A lab-scale approach for the Anion Analysis kit

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In this technical note, we demonstrate the preparation of all reagents needed to run anion analysis and show that they can be used to replicate what was found in the previously available Anion Analysis Kit from SCIEX (Figures 1 and 2).[1]

Due to sales and supply chain impacts, we no longer provide the Anion Analysis Kit (SCIEX, PN A53537). To continue to support scientists performing anion analysis, we instead provide this instruction to prepare all necessary reagents in the lab and demonstrate comparability of results.

The Anion Analysis Kit permits the analysis of small inorganic anions and organic acids, which are often UV-transparent. For this reason, the separation buffer contains a chromophore and detection is achieved with an indirect mode.

The separation method is performed under reverse polarity so that the negatively charged ions migrate toward the anode (the positively charged electrode). In addition, the capillary is dynamically coated with a polycation, which reverses the electro-osmotic flow (EOF) toward the anode, thus reducing the separation time while maximizing migration time reproducibility.

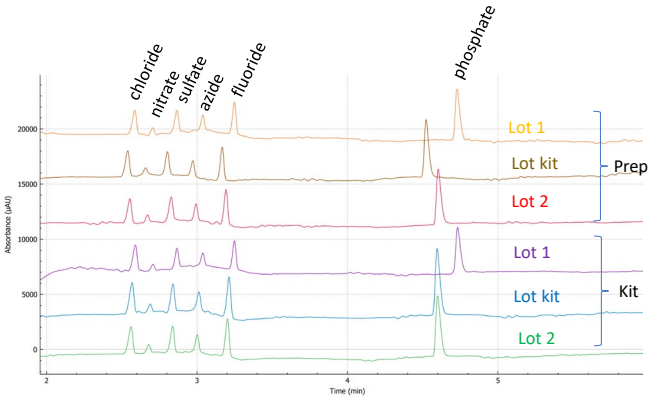

Figure 1. Electropherograms of inorganic test mix. The top 3 traces display the example electropherograms of the inorganic test mix that was prepared with the procedure described in this technical note. The bottom 3 traces display the results of the inorganic test mix acquired from the commercial kit. All samples were analyzed using 3 lots of separation buffer.

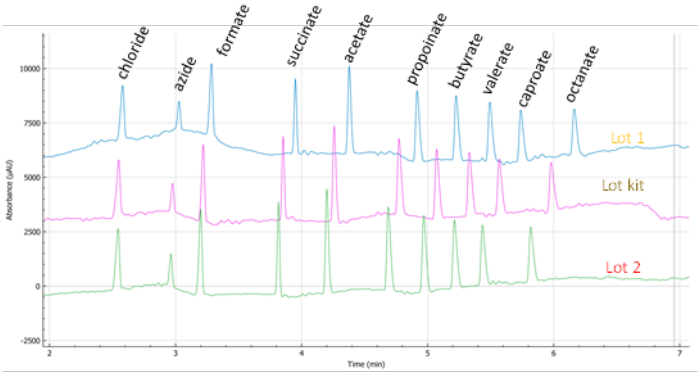

Figure 2. Electropherograms from the kit organic test mix. The traces display the example electropherograms of the organic test mix acquired from the commercial kit. Samples were analyzed using 3 lots of separation buffer.

Key features

- Detailed and complete instructions to prepare anion analysis reagents and test mix standards
- Comparable results with the previously available, commercial Anion Analysis Kit
- Robust and repeatable anion analysis
- Suitable for both inorganic and organic anions
- Indirect absorbance analysis allows for a wide range of sample compatibility

Methods

Materials: The 0.1N NaOH solution (PN 338424) and water (PN C48034) used in this assay were from SCIEX. The 0.1N hydrochloric acid solution was purchased from LabChem (PN LC152204). All other chemicals needed were ordered from Sigma the detailed part number are listed in Table 1.

Table 1. Reagents needed to make anion analysis solutions.

