Your IC results are only as good as your sample vials

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Polymeric sample vials are frequently contaminated with leachable organic or inorganic ions, which originate from the production process or the raw material. These substances can falsify measurement results. The study at hand compares the leaching properties of several vial types from different manufacturers, showing considerable variations in quality and proving the importance of the right choice of vials for ion chromatography (IC). This white paper also provides recommendations and precautions to further reduce leachable contaminants for IC trace analysis.

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Metrohm

Introduction

Polymeric sample vials and sample containers typically are supplied as «sterile», «pyrogen free», etc. These labels are important when it comes to biological or medical samples. «Sterile» vials are free of microorganisms and «pyrogen free» vials do not contain any substances that can induce fever. However, both may still contain organic components or ions.

Organic components including organic acids and other ionic components can diffuse from the plastic material into the solution contacting the surface. These contaminants, which are typically referred to as «extractable» or «leachable», may be introduced to vials during manufacturing as stabilizers or as components of the polymer itself (e.g., traces of fluoride from fluorinated polymers).

In ion chromatography, the major concern for «clean» sample container material is the absence of ions. Any amount of anions or cations added to the sample from the sample container will falsify the analysis result.

The aim of this study is to demonstrate the importance of vial quality and to compare vials from different manufacturers with respect to their leaching properties. The applicability for direct analysis of most sample matrices is pointed out. The comparison of vials shows that Metrohm vials are superior to those from other suppliers when it comes to ion leaching. Moreover, this report presents precautions and an easy procedure to reduce such contaminants even further for accurate trace analysis.

Metrohm Vial Quality

The IC sample vials (6.2743.050, Sample tubes 11 mL and 6.2743.040, Sample tubes 2.5 mL) are based on a polypropylene (PP) material for healthcare applications. Production and packaging is performed under sterile and cleanroom conditions (clean room class 7, according to DIN ISO 14644). This guarantees minimum contamination levels. The vials are tested randomly for leachable anions and cations.

Two tests are performed, the first directly after filling the vial with ultrapure water (see below «Precondition: Ultrapure water»). The second test is done after a leaching time of 12 hours. Leachable inorganic anions, alkali and alkaline earth metal cations as well as ammonium need to be below 1 µg/L each for both initial and 12 hours leaching according to Metrohm specification. Organic acids (glycolate, acetate, propionate, formate, and oxalate) need to be below 1 µg/L in the initial test and below 5 µg/L after 12 hours. The area of each unknown peak needs to be below the peak area of chloride at 1 µg/L in both tests.

Figure 1 shows an overlay of an ion chromatogram from a typical Metrohm vial and a third-party vial. The leaching solutions are injected directly after filling the vials with ultrapure water. For chromatographic conditions, see below the leaching tests. The most critical anions leached from sample vials are chloride, acetate, and formate. The organic acids may be introduced from the atmosphere or they can be leached from the vial polymer directly. The latter results in an increased concentration of acetate and formate after prolonged leaching. Being a ubiquitous anion, chloride contamination typically results from problems in the production process and improper handling of the vials.

Table 1. Requirements for vial cleanliness

Ions	Required concentration limit
Inorganic anions (F^-, Cl^-, NO_2^-, Br^-, NO_3^-, HPO_4^{2-}, SO_4^{2-}), initial contamination and after 12 hours leaching	< 1 µg/L
Cations (Li^+, Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+}), initial contamination and after 12 hours leaching	< 1 µg/L
Organic acids initial contamination	< 1 µg/L
Organic acids after 12 hours leaching	< 5 µg/L

