A lab-scale approach for the Cation Analysis Kit

Fang Wang, Philip Hoang, and Quincy Mehta
SCIEX, USA

In this technical note, we demonstrate the preparation of all reagents necessary to run cation analysis and show that they can be used to generate results equivalent to those acquired with the previously available Cation Analysis Kit from SCIEX (Figures 1 and 2).[1]

Due to discontinuance, SCIEX no longer provides the Cation Analysis Kit (PN: A53540).[1] To continue to support our customers performing cation analysis, we instead provide the instructions here to prepare all necessary reagents and demonstrate the comparability of the results between the commercial and lab-produced test kits.

The Cation Analysis Kit supports the analysis of small, inorganic cations and aliphatic amines, 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 normal polarity so that the positively charged ions migrate toward the cathode (the negatively charged electrode). The capillary is dynamically coated with a polycation and later with a polyanion, which directs the electroosmotic flow (EOF) toward the cathode, thus reducing the separation time while maximizing migration time reproducibility.

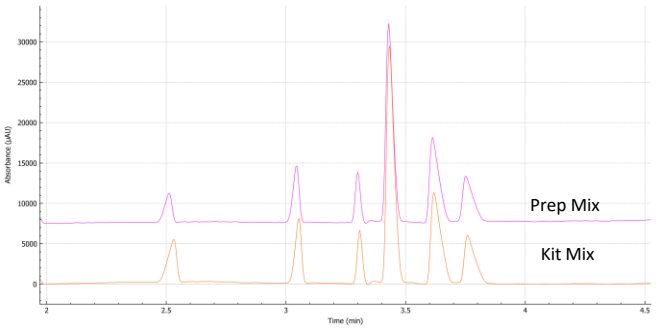


Figure 1. Electropherograms comparison between the prepared and the kit cation test mix. The top trace displays the example electropherogram of the cation text mix prepared using the procedure described in this technical note. The bottom trace displays the results of the cation test mix from the commercial kit. Both were analyzed using reagents from the kit.

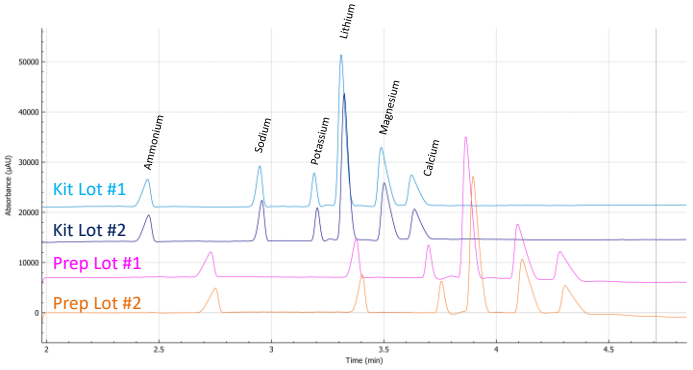


Figure 2. Electropherogram comparison of the cation test mix generated using prepared separation buffers and separation buffers from the kit. The top 2 traces display the example electropherograms of the cation test mix analyzed using 2 lots of commercial kits. The bottom 2 traces display the example electropherograms of the cation test mix separated with 2 lots of prepared reagents.

Key features

- Detailed and complete instructions to prepare the cation analysis reagents and test mix standards
- Comparable results with the previously available, commercial Cation Analysis Kit
- Robust and repeatable cation analysis
- 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). Polyacrylic acid (~ MW. 450,000) was purchased from both Sigma-Aldrich and Polysciences to evaluate the impact of raw material on separation performance. All other chemicals needed were ordered from Sigma-Aldrich with the detailed part numbers listed in Table 1.

Table 1. Reagents are needed to make the cation analysis solutions.