Chemical Name	PN
Sodium nitrate	*S5506-250G*
Sodium sulfate	*238597-25G*
Sodium fluoride	*201154-100G*
Sodium azide	*71289-5g*
Potassium chloride	*P5405-250g*
Potassium dihydrogen phosphate	*1048730250*
Sodium phosphate dibasic	*S0876-500g*
Sodium hydroxide	*S5881-500G*
Sodium formate	*71539-500G*
Succinic acid	*S3674-100G*
Sodium acetate trihydrate	*S8625-250G*
Propionic acid	*402907-100ML*
Butyric acid	*B103500-5ML*
Valeric acid	*240370-5ML*
Hexanoic acid	*153745-2.5G*
Octanoic acid	*C2875-10ML*
Sodium carpylate	*C5038-100G*
Bis-tris	*B7535-25G*
2,3 Pyridinedicarboxylic acid	*P63204-25g*
Polyethylenimin (50% wt)	*181978-5G*
Ethylene glycol	*324558-100ML*

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Instrumentation: The PA 800 Plus system was from SCIEX (Framingham, MA). The system was configured with a UV detector with a 230 nm filter (SCIEX, PN 144433) and a 254 nm filter (SCIEX, PN 144438). Analysis was performed using a 50 cm effective length (60 cm total) bare-fused silica capillary (BFS capillary) with 75 µm ID (SCIEX, PN 338454) with an 800 µm aperture (SCIEX, PN 144711). Instrument control was achieved using the 32 Karat software package. After the experiment was completed, data were exported into ASCII format and imported into BioPhase 8800 software for processing.

Reagent preparation: All reagent solutions listed below were prepared using a graduated cylinder with the proper volume unless stated otherwise.

Polyethylenimin (1% wt) stock solution preparation: Dilute 200 mg of the polyethylenimin (50% wt) into a final volume of 10 mL with filtered deionized water. Mix well.

Anion coating: In a graduated cylinder with 70 mL of filtered deionized water, while stirring, add 1165 mg of bis-tris, 460 mg of 2,3 pyridinedicarboxylic acid and 100 µL of the prepared polyethylenimin (1%wt) stock solution. Stir until all solids are fully dissolved. Add water until the total volume reaches 100 mL.

Conditioner-Na: 0.1N NaOH solution.

Anion acid rinse: 0.1N HCl solution.

Anion internal standard: Dissolve 50 mg of sodium caprylate into 10 mL filtered deionized water.

Anion separation buffer: In a graduated cylinder with 70 mL of filtered deionized water, while stirring, add 3450 mg of ethylene glycol with a pipette, 1048.5 mg of bis-tris, 414 mg of 2,3 pyridinedicarboxylic acid and 100 µL of the prepared polyethylenimin (1% wt) stock solution. Stir until all solids are fully dissolved. Add water until the total volume reaches 100 mL.

Test mix stock solution: In a 15 mL centrifuge tube with volume markers, add 7 mL of filtered deionized water and 100 mg of sodium azide and sonicate to dissolve all solid into solution. Add water until the total volume reaches 10 mL. Cap the tube and invert multiple times to mix the solution well. Repeat this process for the following chemicals: 1) potassium chloride, 2) sodium formate, 3) sodium acetate trihydrate, 4) succinic acid, 5) propionic acid, 6) butyric acid, 7) valeric acid, 8) hexanoic acid, 9) sodium nitrate, 10) sodium sulfate, 11) sodium fluoride, 12) potassium dihydrogen phosphate and 13) sodium phosphate dibasic. This process yields a total of 14 stock solutions.

Anion organic test mix: In a graduated cylinder with 70 mL of filtered deionized water, while stirring, add 2 mL of 0.1N sodium hydroxide and the test mix stock solutions. The following amounts of test mix stock solutions must be added: 154 µL of sodium azide, 414 µL of potassium chloride, 295 µL of sodium formate, 454 µL of sodium acetate, 200 µL of succinic acid, 200 µL of propionic acid, 200 µL of butyric acid, 200 µL of valeric acid, and 200 µL of hexanoic acid. Then, add 2 µL of octanoic acid directly from the reagent bottle. Mix everything well. Remove the stir bar and add water until the total volume reaches 100 mL. ***Note*: The water solubility of octanoic acid is 6.8 mg/100 mL. Therefore, the compound must be added directly to the final test mix. After the 100 mL final volume is adjusted, transfer the liquid into a plastic bottle and mix with sonication to avoid erroneous analysis (wide peaks). The solubility is not enough for a 2-step dilution procedure.**

Anion inorganic test mix: In a graduated cylinder with 70 mL of filtered deionized water, while stirring, add in 200 µL of sodium azide, 419 µL of potassium chloride, 275 µL of sodium nitrate,

288 µL of sodium sulfate, 200 µL of sodium fluoride, 136 µL of potassium dihydrogen phosphate and 568 µL of sodium phosphate dibasic. Stir until all solids are fully dissolved. Add water until the total volume reaches 100 mL.