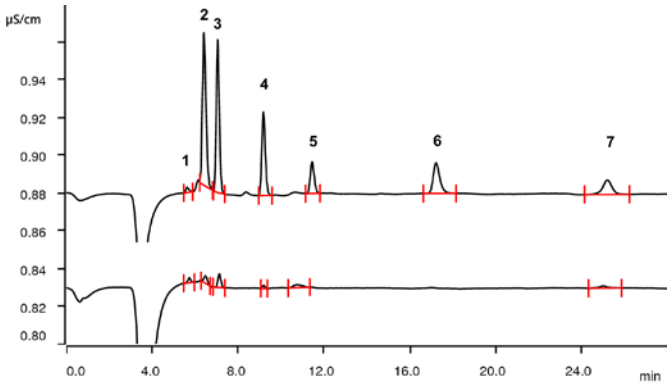

Figure 1. Overlay of initial leaching solutions of a Metrohm vial (lower trace) and a vial from another supplier (upper trace). Anions: 1 fluoride, 2 acetate, 3 formate, 4 chloride, 5 nitrite, 6 nitrate, 7 sulfate

Table 2. Results initial anion contamination

	RT [min]	Anions	Requirement [µg/L]	Metrohm [µg/L]	Within limits	Other supplier [µg/L]	Within limits
1	5.6	Fluoride	< 1 µg/L	< 0.10	Yes	0.55	Yes
2	6.4	Acetate	< 1 µg/L	0.28	Yes	5.80	No
3	7.1	Formate	< 1 µg/L	0.61	Yes	2.54	No
4	9.2	Chloride	< 1 µg/L	< 0.10	Yes	1.47	No
5	11.5	Nitrite	< 1 µg/L	< 0.10	Yes	1.08	No
6	17.2	Nitrate	< 1 µg/L	n.d.*	Yes	1.27	No
7	25.2	Sulfate	< 1 µg/L	< 0.10	Yes	0.68	Yes

*not detected

In Figure 2, the same vial types are compared for cation contamination as in Figure 1. The critical cations are mainly sodium, ammonium, and calcium. Especially ammonium often contaminates vials through atmospheric effects. Sodium on the other hand is as ubiquitous as chloride.

The results of the comparison of initial anion and cation leachates demonstrate the quality of Metrohm vials, which is due to the superior production process. All contamination levels are within the specifications. Meanwhile, most anions and cations of interest exceed the required levels in vials from another supplier. These vials are not recommended for sub-mg/L applications.

The cleanliness of Metrohm sample vials permits direct sample analysis for almost all applications. For direct analysis of concentrations in the low µg/L range as well as for preconcentration, it is recommended to follow the procedure described below (prerinsing of the vials with ultrapure water or the sample itself).

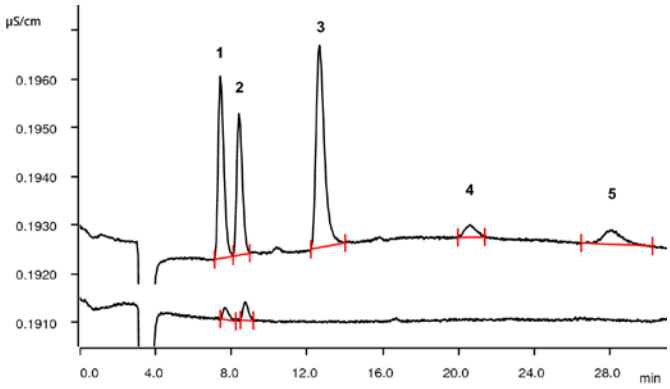

Figure 2. Overlay of initial leaching solutions of a Metrohm vial (lower trace) and a vial of a other supplier (upper trace). Cations: 1 sodium. 2 ammonium, 3 potassium, 4 magnesium, 5 calcium

Table 3. Results initial cation contamination

	RT [min]	Cations	Requirement [µg/L]	Metrohm [µg/L]	Within limits	Other supplier [µg/L]	Within limits
1	5.6	Sodium	< 1 µg/L	0.17	Yes	1.89	No
2	6.4	Ammonium	< 1 µg/L	0.13	Yes	1.01	No
3	7.1	Potassium	< 1 µg/L	n.d.	Yes	4.41	No
4	9.2	Magnesium	< 1 µg/L	n.d.	Yes	<0.10	Yes
5	11.5	Calcium	< 1 µg/L	n.d.	Yes	0.18	Yes