Chemical Name	PN
Polyacrylic acid (~ MW. 450,000)	*181285-5G*
Lithium chloride	*793620-100G*
Lithium hydroxide	*909025-100G*
Propyl-4-hydroxybenzoate (NaCl)	*91359-100MG*
Potassium chloride	*P5405-250g*
4-Aminopyridine	*275875-5G*
Malic acid	*240176-50G*
18-Crown-6	*186651*
Sodium chloride	*S9625*
Calcium chloride	*C4901-100G*
Magnesium nitrate	*237175-100G*
Ammonium chloride	*213330-25G*
Polyethyleneimine (50% wt)	*181978-5G*

Instrumentation: The PA 800 Plus system was from SCIEX (Framingham, MA). The system was configured with a UV detector with a 200 nm filter (SCIEX, PN: 144430). Analysis was performed using a 50 cm effective length (60 cm total) bare-fused silica capillary (BFS capillary) with a 75 µm ID (SCIEX, PN: 338454) and 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 the BioPhase 8800 software for processing.

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

Polyethyleneimine (1% wt) stock solution preparation: Dilute 200 mg of the polyethyleneimine (50% wt) to a final volume of 10 mL with filtered deionized water. Use a stir bar to mix well.

Polyacrylic acid (~ MW. 450,000) stock solution preparation (0.5% wt): Weigh 50 mg of polyacrylic acid (~ *MW. 450,000*) into a glass vial and add 10 mL of filtered deionized water. Cap the glass vial and seal it well. Put the glass vial onto a rotator and allow the polymer to slowly dissolve minimum overnight.

Propyl-4-hydroxybenzoate (NaCl) stock solution preparation: Weigh out 25 mg of propyl-4-hydroxybenzoate (NaCl) and dissolve the solid in 10 mL of filtered deionized water in a 15 mL conical tube. Cover the conical tube and mix well by inverting it multiple times.

Rinse solution: CE-grade water (PN: C48034) from SCIEX.

Cation coating A: In a graduated cylinder, add 105 µL of the prepared polyethyleneimine (1% wt) stock solution. Add water until the total volume reaches 100 mL.

Cation coating B: Mix 1 mL of the prepared polyacrylic acid (~ *MW. 450,000*) stock solution (0.5% wt) with 99 mL of filtered deionized water.

Cation separation buffer: In a graduated cylinder with 70 mL of filtered deionized water, while stirring, add 1 mL of propyl-4-hydroxy-benzoate (NaCl) stock solution, 188 mg of 4-aminopyridine, 254 mg of malic acid, 450 mg of 18-crown-6 and 260 µL of prepared cation coating B. Cover with parafilm and invert to mix until all solids are fully dissolved. After inverting, add water until the total volume reaches 100 mL. Note: 18-crown-6 is a hygroscopic crystalline solid. Ensure that the solid is weighed quickly to minimize its absorption of moisture.

Conditioner-Li: In a graduated cylinder, add 420 mg of lithium hydroxide to 70 mL of filtered deionized water. Cover with parafilm and invert to mix until all solids are fully dissolved. Add water until the total volume reaches 100 mL.

Conditioner-Na: 0.1N NaOH solution (SCIEX, PN: 338424)

Cation internal standard: In a graduated cylinder, add 85 mg of lithium chloride to 7 mL of filtered deionized water. Cover with parafilm and invert to mix until all solids are fully dissolved. Add water until the total volume reaches 10 mL.

Test mix stock solution: Add 100 mg of sodium chloride to 7 mL of filtered deionized water in a 15 mL centrifuge tube and sonicate to dissolve all solids into the solution. Add water until the total volume reaches 10 mL. Cap the tube and invert it multiple times to mix the solution well. Repeat this process for the following 5 chemicals: 1) potassium chloride, 2) calcium chloride, 3) lithium chloride, 4) magnesium nitrate, and 5) ammonium chloride. This process yields a total of 6 stock solutions.

Cation test mix: In a graduated cylinder with 70 mL of filtered deionized water, while stirring, add 508 µL of sodium chloride, 382 µL of potassium chloride, 555 µL of calcium chloride, 1220 µL of lithium chloride, 2100 µL of magnesium nitrate and 630 µL of ammonium chloride. Cover and invert to mix until all solids are fully dissolved. Add water until the total volume reaches 100 mL.