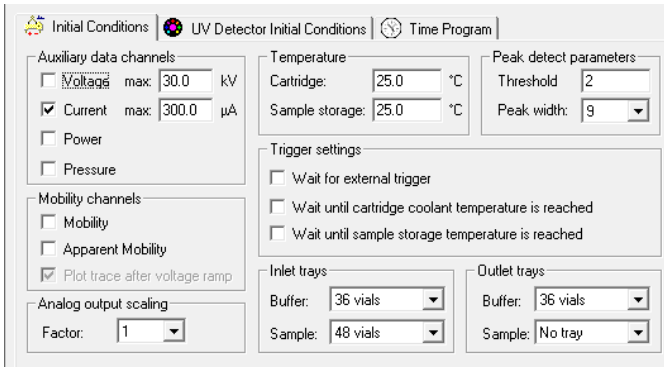
Rinse solution: CE grade water (PN C48034).

Sample preparation:

Depending on the concentration of the analytes, the sample should be injected as-is or diluted. Dilution should be done so that the final concentration of the sample anions is between 5 ppm and 200 ppm. Special care should be taken to verify the pH of the sample, which should be above pH 5. A 50mM NaOH solution can be used to dilute the sample and adjust its pH value.

The anion internal standard (I.S.) consists of 30mM sodium octanoate (sodium caprylate), which is the equivalent of 4296 ppm of octanoate ion. The I.S. can be used in the quantification of the sample anions. To use it, dilute the I.S. by a factor of 50 with the sample. For example, mix 4 µL of I.S. with 200 µL of sample to yield 86 ppm of octanoate ion.

A) Initial conditions for all anion separation methods



B) Detector conditions for all anion separation methods

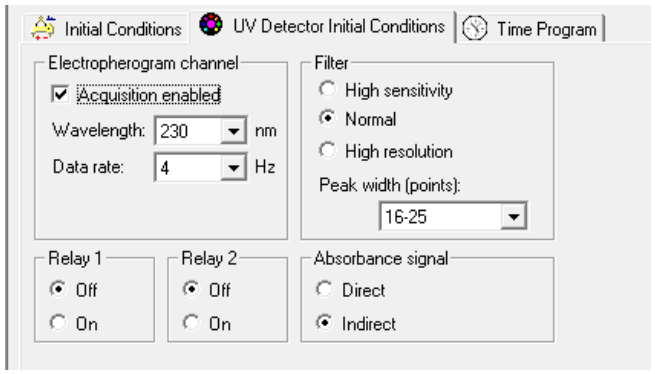
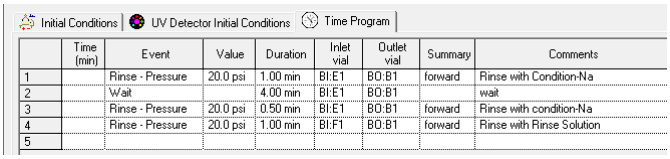


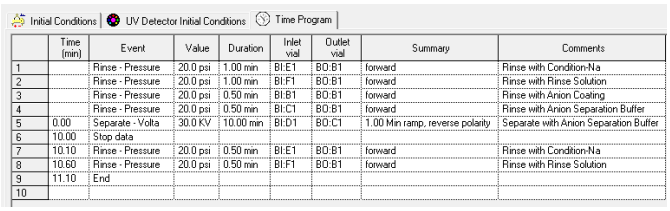
Figure 3. Initial conditions and detector conditions for all anion analysis methods. Some ions, such as nitrate, absorb at short wavelengths. In these situations, the wavelength (nm) shown in panel B should be set to 254 nm.