*not detected

Leaching procedure for trace analysis

Precondition: Ultrapure water

In ion chromatography, the use of ultrapure water is a must to achieve lowest detection limits and to prolong the lifetime of the analytical column. The used ultrapure water is water with a resistivity at 25 °C of > 18.2 MΩ·cm and a TOC of < 5 µg/L. This corresponds to the requirements of Grade I water according to ISO 3696 and ASTM D1193.

The required water quality is reached with modern water purification systems only. Double distilled water or «ion exchange» water does not fulfill these requirements. Ultrapure water has to be used immediately, especially when aiming for trace analysis. Storage of ultrapure water allows absorption of gases from the ambient air. Such gases, like CO_2, NH_3, or others, will increase the contamination in the vial and result in increased conductivity and blank values.

Metrohm IC systems can be connected with an **ELGA Purelab® flex 5 or flex 6** to obtain the highest water purity for eluent production and for liquid handling. Any ionic contamination in the eluent or liquid handling water will interfere with the results for the respective ions.

On the other hand, ionic contamination in liquid handling water, which flows through the preconcentration column, will yield in results that are too high. All leaching steps are performed using fresh ultrapure water directly from the purification system.

Figure 3. Metrohm IC system with 941 Eluent Production Module, 858 Professional Sample Processor, and ELGA Purelab® flex 6 to obtain the highest water purity for eluent production and for liquid handling

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Leaching and IC analysis of 11 mL Vials (6.2743.050)

The general procedure includes multiple rinsing steps with either the sample or ultrapure water. The vial finally filled with sample should be covered immediately with a stopper (6.2743.070, Stopper with perforation) to avoid later contamination by dust, air, etc.

The following study of the Metrohm 11 mL vials requires a proper lab environment. The operator has to work with gloves that are tested for lowest ion contamination. Besides a leaching test (**see Application Note AN-S-304**), the determination of total anions and sulfur in the gloves by combustion IC (**see Application Note AN-CIC-004**) is recommended. In trace level determinations of anions and cations, glass tools must not be used for sample handling. The glass surface acts as an ion exchanger and can bind or release ions from or to the liquid. Most recommended for these analyses is a cleanroom environment.

Leaching procedure: The vials are initially rinsed 3 times with 10 mL ultrapure water. The fourth refill with 10 mL ultrapure water is covered with a stopper immediately after filling. The vial is leached for 12 hours and subsequently analyzed for trace anions, organic acids, and cations.

The anion analyses are performed with a ProfIC Vario 9.1 system applying intelligent Preconcentration Technique with Matrix Elimination (MiPCT-ME). The anions are determined on a Metrosep A Supp 7 - 250/4.0 column applying conductivity detection after sequential suppression. Inline Eluent Production is performed with a 941 Eluent Production Module.

The eluent concentrates need to be manufactured from ultrapure chemicals. Commercially available concentrates cannot be used for this purpose. The ultrapure water for the dilution of the eluent concentrate is delivered directly from an ELGA Purelab® flex 6 to ensure optimum conditions. The chromatographic conditions are given below, in Table 4.

Table 4. Chromatographic conditions

Analytical column	Metrosep A Supp 7 - 250/4.0
Preconcentration column	Metrosep A PCC 2/4.0
Eluent	3.6 mmol/L sodium carbonate
Flow	0.8 mL/min
Column temperature	45 °C
Preconcentration volume	1000 µL
Suppressor regeneration	100 mmol/L sulfuric acid

