



Cation Analysis

User's Guide

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Table of Contents

Using the Cation Analysis Kit, 1

Introduction, 1

Safety, 1

Materials and Reagents, 2

Storing Kit Components, 2

Cleaning Vial Caps, 3

Turning on the UV Lamp, 3

Cleaning the Capillary Interface, 3

Installing the Capillary Inside the Cartridge, 3

Conditioning a New Capillary, 4

Storing the Capillary, 4

Preparing the Buffer Trays, 5

Preparing the Sample, 6

Running Methods, 7

Checking System Performance with the Cation Test Mix, 10

Integration Parameters, 11

Troubleshooting, 12

APPENDIX A: Filter Installation, A-1

Installing the 200 nm Filter, 1

APPENDIX B: System Configuration, B-1

Configuring the P/ACE MDQ System, B-1

Activating Caesar Integration in 32 Karat, B-3

Using the Cation Analysis Kit

Introduction

The Beckman Coulter Cation Analysis Kit contains the supplies necessary for the separation and quantitation of cations, using the P/ACE MDQ Capillary Electrophoresis system. Each cation kit yields approximately 500 tests.



NOTE

The P/ACE MDQ must be equipped with a UV detector and a 200 nm filter to perform this assay.

This kit permits 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 in indirect mode.

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



IMPORTANT

The main focus for this application is in the biopharma market. This product can also be used for environmental testing and food and beverage markets, among other areas. This product is **for laboratory use only**. It is not for use in diagnostic procedures. No clinical decision or patient notification may be made based on results using this research assay.

Safety

Refer to the Material Safety Data Sheets (MSDS) information, available at www.beckmancoulter.com, regarding the proper handling of materials and reagents. Always follow standard laboratory safety guidelines.

Materials and Reagents

Contents of this Kit (Reorder # A53540)

Component	Quantity
Cation Coating A	1
Cation Coating B	1
Cation Separation Buffer	1
Conditioner — Na	1
Conditioner — Li	1
Cation Internal Standard	1
Cation Test Mix	1
Capillary, 50 cm, 75 μ m I.D.	3 pieces
Rinse Solution	2
Ion Analysis Insert	1

Materials Required but not Included in this Kit

Description	Part Number
2 mL glass vials (pack of 100)	144980
Vial caps for 2 mL glass vials, red caps (pack of 100)	144648
Filter 200 nm (see note below)	144430
PCR vial holders (pack of 50)	144657
PCR vial springs (pack of 10)	358821
PCR vials (pack of 50)	144709
PCR vials gray caps (pack of 50)	144656
Adequate pipettes and pipette tips	



NOTE

A 200 nm filter, for use with the UV Detector, is provided as one of four standard filters on P/ACE MDQ UV Systems. Please check the condition of this filter as instructed in the *P/ACE MDQ Installation and Maintenance Guide* (A36419).

Storing Kit Components

Upon receipt, store all components at room temperature and away from direct sunlight.

Cleaning Vial Caps



NOTE

The vial caps may contain impurities that can be detected with the Cation Analysis Kit. Therefore, wash the caps before use.

1. Using a clean beaker, rinse the caps twice with double-deionized (DDI) water. Do not use soap.
2. Let the caps soak in DDI water for at least one hour, making sure the caps are completely submerged.
3. Remove the caps from the water.
4. To dry the caps, either place them in an oven set at 55°C for two hours, or allow them to dry overnight at room temperature covered by clean, lint-free laboratory wipes, such as Kimwipes.



NOTE

The vial caps become compressed and lose elasticity during use, which can lead to pressure failures and current leakage errors. Therefore, reusing caps is not recommended.

Turning on the UV Lamp

Turn on the UV lamp and allow the system to warm up for at least 30 minutes prior to experimentation.

Cleaning the Capillary Interface

Carefully clean the system electrodes and interface block as described in the **Maintenance Procedure** section of the instrument manual. Repeat this general maintenance procedure after every 24 hours of operation.

Installing the Capillary Inside the Cartridge

1. Install a 75 μm I.D., 60.2 cm long (50 cm from injection site to detector) fused-silica capillary into a capillary cartridge using the *Capillary Cartridge Rebuild Procedure* (144655).
2. Use an 800 μm aperture in the cartridge. This aperture is labeled with an “8.”
3. After the capillary has been installed in the cartridge, insert the cartridge in the P/ACE MDQ system.
4. Close the cartridge cover and tray cover.

Conditioning a New Capillary

After installing a new capillary, rinse the capillary for one minute with Conditioner — Li. Wait four minutes, then rinse for 30 seconds with Conditioner — Li. Rinse for one minute with Rinse Solution. Use 20 psi of pressure for all rinses.

Storing the Capillary

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.

After a long storage period, or at the start of each day, condition the capillary using the **Capillary Conditioning** method described in the **Running Methods** section of this manual.