A) Time program for capillary conditioning method



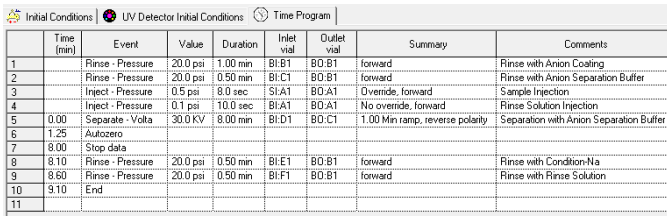
	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	20.0 psi	1.00 min	BI:E1	BO:B1	forward	Rinse with Condition-Na
2		Wait		4.00 min	BI:E1	BO:B1		wait
3		Rinse - Pressure	20.0 psi	0.50 min	BI:E1	BO:B1	forward	Rinse with condition-Na
4		Rinse - Pressure	20.0 psi	1.00 min	BI:F1	BO:B1	forward	Rinse with Rinse Solution
5								

B) Time program for conditioning method



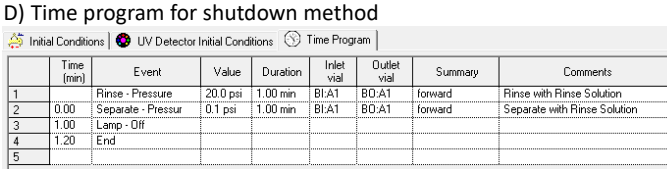
	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	20.0 psi	1.00 min	BI:E1	BO:B1	forward	Rinse with Condition-Na
2		Rinse - Pressure	20.0 psi	1.00 min	BI:F1	BO:B1	forward	Rinse with Rinse Solution
3		Rinse - Pressure	20.0 psi	0.50 min	BI:B1	BO:B1	forward	Rinse with Anion Coating
4		Rinse - Pressure	20.0 psi	0.50 min	BI:C1	BO:B1	forward	Rinse with Anion Separation Buffer
5	0.00	Separate - Volta	30.0 KV	10.00 min	BI:D1	BO:C1	1.00 Min ramp, reverse polarity	Separate with Anion Separation Buffer
6	10.00	Stop data						
7	10.10	Rinse - Pressure	20.0 psi	0.50 min	BI:E1	BO:B1	forward	Rinse with Condition-Na
8	10.60	Rinse - Pressure	20.0 psi	0.50 min	BI:F1	BO:B1	forward	Rinse with Rinse Solution
9	11.10	End						
10								

C) Time program for separation method



	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	20.0 psi	1.00 min	BI:B1	BO:B1	forward	Rinse with Anion Coating
2		Rinse - Pressure	20.0 psi	0.50 min	BI:C1	BO:B1	forward	Rinse with Anion Separation Buffer
3		Inject - Pressure	0.5 psi	8.0 sec	SI:A1	BO:A1	Override, forward	Sample Injection
4		Inject - Pressure	0.1 psi	10.0 sec	BI:A1	BO:A1	No override, forward	Rinse Solution Injection
5	0.00	Separate - Volta	30.0 KV	8.00 min	BI:D1	BO:C1	1.00 Min ramp, reverse polarity	Separation with Anion Separation Buffer
6	1.25	Autozero						
7	8.00	Stop data						
8	8.10	Rinse - Pressure	20.0 psi	0.50 min	BI:E1	BO:B1	forward	Rinse with Condition-Na
9	8.60	Rinse - Pressure	20.0 psi	0.50 min	BI:F1	BO:B1	forward	Rinse with Rinse Solution
10	9.10	End						
11								

D) Time program for shutdown method



	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	20.0 psi	1.00 min	BI:A1	BO:A1	forward	Rinse with Rinse Solution
2	0.00	Separate - Pressur	0.1 psi	1.00 min	BI:A1	BO:A1	forward	Separate with Rinse Solution
3	1.00	Lamp - Off						
4	1.20	End						
5								

Figure 4. Time program conditioning and separation methods in 32 Karat software. A) Conditioning of a brand-new capillary. B) Conditioning at the beginning of a sequence. C) Separation of the standards in the kit. D) Shutdown and capillary storage.