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 cations is between 1 and 50 ppm. Special care should be taken

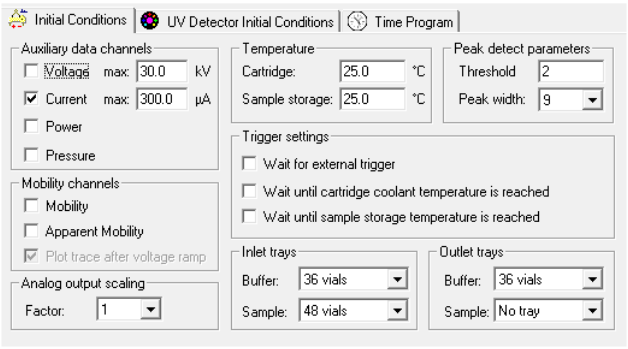
to verify the pH of the sample, which should be slightly acidic. Optional, If the sample pH is not acidic, adjust the pH of the sample by adding 3mM HCl or nitric acid.

The cation internal standard (I.S.) consists of 0.20M lithium chloride (LiCl), which is equivalent to 1388 ppm of lithium-ion. The I.S. can be used in the quantification of the sample cations. 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 28 ppm of lithium-ion.

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

Instrument preparation: Set up and configure the instrument

A) Initial conditions for all cation separation method



B) Detector conditions for all cation separation method

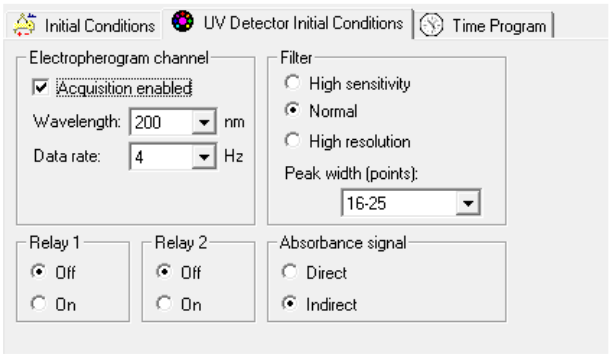


Figure 3. Initial conditions and detector conditions for all cation analysis methods. Some ions, such as nitrate, absorb at short wavelengths. In these situations, the wavelength (nm) shown in panel B should be set to 254 nm.

with the UV detector and 200 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) and is followed by the separation method for the sample and standards (Figure 4C) and the shutdown method (Figure 4D).

A) Time program for new capillary conditioning method

Initial Conditions | UV Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	20.0 psi	1.00 min	BI:F1	BO:B1	forward	Rinse with Conditioner Na
2		Wait		4.00 min	BI:F1	BO:B1		waite for 4 mins
3		Rinse - Pressure	20.0 psi	30.00 min	BI:F1	BO:B1	forward	Rinse with Conditioner Na
4								

B) Time program for condition method

Initial Conditions | UV Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	20.0 psi	1.00 min	BI:F1	BO:B1	forward	Rinse with Conditioner Na or Li
2		Rinse - Pressure	20.0 psi	1.00 min	BI:A4	BO:B1	forward	Rinse with Rinse solution
3		Rinse - Pressure	20.0 psi	1.00 min	BI:B1	BO:B1	forward	Rinse with Cation Coating A
4		Rinse - Pressure	20.0 psi	2.00 min	BI:C1	BO:B1	forward	Rinse with Cation Coating B
5		Rinse - Pressure	20.0 psi	1.50 min	BI:D1	BO:B1	forward	Rinse with Cation Separation buffe
6	0.00	Separate - Voltag	30.0 KV	5.00 min	BI:E1	BO:C1	1.00 Min ramp, normal polarity	voltage with Cation Separation bu
7	5.00	Stop data						
8	5.10	Rinse - Pressure	20.0 psi	0.50 min	BI:F1	BO:B1	forward	Rinse with Conditioner Na or Li
9	5.60	Rinse - Pressure	20.0 psi	0.50 min	BI:B4	BO:B1	forward	Rinse with Rinse solution
10	6.10	End						
11								