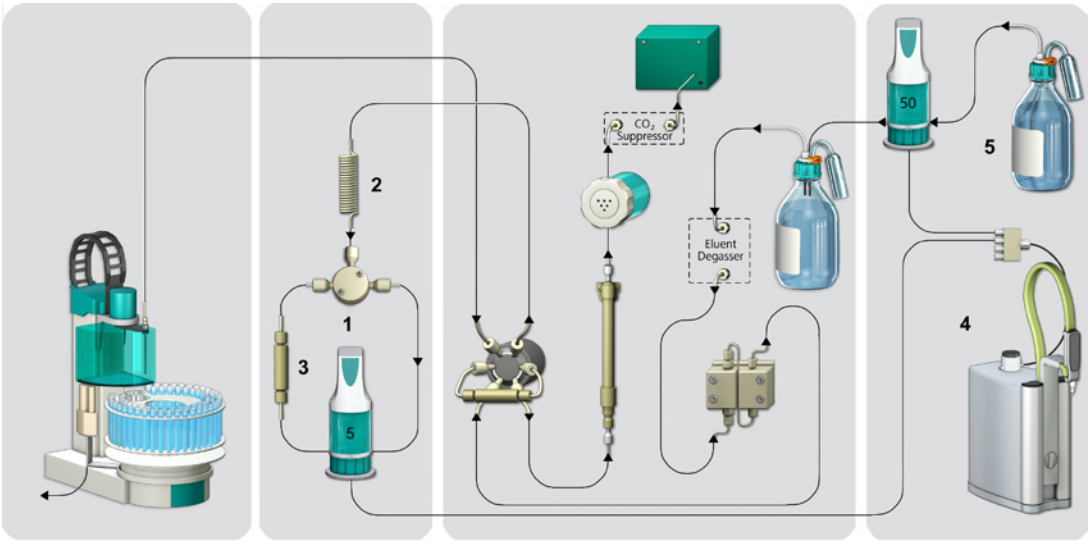

Figure 4. Schematic setup of the ProfIC Vario 9.1 system with sequential anion suppression. 1: Liquid handling for preconcentration, 2: buffer tubing, 3: ion trap column, 4: ELGA Purelab® flex 6, 5: eluent concentrate and Dosino for Inline Eluent Production

Figure 4 shows the graphical setup of the applied ProfIC Vario 9.1 system. The Dosino for Preconcentration/Matrix Elimination Part (1) pulls the exact volume to be preconcentrated from the sample vial into the sample path. Subsequently, this volume is transferred to the buffer tubing (2). After switching the injector, the Dosino doses the sample and the respective volume of ultrapure water for Matrix Elimination onto the preconcentration column. The water for Matrix Elimination is polished by delivery through an ion trap column (3).

Figure 5 shows a chromatogram (red) of a Metrohm 11 mL vial after 3-times rinsing. The fourth addition of ultrapure water is analyzed. For comparison, an injection of a 1 µg/L standard solution (black, requirement level) is added. The leached anions are obviously far below the limit concentrations. This proves that this vial type is suitable for the analysis in the low µg/L range.

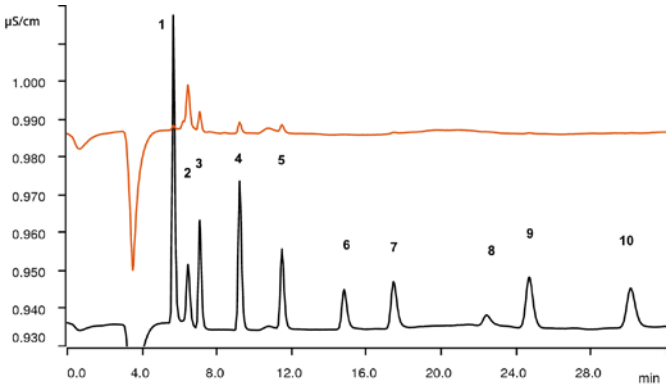

Figure 5. Overlay of chromatograms of ultrapure water after 3-times rinsing (red) and a 1.0 µg/L standard solution of standard anions including acetate and formate (black)