PA 800 Plus system method: Figure 3 shows the initial conditions (3A) and UV detector initial conditions (3B). Figure 4 shows the time program to condition a brand-new capillary (4A), the method to condition at the beginning of a sequence (4B), the method to separate the standard in the kit (4C) and assay/instrument shutdown (4D).

Instrument preparation:

Set up and configure the instrument with the UV detector and 230 nm filter. Refer to the PA 800 Plus Pharmaceutical Analysis System Maintenance Guide for additional information.[2]

If installing a new capillary, run the new capillary rinse condition (Figure 4A), before running a sequence. Otherwise create a sequence that starts with the conditioning method (Figure 4B),

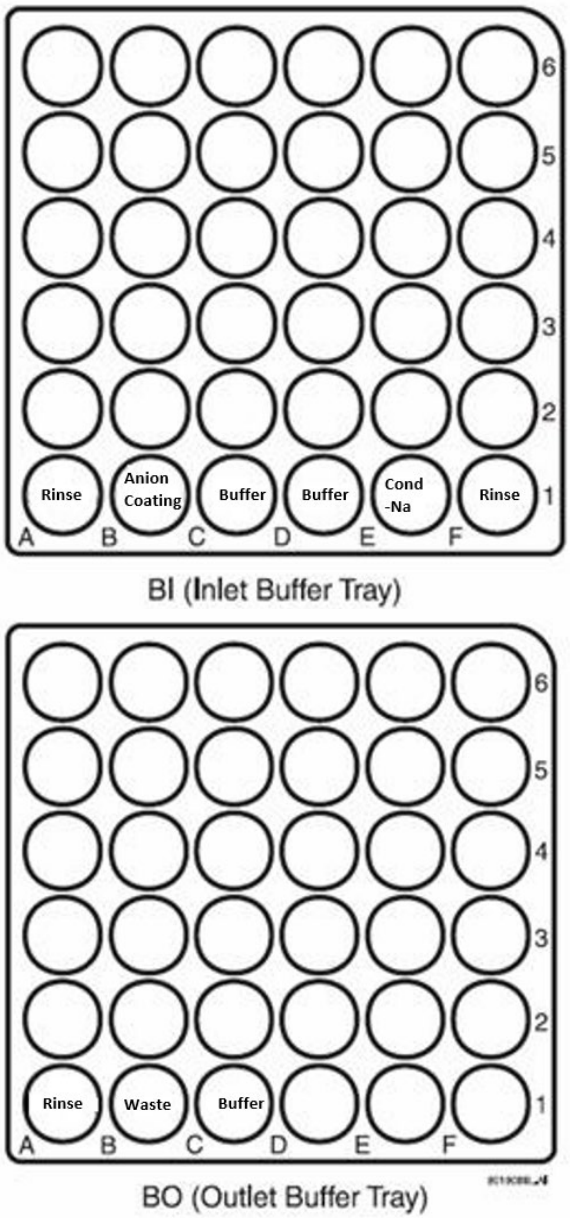


Figure 5. Buffer tray configuration for anion analysis. Fill each vial listed, except waste, with 1.5 mL of the specified solution. Fill the waste vial in the outlet buffer tray with 0.7 mL of CE-grade water. Note: When analyzing fluoride and/or phosphate ions, fill a vial with the anion acid rinse and place it in position F1 of the inlet buffer tray. The use of the anion acid rinse is required when running the anion inorganic test mix to avoid peak tailing.

followed by the separation method for sample and standards (Figure 4C) and the shutdown method (Figure 4D).

Prepare buffer trays according to the buffer tray map shown in Figure 5 and adjust the buffer vial numbers based on the sequence created. Each buffer vial is good for 12 injections or as demonstrated to be appropriate for the samples to be analyzed. After use, store the capillary on the instrument or in the original capillary storage box, with both ends submerged in rinse solution. Do not allow the capillary ends to dry because the capillary may become plugged or damaged if stored incorrectly.

Data processing: Data files were exported in ASCII format and imported into the data analysis software modules of the BioPhase software.