C) Time program for separation method

Initial Conditions | UV Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	20.0 psi	0.50 min	BI:B1	BO:B1	forward	Rinse with Cation coating A
2		Rinse - Pressure	20.0 psi	0.50 min	BI:C1	BO:B1	forward	Rinse with Cation coating B
3		Rinse - Pressure	20.0 psi	1.50 min	BI:D1	BO:B1	forward	Rinse with Cation Separation buffer
4		Wait		0.20 min	BI:A1	BO:A1		water dip
5		Inject - Pressure	0.5 psi	5.0 sec	SI:A1	BO:A1	Override, forward	Inject sample
6		Inject - Pressure	0.1 psi	10.0 sec	BI:A4	BO:A1	No override, forward	inject Rinse solution
7	0.00	Separate - Voltag	30.0 KV	5.00 min	BI:E1	BO:C1	1.00 Min ramp, normal polarity	Separate with Cation Separation bu
8	2.00	Autozero						auto zero
9	5.00	Stop data						stop data
10	5.10	Rinse - Pressure	20.0 psi	0.50 min	BI:F1	BO:B1	forward	Rinse with Conditioner-Na or Li
11	5.60	Rinse - Pressure	20.0 psi	0.50 min	BI:B4	BO:B1	forward	Rinse with Rinse solution
12	6.10	End						
13								

D) Time program for shutdown method

Initial Conditions | UV Detector Initial Conditions | Time Program

	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 - Pressu	0.1 psi	1.00 min	BI:A1	BO:A1	forward	Rinse with Rinse Solution
3	1.00	Lamp - Off						lamp off
4	1.20	End						
5								

Figure 4. Time programs for conditioning and separation methods for cation analysis 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.

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 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 might 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 8800 software.