Table 5. Results

	RT [min]	Ions	Requirement [µg/L]	Concentration [µg/L]	Within limits
1	5.6	Fluoride	< 1 µg/L	< 0.10	Yes
2	6.5	Acetate	< 5 µg/L	0.52	Yes
3	7.1	Formate	< 5 µg/L	0.16	Yes
4	9.2	Chloride	< 1 µg/L	< 0.10	Yes
5	11.5	Nitrite	< 1 µg/L	0.15	Yes
6	14.8	Bromide	< 1 µg/L	n.d.*	Yes
7	17.4	Nitrate	< 1 µg/L	n.d.	Yes
8	22.4	Phosphate	< 1 µg/L	n.d.	Yes
9	24.7	Sulfate	< 1 µg/L	n.d	Yes
10	30.1	Oxalate	< 1 µg/L	n.d.	Yes

*not detected

The cations are determined applying sequentially suppressed cation chromatography with a ProfIC Vario 15 system (Partial-Loop Injection Technique, MiPT). Ultrapure water supply for eluent production and liquid handling is provided directly from an ELGA Purelab® flex 6. This ensures lowest contamination levels and therefore lowest blank values in the eluent and sample handling.

In Figure 6, the schematic setup of a ProfIC Vario 15 system with sequential suppression is presented. The liquid handling Dosino (1) pulls the sample from the vial bypassing the injector into the buffer tubing (2). After switching the injector to the «fill» position, the Dosino loads the 250 µL loop with volumes of exactly 4–200 µL. The sample volume is set to the maximum of 200 µL to reach highest sensitivity.

Table 6. Chromatographic conditions

Analytical column	Metrosep C Supp 2 - 250/4.0
Eluent	5.0 mmol/L nitric acid 50 µg/L Rb^+
Flow	1.0 mL/min
Column temperature	45 °C
Injection volume	200 µL
Suppressor regeneration	70 mmol/L sodium hydrogen carbonate 70 mmol/L sodium carbonate

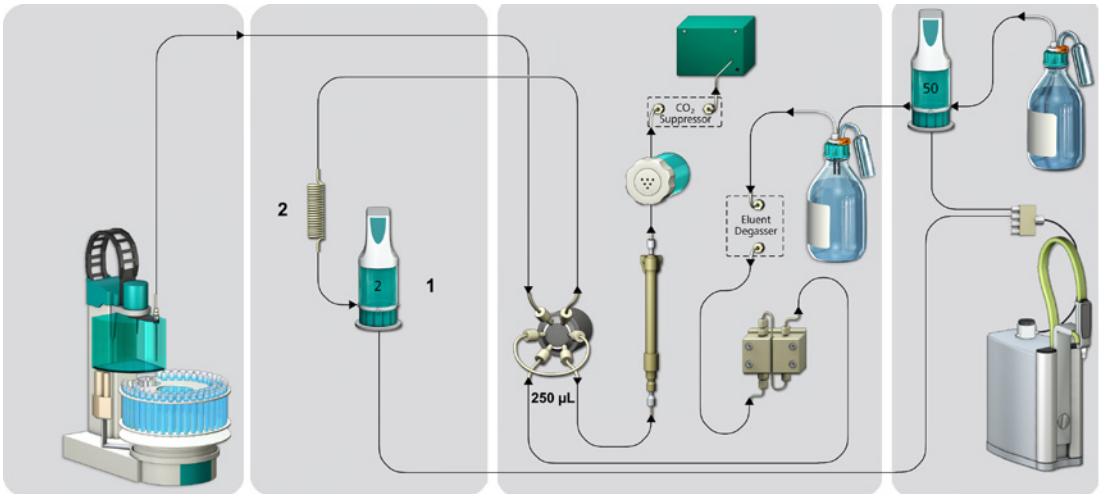

Figure 6. Schematic setup of the ProfIC Vario 15 system with sequential cation suppression. 1: Liquid handling Dosino for partial-loop injection, 2: Buffer tubing

Figure 7 shows a chromatogram (red) of a Metrohm 11 mL vial after 3-times rinsing. The fourth addition of ultrapure water is analyzed. For comparison, an injection of a 1 µg/L standard solution (black, requirement level) is added. The leached cations are obviously far below the limit concentrations. This proves that this vial type is suitable for analysis in the low µg/L range.