Results and Discussion

Experimental design: Two different scientists followed the above protocol and prepared 2 lots of separation buffer. The test mix was only prepared by 1 scientist and tested using all 3 lots of separation buffer and buffer solutions. We evaluated the previously available commercial kit and the prepared lots 1 and 2 of the separation buffer in a single sequence. Therefore, all samples were run on the same cartridge and on the same system to demonstrate the consistency and the reliability of the preparation protocol. Triplicate injections were performed with the test mix prepared in the lab and the test mix acquired from the kit.

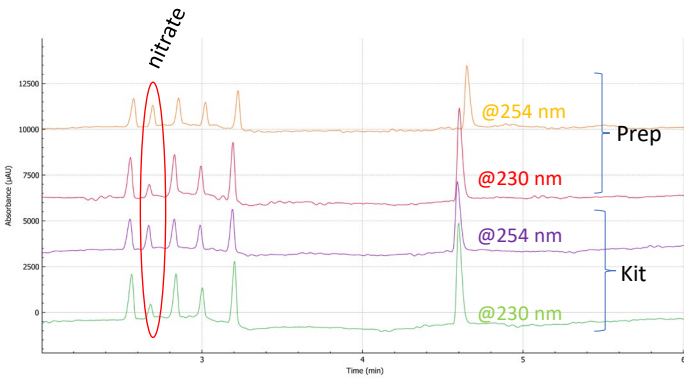


Figure 6. Electropherograms from the inorganic test mix with the 2 detection wavelengths using a single prepared buffer lot. The top 2 electropherograms display the results of the inorganic test mix prepared according to the procedure described in this technical note. The bottom 2 traces display the results of the inorganic test mix acquired from the commercial kit.

Table 2. Signal comparison for nitrate ions at 230 and 254 nm. Average values were calculated from 6 injections of both the prepared and commercially available inorganic test mixes.

Wavelength	Average height	Average S/N P-P	Average S/N RMS
230 nm	*593.85*	*6.30*	*25.04*
254 nm	*1145.61*	*10.48*	*41.43*

Inorganic test mix detection wavelength: As noted in the original kit application guide,[1] a detection wavelength of 254 nm provides an optimal detection signal for ions that absorb in the short UV range, such as nitrate. The inorganic test mix was analyzed with both the 230 and 254 nm filters. The results are shown in (Figure 6). When detecting at 254 nm, the nitrate ions show improved peak height and a 4x improvement in the signal-to-noise ratio (Table 2).

Comparability of the separation buffers: The separation performance and repeatability of the separation buffer from the commercial kit and the 2 lots prepared in the lab were compared. The test mix from the commercial kit was analyzed using 3 lots of buffers in a single sequence. Electropherograms of the inorganic test mix are shown in Figure 1. Similar profiles were observed for the prepared inorganic test mix and that from the kit. Additionally, the sample was analyzed using the different separation buffers, which showed comparable quantification results (Tables 3 and 4). The % difference between the kit buffer and the 2 lots of prepared buffers in average peak MT was less than 4% and the average corr. area% were less than 7% for all ions included.

Table 3. Inorganic test mix from kit separation using the kit buffer.

	Average MT	%RSD MT	Average corr. area%	%RSD corr. area%	Resolution
Chloride	2.61	1.16	23.14	12.08	NA
Nitrate	2.73	1.37	4.38	22.96	2.37-3.14
Sulfate	2.89	1.46	14.32	3.54	3.37-4.25
Azide	3.08	1.76	9.83	27.00	3.49-4.95
Fluoride	3.29	1.98	20.39	4.45	4.13-5.53
Phosphate	4.82	4.04	27.95	9.36	25.87-34.14

Table 4. Inorganic test mix from kit separation using the prepared buffer. Average values were calculated from 6 injections and with 2 prepared buffer lots.

	Average MT	%RSD MT	Average corr. area%	%RSD corr. area%	Resolution
Chloride	2.58	0.60	21.50	7.80	0-0
Nitrate	2.70	0.67	4.16	15.27	2.35-2.83
Sulfate	2.85	0.72	15.54	6.53	3.37-3.98
Azide	3.02	0.81	10.54	6.87	3.42-3.94
Fluoride	3.22	0.96	19.68	3.35	4.34-4.89
Phosphate	4.64	1.79	28.58	4.05	25.84-29.48

Table 5. Analysis results of organic test mix using the kit buffer.