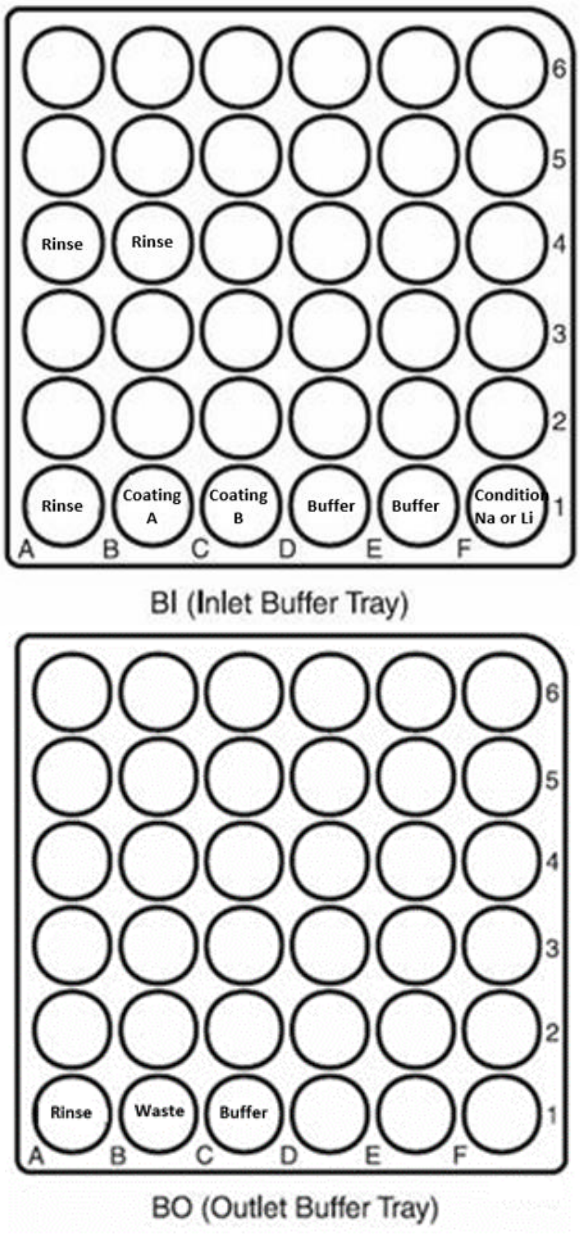


Figure 5. Buffer tray configuration for cation analysis. Fill each vial listed, except waste, with 1.5 mL of the specified solution. Fill the waste vial in the outlet buffer tray with 0.7 mL of CE-grade water. Note: Small amounts of sodium or lithium can be detected when using Conditioner-Na (0.1N NaOH) or Conditioner-Li (0.1N LiOH), respectively, as the conditioner solution. Select the conditioner solution loaded into the buffer inlet vial at position F1 to minimize carryover of the analyte of interest. If the sodium ion is an analyte of interest, use Conditioner-Li; if the lithium-ion is an analyte of interest, use Conditioner-Na.

Results and discussion

Experimental design: Two different scientists followed the above protocol and prepared 2 lots of separation buffers. 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 reliability of the preparation protocol. Triplicate injections were performed for each combination of sample and separation buffer.

Comparability of prepared cation test mix: The cation test mixes prepared in the lab and from the commercial kit were analyzed using all buffers from the kit in 1 sequence. Comparable results were observed. Figure 1 shows an example of the electropherograms and Tables 2 and 3 show the quantification of ions for the 2 samples. The results from the kit test mix are shown in Table 2 and the results from the prepared test mix are shown in Table 3. The % difference in the average peak migration time between the kit test mix and the prepared test mix was less than 0.2%. The average corr. area% was less than 3% for all ions included (Figure 1 and Tables 2 and 3)

Table 2. Analysis results for the cation test mix from the kit with reagents from the kit.

	Average MT	%RSD MT	Average corr. area%	%RSD corr. area%	Resolution
Ammonium	2.45	0.09	11.45	0.50	0.00
Sodium	2.96	0.10	10.54	1.20	8.69
Potassium	3.20	0.20	6.59	1.65	5.51
Lithium	3.32	0.23	41.85	0.68	2.41
Magnesium	3.50	0.20	19.21	0.39	2.67
Calcium	3.63	0.19	10.37	0.70	1.75

Table 3. Analysis results for the cation test mix prepared in the lab with reagents from the kit.

	Average MT	%RSD MT	Average corr. area%	%RSD corr. area%	Resolution
Ammonium	2.45	0.09	11.43	0.37	0.00
Sodium	2.96	0.07	10.46	0.32	8.74
Potassium	3.20	0.10	6.56	0.67	5.56
Lithium	3.32	0.09	41.27	0.35	2.45
Magnesium	3.50	0.09	19.64	0.26	2.68
Calcium	3.64	0.12	10.64	0.53	1.76

Impact of coating solution B: As shown in Figure 1, when the cation test mix was analyzed using buffers prepared in the lab, the migration times for all ions were delayed compared to using the kit buffers. First, lot-to-lot variation was evaluated, and the results indicate that the shift is larger than the lot-to-lot variations for both the prepared buffers and the kit buffers.

Based on the separation mechanism of the kit, a matrix condition involving coating solution A, coating solution B, and separation buffer was analyzed in the same sequence mix and the migration times of the ammonium ion (first peak) and the calcium ion (last peak) were used as key indicators for the separation. As shown in Table 4, the source of cation coating B directly controls the migration time of all the peaks. When the cation coating B that was prepared in the lab was used, all ions migrated later.

Table 4. Experimental design and results to understand migration time shift in the prepared buffers.

