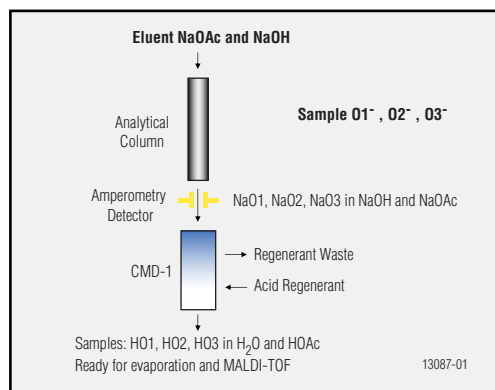


Figure 5. Diagram showing use of a carbohydrate membrane desalter prior to MADLI-TOF mass spectrometry.

USE OF TWO MEMBRANE-BASED ION-EXCHANGE SUPPRESSORS PRIOR TO ESI-MS DETECTION

- First suppressor neutralizes and desalts the column effluent
- Second suppressor replaces the analyte counterion with selected ions
 - Increases sensitivity in ESI for some analytes
 - Decreases sensitivity in ESI for other ions

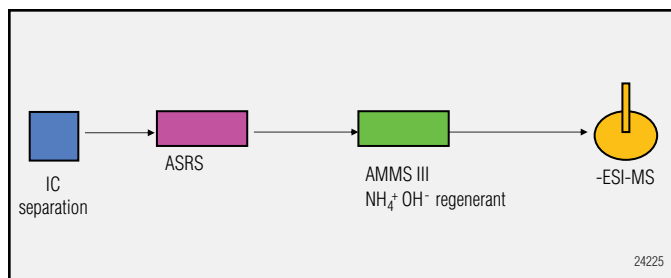


Figure 6. Flow diagram showing the use of two membrane-based ion-exchange suppressors prior to ESI-MS detection.

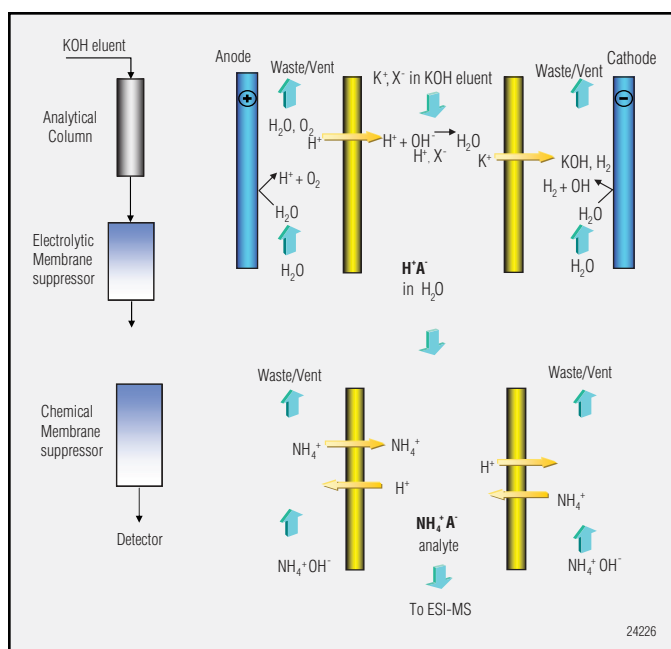


Figure 7. Schematic of two membrane suppressors used in series.

Figure 6 shows the system utilizing two suppressors in series. Using this arrangement, the eluent cation K^+ , and other cations, are removed in the first suppressor. The replacement cation H^+ neutralizes the OH^- from the eluent. The acid-form analytes enter the second suppressor in a background of water. The H^+ is then exchanged with the cation provided in the second suppressor, with the goal of improving sensitivity. The replacement cation chosen depends on the properties of the analytes and matrix anions.

Figure 7 provides details of the electrolytic suppressor and the chemical suppressor. The H^+ for the exchange with cations in the analytical stream is provided by the electrolysis of water. The water is provided from an exterior source such as a pressurized bottle, because the normal recycle of eluent to the regenerant chambers is unavailable after passing through the mass spectrometer. The cation, such as NH_4^+ , Na^+ , or TBA^+ , for the salt conversion of the analytes in the AMMS III suppressor is supplied by reservoir.

IMPROVED ESI-MS DETECTION OF WEAK ACIDS IN THE SALT FORM

Figure 8 shows the fragmentation of two weak carboxylic acids, acetate and propionate, when they are in acid form. The fragmentation of chloroacetate to chloride is independent of salt form and reproducible. The conditions for this chromatogram are provided in Table 2. The extent of fragmentation of weak carboxylic acids is dependent on the

concentration of non- H^+ cations exiting the suppressor. This means that quantification of weak acids is affected by the operation of the suppressor as evidenced by background conductivity. When the suppressor is operating with a background conductivity of less than approximately 0.5 μS , fragmentation of weak acids is observed, affecting quantification. In the chromatograms shown in figure 8, the background conductivity exiting the SRS[®] suppressor was 0.4 μS .

This fragmentation only occurs when the acid analytes are in the acid form. This effect is remedied by converting the weak acid analytes to a salt form using a cation-exchange membrane device, the AMMS III, as shown in Figure 7. The use of the SRS and AMMS III suppressors in series is convenient since the SRS removes involatile eluent ions and counterions while the AMMS III converts the analytes to a desirable salt form.