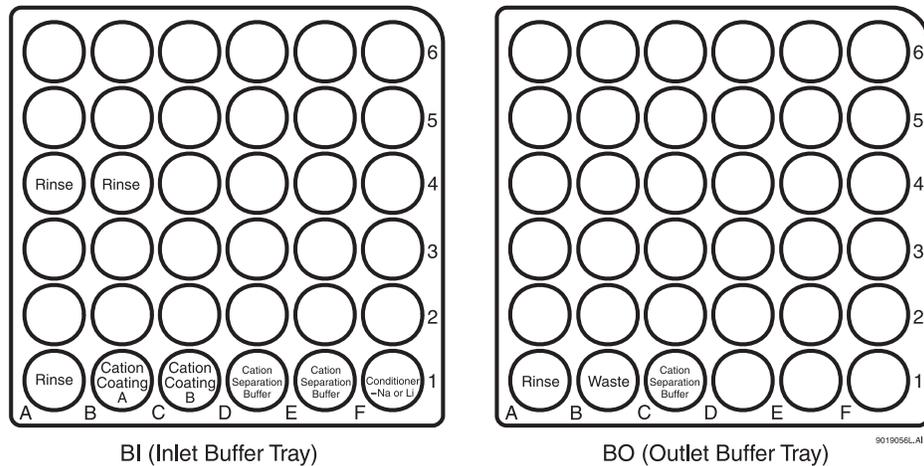


Do not share capillaries between applications. If the capillary has been used for anion analysis, do not use it for cation analysis.

Preparing the Buffer Trays

1. Fill 2 mL vials with 1.4 mL of each reagent and position in the buffer trays, as shown in Figure 1.
2. In the **Waste** position, place a vial filled with 700 μ L of Rinse Solution.
3. Close each vial with a clean red cap.

Figure 1. Buffer tray configurations for performing cation analysis



4. Load the Inlet Buffer and Outlet Buffer trays inside the P/ACE MDQ system.
5. Replace all vials after twenty runs or after 24 hours inside the instrument. The increment option in the method can be used to automatically increment the vials every twenty runs on both buffer trays.



NOTE

A small amount of sodium can be detected when using Conditioner—Na (0.1 M NaOH). Therefore, when analyzing for sodium, fill the buffer inlet vial at position F1 with Conditioner—Li (0.1 M LiOH) to minimize sodium carryover. However, a small amount of lithium may then be detected.



NOTE

The rinse solution used in this kit is ultra-purified water specifically for capillary electrophoresis analysis of ions.

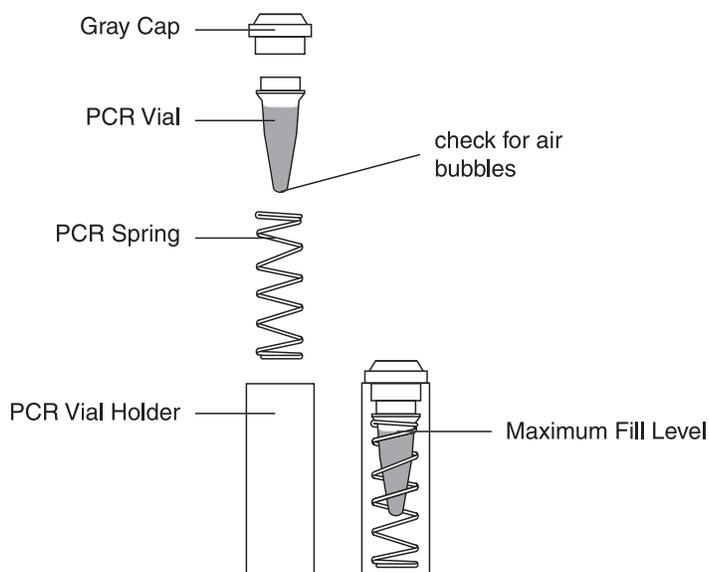
Preparing the Sample

Depending upon 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, by adding 3 mM HCl or nitric acid.

The Cation Internal Standard (I.S.) consists of 0.20 M lithium chloride (LiCl), which is equivalent to 1,388 ppm of lithium ion. The Cation I.S. can be used in the quantitation of the sample cations. To use it, dilute the Cation 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.

Fill a PCR vial with 200 μL of test or sample mix. Make sure there are no air bubbles at the bottom of the PCR vial. Air bubbles can affect the sample injection. Place the PCR vial on a PCR holder equipped with a vial spring. Seal the PCR vial with a clean gray cap and place it in the inlet sample tray.

Figure 2. PCR vial setup



Running Methods

Three methods are required for performing cation analysis:

- Cation Capillary Conditioning
- Cation Separation
- Cation Shutdown

All three methods utilize the same **Initial Conditions** (Figure 3) and **UV Detector Settings** (Figure 4). However, each method has a different **Time Program** (Figures 5, 6, and 7). Save all three methods, with their corresponding names, in the 32 Karat folder.