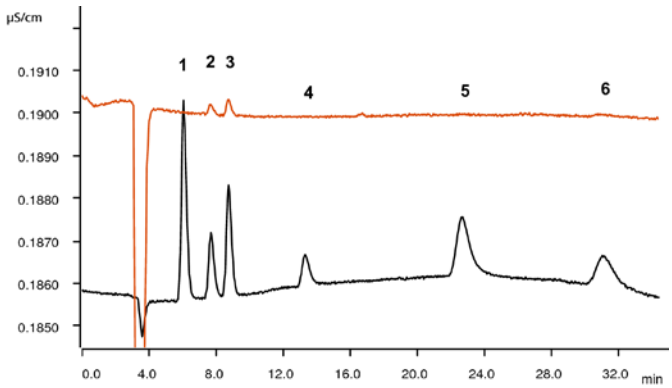

Figure 7. Overlay of chromatograms of ultrapure water after 3-times rinsing (red) and a 1.0 µg/L standard solution of standard cations (black)

Table 6. Results

	RT [min]	Ions	Requirement [µg/L]	Conc. [µg/L]	Within limits
1	5.1	Lithium	< 1 µg/L	n.d.*	Yes
2	7.7	Sodium	< 1 µg/L	0.17	Yes
3	8.8	Ammonium	< 1 µg/L	0.13	Yes
4	13.3	Potassium	< 1 µg/L	n.d.	Yes
5	22.6	Magnesium	< 1 µg/L	n.d.	Yes
6	31.0	Calcium	< 1 µg/L	n.d.	Yes

*not detected

Leaching test of different sample vials for trace cation analysis

This test series compares vial types with different geometries for trace cation analysis. Besides the Metrohm 11 mL standard vials, a 125 mL PE bottle, a sample tube, and a cell culture flask were tested.

Tested vials:

- 6.2743.050 Sample tubes 11 mL
- 6.2743.070 Stopper with perforation
- 6.1627.000 PE bottle, 125 mL for IC
- 50 mL Sample tube
- Cell culture flask

Test procedure:

In this trial, the same prerinsing procedure as above is applied for all vial types. The rinsing volume is adjusted according to the typical values used with the respective type of vial. Cations are determined applying sequentially suppressed cation chromatography with a ProfIC Vario 9.1 system (intelligent Preconcentration Technique with Matrix Elimination, MiPCT-ME).

Ultrapure water supply for eluent production and liquid handling provided is directly from an ELGA Purelab® flex 6. This ensures lowest contamination levels and therefore lowest blank values in the eluent and sample handling. The chromatographic conditions are listed in Table 7 on the following page. The system setup is the same as given in Figure 4. Analytical column and suppressor are selected for cation analysis.

Table 7. Chromatographic conditions

Analytical column	Metrosep C Supp 1 - 250/4.0
Preconcentration column	Metrosep C PCC 1 HC/4.0
Eluent	4.0 mmol/L nitric acid 200 µg/L Rb^+
Flow	1.0 mL/min
Column temperature	45 °C
Injection volume	2000 µL
Suppressor regeneration	70 mmol/L sodium hydrogen carbonate 70 mmol/L sodium carbonate

Figure 8 shows the overlay of chromatograms of the four vial types (6.2743.050 11 mL Sample vial, green; 6.1627.000 Bottle 125 mL for IC, yellow; 50 mL sample tube, red; cell culture flask, blue). The black chromatogram corresponds to a standard solution of 25 ng/L of each cation.