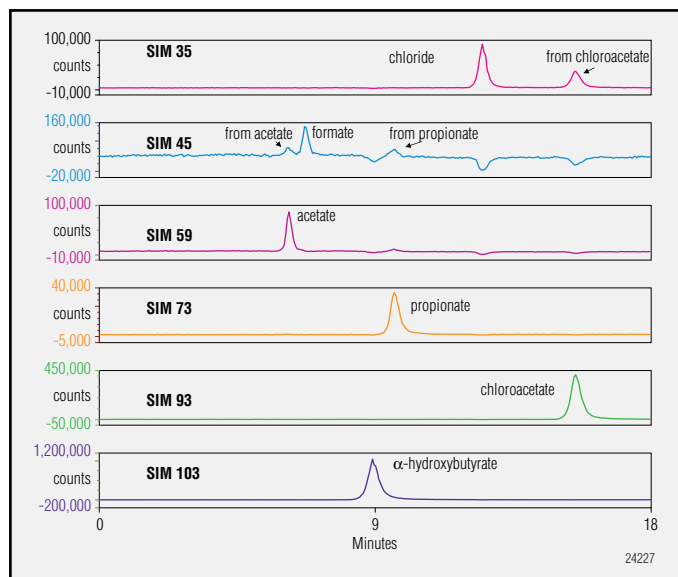


Figure 8. SIM chromatograms showing the fragmentation of aliphatic carboxylic acids. System used is shown in Figure 1.

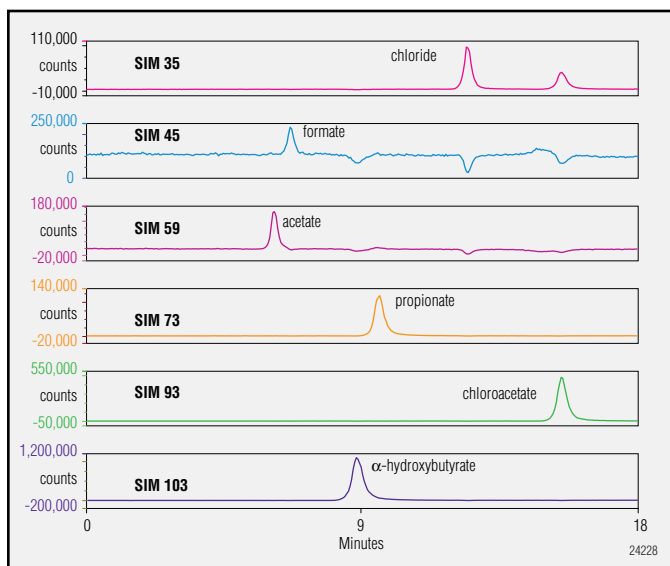


Figure 9. SIM chromatograms showing no fragmentation of aliphatic carboxylic acids. System used is shown in Figure 6.

Table 2. Conditions for Figures 8 and 9

Column	IonPac AS15, 3 × 150 mm
Suppressors	ASRS ULTRA II, 2-mm, external water, 40 mA; AMMS III
Regenerant	3.5 mM ammonium hydroxide, tetrabutylammonium hydroxide, sodium hydroxide
Eluent	KOH, 8 mM for 0-8 min then 8 to 30 mM to 15 min, using eluent generator
Injection volume	25 μL
Flow rate	0.4 mL/min
Detection	ESI-MS using MSQ™ Plus single quadrupole mass spectrometer
Post-column solvent	90/10 methanol/water, 0.2 mL/min
MS conditions	-ESI, 400 °C, 3 kV
Analyses: All 1 mg/L	Cone voltage (V)
Chloride	50
Acetate	40
Formate	40
α -Hydroxybutyrate	50
Propionate	40
Chloroacetate	40

Acid Analyte (all 1 mg/L, 25 μL)	pK _a	Area counts H ⁺ form	S/N H ⁺ form	% Recovery n=5	Area counts NH ₄ ⁺ form	S/N NH ₄ ⁺ form	% Recovery n=5
HCl	<0	28,000	95	106	13,500	83	102
Formic	3.75	20,400	4	110	15,700	10	102
Acetic	4.76	17,500	59	76	39,150	50	105
Propionic	4.87	12,900	60	51	74,280	360	107
α -Hydroxybutyric	3.65	46,500	120	94	254,000	258	106
Chloroacetic	2.87	19,200	150	95	90,180	35	105

The data in Table 3 shows raw peak area counts, signal-to-noise ratio and recovery for the test acids listed. Percent recovery is calculated as $100(PA5-PA1/PA1)$ where PA5 is the peak area in the fifth injection and PA1 is the peak area in the first injection. During system startup with the system shown in Figure 1, the peak areas of the acetate and propionate decrease over time due to fragmentation of the acid form. As a higher percentage of counterions are replaced by H⁺ the extent of fragmentation increases, as seen in the increase of peaks at m/z 45. At full suppression, the appearance of the additional peaks at m/z 45 also makes peak integration less reliable, as shown in Figure 9. The fragmentation of chloroacetate is stable over time, and has a higher s/n in the acid form as compared to the ammonium form. Conversion of the weak acids to a salt form minimizes fragmentation and insures good reproducibility

SUMMARY

Ion chromatography suppressors are ion-exchange-based devices that are used to both neutralize the eluent and replace counterions. By using the electrolytic SRS and chemical AMMS III suppressors in series, the salt form of analytes can be changed after the eluent is desalted to improve detection by ESI-MS. The SRS removes involatile eluent ions and counterions before the AMMS III converts the analytes to a more desirable salt form.

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