	Average MT	%RSD MT	Average corr. area%	%RSD corr. area%	Resolution
Chloride	2.53	0.00	14.40	6.42	0-0
Azide	2.94	0.08	5.17	1.18	8.47-8.75
Formate	3.17	0.08	13.83	0.22	5.27-5.33
Succinate	3.77	0.05	9.81	5.31	14.61-14.67
Acetate	4.15	0.10	12.59	1.86	9.15-9.21
Propoinate	4.62	0.05	11.30	4.08	9.20-9.33
Butyrate	4.90	0.10	9.70	0.45	4.75-4.87
Valerate	5.13	0.05	8.71	1.87	3.88-4.06
Caproate	5.35	0.09	7.78	4.08	3.55-3.64
Octanate	5.72	0.07	6.71	1.04	5.88-5.99

Table 6. Analysis results of organic test mix using the prepared buffer. Average values were calculated from 6 injections and with 2 prepared buffer lots.

	Average MT	%RSD MT	Average corr. area%	%RSD corr. area%	Resolution
	Average of MT	%RSD for MT	Average of Corr. Area%	%RSD for Corr. Area%	Resolution
Chloride	2.56	0.75	14.94	3.33	0-0
Azide	3.00	1.03	5.85	8.91	8.59-9.24
Formate	3.24	1.20	13.69	2.40	5.23-5.68
Succinate	3.88	2.05	9.54	1.69	15.03-15.82
Acetate	4.29	1.82	12.73	1.47	9.27-10.17
Propoinate	4.80	2.05	10.98	2.32	9.42-10.40
Butyrate	5.10	2.16	9.68	1.86	4.92-5.40
Valerate	5.43	2.05	8.66	2.03	4.31-4.49
Caproate	5.59	2.36	7.44	2.71	3.60-4.02
Octanate	6.00	2.55	6.40	6.77	5.90-6.83

Electropherograms of the organic test mix from the commercial kit are shown in Figure 2. The sample analyzed using the different separation buffers showed comparable quantification results (Tables 5 and 6). The % difference between the kit buffer and the 2 lots of prepared buffers in average peak MT was less than 5% and the average corr. area% was less than 5.0% for all ions except azide. Due to the high absorbance of azide at short

UV wavelengths, the reported corr. area% was the smallest compared to all other peaks, resulting in a relatively high % difference of 10%.

Comparability of prepared inorganic test mix: The inorganic test mixes prepared in the lab and from the commercial kit were analyzed using the same lot of prepared separation buffer. Comparable results were achieved in both test mix standards.

The % difference between the kit test mix and the prepared test mix in average peak MT was less than 0.5% and the average corr. area% was less than 5% for all ions included. (Figure 1 and Tables 4 and 7)

Table 7. Analysis results of inorganic test mix prepared following the technical note instructions.

	Average MT	%RSD MT	Average corr. area%	%RSD corr. area%	Resolution
chloride	2.55	0.41	20.01	4.33	0-0
Nitrate	2.67	0.47	4.89	4.72	2.39-2.53
Sulfate	2.83	0.44	16.44	7.78	3.48-3.54
Azide	2.99	0.43	10.29	2.16	3.54-3.59
Fluoride	3.19	0.39	19.95	1.60	4.36-4.45
Phosphate	4.59	0.50	28.41	3.66	26.62-27.63

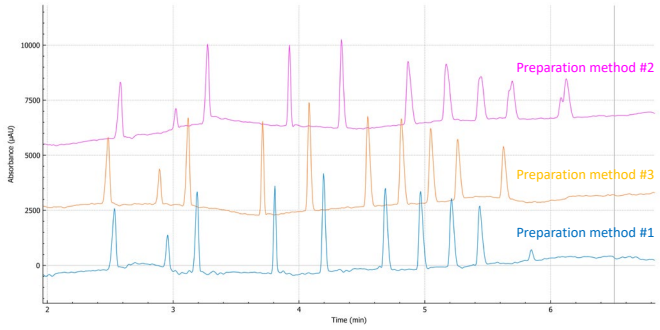


Figure 7. Electropherograms of organic test mix analyzed with kit buffer and using 3 different preparation methods. The top trace (preparation method #2) was prepared by adding the octanoic acid directly into solution and shaking to mix. The middle trace (preparation #3; final preparation) was prepared by adding the octanoic acid directly and sonicating to mix. The bottom trace (preparation trace #1) was prepared using an octanoic acid stock solution.