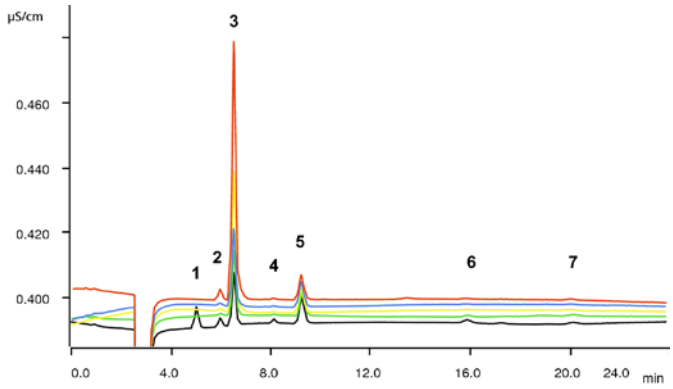

Figure 8. Overlay of chromatograms of the four tested vials as well as a standard injection of 25 ng/L of each cation (black chromatogram). Cations: 1 lithium, 2 sodium, 3 ammonium, 4 potassium, 5 rubidium (from the eluent), 6 magnesium, 7 calcium.

Figure 9 summarizes the results of the cation leaching test. All tested vial types leach out cation concentrations within the required maximum concentration for the cations (1 µg/L, red grid line). These results indicate that all of these vial types can be used for trace cation analysis. The most critical component is ammonium for the cell culture flask.

Whether the elevated levels indeed stem from leached ammonium or from ambient ammonium is very difficult to assess as ammonium may be introduced from ambient air. Trace analysis of ammonium therefore requires special attention.

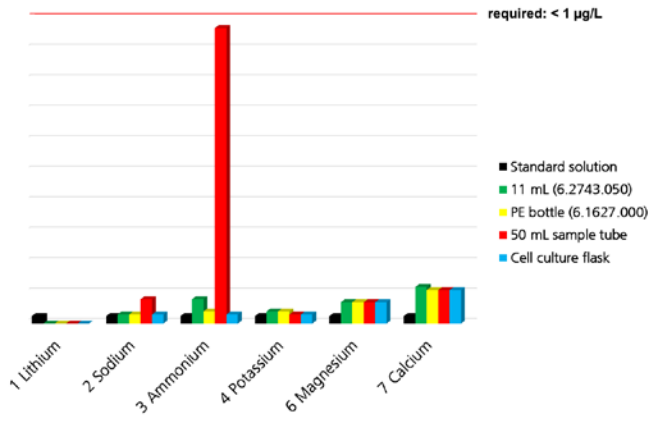

Figure 9. Visualization of the leaching results for the 4 sample vial types. The red grid line indicates the maximum allowable leached cation concentrations. The black bars correspond to the standard solution of 25 ng/L of all cations.

Conclusions

Besides the purity of the water used for eluent preparation and sample handling, the level of ionic contamination of sample containers is crucial for high accuracy and reliability in trace ion analysis (lower µg/L range). The contamination level depends on the material type, the manufacturing process, and last – but by far not least – the storage conditions. Sample containers need to be stored in a closed compartment, free of dust and aggressive gases (e.g., ammonia, hydrochloric acid, etc.).

For trace analysis, it is strongly recommended to prerinse the sample container with the respective sample. For ultratrace analysis (sub-µg/L range), we recommend to leach the sample containers over a longer time and keep them in the leaching solution (typically ultrapure water) until they are used.

Clean sample containers are only the first step. The remaining manual sample handling needs to be high-quality as well. Use ultra-clean tools (pipettes, volumetric flasks, stoppers, etc.). Wear gloves of high quality. A leach test can help finding the appropriate product. Avoid any glass, especially for trace analysis of cations.

Even for higher concentration analyses, contamination caused by sample containers should not be underestimated. Proper storage of the containers ensures the quality of the results. Checking the sample path for carry-over on a regular basis is recommended. Metrohm sample vials and sample bottles are tested frequently to ensure a constant, high purity and quality.

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