Coating A source	Coating B source	Separation buffer source	MT for ammonium	MT for calcium
Kit	Kit	Kit	2.45	*3.64*
Kit	Prep	Prep	2.81	4.52
Prep	Prep	Kit	2.73	4.29
Prep	Prep	Prep	2.74	4.33
Prep	Kit	Prep	2.56	3.81

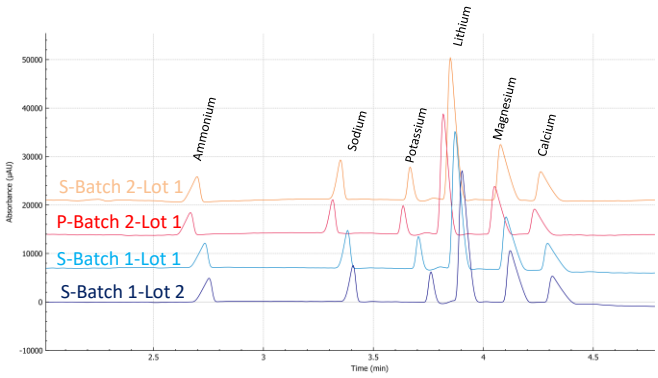


Figure 6. Electropherograms of the cation test mix were analyzed with 4 sets of buffer solutions (coating A, coating B, and separation buffer). The top 2 traces are from buffers made as batch 2 using polyacrylic acid (~ MW. 450,000) from Sigma-Aldrich (S) and Polysciences (P). The bottom 2 traces are from buffers prepared by 2 different analysts using polyacrylic acid (~ MW. 450,000) from Sigma-Aldrich (S).

Since polyacrylic acid (~ MW. 450,000) is the sole component of cation coating B, the same compound from a different vendor was purchased and evaluated. Polyacrylic acids (~ MW. 450,000) from Sigma-Aldrich and Polysciences were used to prepare new batches of cation coating B in parallel. All separation buffers were prepared again and used for the cation test mix separation. Results were comparable to previously prepared buffers and show a similar delay in absolute ion migration time compared to the results acquired using all buffers and solutions from the previously available commercial kits (Figure 6). A previous study (data not shown) for a similar application showed that molecular weight distribution plays an important role in the migration time and peak resolution of an analyte. Despite both vendors reporting the same molecular weight [3-4], we hypothesize that this phenomenon can explain the delayed migration times observed here. Additional characterization is needed to test this hypothesis.

Table 5. Analysis results for the cation test mix from the kit with reagents prepared in the lab.

	Average MT	%RSD MT	Average corr. area%	%RSD corr. area%	Resolution
Ammonium	2.70	1.45	11.28	1.76	0.00
Sodium	3.35	1.41	10.34	1.27	10.03
Potassium	3.67	1.74	6.63	1.96	6.51
Lithium	3.84	1.38	41.61	0.55	2.96
Magnesium	4.06	1.34	19.47	0.93	2.88
Calcium	4.25	1.42	10.67	2.02	2.02

Comparability of the quantification results on cation test mix: Despite migration time differences using the prepared separation buffers, the quantification of the ions in the test mix was consistent between the kit and the prepared buffers (Tables 2 and 5). Consistent with earlier observations, the solutions prepared in the lab showed improved resolution for all ions in the cation test mix.

Additionally, great repeatability and robustness were demonstrated in the preparation procedure. Four different batches of solutions (coating A, coating B, and separation buffer) prepared using polyacrylic acid (~ MW. 450,000) from 2 different vendors and prepared by 2 different analysts on 2 different days using were used to analyze the cation test mix. Results were comparable across 14 injections. %RSD was less than 2% for peak migration time and corrected area % for all ions (Table 5).

Conclusions

- With the instructions provided, a lab-scale Cation Analysis Kit was successfully created

- No method modifications were needed to use the lab-scale cation analysis reagents to replace the use of the previously available commercial Cation Analysis Kit (PN: A53540)
- The lab-scale preparation of the reagents provided comparable results to the commercial kit

References

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

Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
Phone 508-383-7700
sciex.com

International Sales
For our office locations please call the division headquarters or refer to our website at
sciex.com/offices