Comparability of the prepared organic test mix: The preparation of the organic test mix was not as straightforward as that of the inorganic mix. Three different ways of mixing were evaluated, and variation was observed in the signal intensity and peak shape of the octanate ions (Figure 7). In the first preparation method, a stock solution of 100 mg in 10 mL was prepared the same way as other components and further diluted to make the organic test mix. Using this method, the peak intensity was significantly lower than expected (Figure 7). As noted in the section for organic test mix preparation, the low peak intensity was attributed to the low solubility of octanoic acid in water. In the second preparation method, the octanoic acid was directly added into the final solution and mixed by inverting the volumetric flask several times. The relative corrected peak area matched the expected peak area, however, the peak shape for the octanate ion was asymmetric with a split peak top. In the third preparation method, the solution was sonicated for 10-15 mins after the octanoic acid was added and the volume was adjusted. The third preparation yielded results that matched the expected peak intensity and had good peak shape.

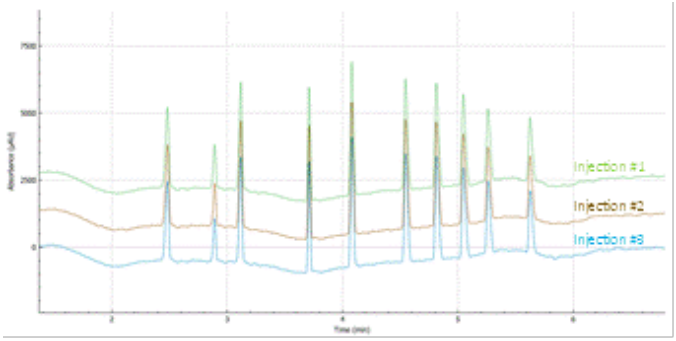


Figure 8. Electropherograms of final preparation organic text mix from three replicate injections.

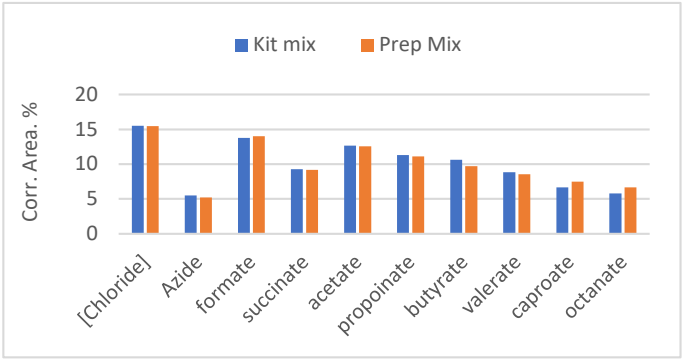


Figure 9. Corrected area % for each ion in the organic test mix from the kit (blue) and the final preparation (orange).

The organic test mixes from the final preparation and those from the commercial kit were analyzed using the separation buffer from the kit. The final preparation showed great assay repeatability between injections (Figure 8) and comparable quantification to the test mix from the commercial kit (Figure 9).

The % differences between the kit test mix and prepared test mix in average peak MT was less than 0.5% and the average corr. area% was less than 10% for all ions included (Figures 2 and 9).

Conclusions

- With the instructions provided, a lab-scale Anion Analysis Kit was created successfully
- No method modifications were needed to use the lab-scale anion analysis reagents to replace the use of the previously available commercial Anion Analysis Kit
- The lab-scale preparation of the reagents provided comparable results compared to the commercial kit

References

1. SCIEX Anion Analysis Kit Instruction Guide, SCIEX, A49108AESCIEX Anion Analysis Kit Instruction Guide, SCIEX, A49108AE
2. PA 800 Plus Pharmaceutical Analysis System, system maintenance Guide, SCIEX, RUO-IDV-05-5519-B

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