NOTE

These three methods can be downloaded from www.CELeader.com/downloads.

Figure 3. Initial conditions for **Cation Capillary Conditioning**, **Cation Separation**, and **Cation Shutdown** methods

Section	Parameter	Value
Auxiliary data channels	Voltage	max: 30.0 kV
	Current	max: 300.0 µA
	Power	
	Pressure	
Mobility channels	Mobility	
	Apparent Mobility	
	Plot trace after voltage ramp	checked
Analog output scaling	Factor	1
Temperature	Cartridge	25.0 °C
	Sample storage	25.0 °C
Peak detect parameters	Threshold	2
	Peak width	9
Trigger settings	Wait for external trigger	unchecked
	Wait until cartridge coolant temperature is reached	checked
	Wait until sample storage temperature is reached	checked
Inlet trays	Buffer	36 vials
	Sample	48 vials
Outlet trays	Buffer	36 vials
	Sample	No tray

Figure 4. UV Detector initial conditions for Cation Capillary Conditioning, Cation Separation, and Cation Shutdown methods

The screenshot shows the 'UV Detector Initial Conditions' window. It has three tabs: 'Initial Conditions', 'UV Detector Initial Conditions', and 'Time Program'. The 'UV Detector Initial Conditions' tab is active. It contains several control panels:

- Electropherogram channel:** 'Acquisition enabled' is checked. 'Wavelength' is 200 nm. 'Data rate' is 4 Hz.
- Filter:** 'Normal' is selected. 'Peak width (points)' is 16-25.
- Relay 1:** 'Off' is selected.
- Relay 2:** 'Off' is selected.
- Absorbance signal:** 'Indirect' is selected.

Figure 5. Time program for Cation 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 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 Buffer.
6	0.00	Separate - Voltage	30.0 KV	5.00 min	BI:E1	BO:C1	1.00 Min ramp, normal polarity	Separation.
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								

Figure 6. Time program for the Cation 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	Sample injection.
6		Inject - Pressure	0.1 psi	10.0 sec	BI:A4	BO:A1	No override, forward	Water injection.
7	0.00	Separate - Voltage	30.0 KV	5.00 min	BI:E1	BO:C1	1.00 Min ramp, normal polarity	Voltage Separation.
8	2.00	Autozero						
9	5.00	Stop data						
10	5.10	Rinse - Pressure	20.0 psi	0.50 min	BI:F1	BO:B1	forward	Rinse with Conditioner-Na or Conditioner-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								

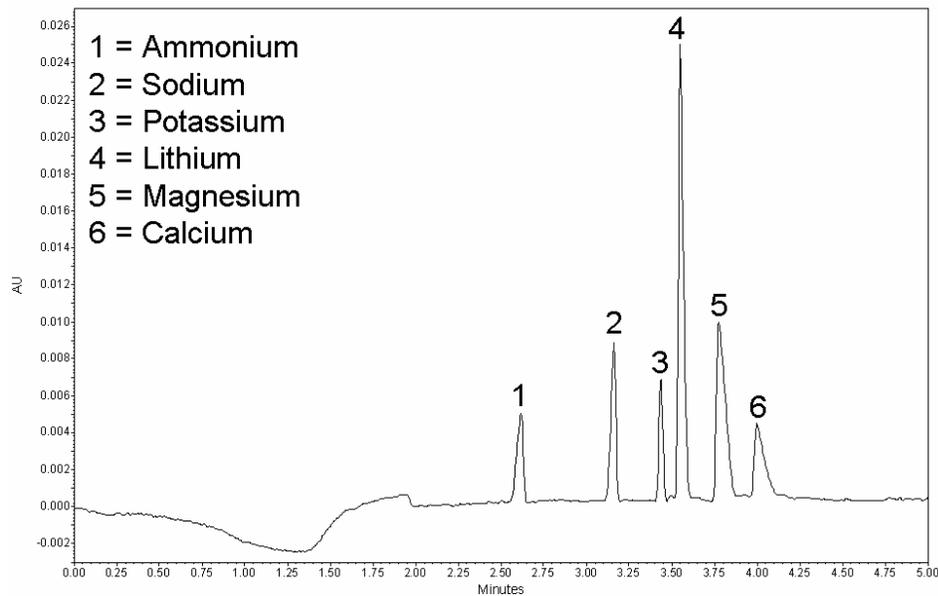
Figure 7. Time program for the **Cation Shutdown** method

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

Checking System Performance with the Cation Test Mix

To check the P/ACE MDQ system performance, run the Cation Test Mix after performing the Capillary Conditioning method. Compare the electropherogram obtained with the one shown in Figure 8. The electrical current during the separation should be stable around +33 μA . A positive electrical value indicates that normal polarity was used in the separation.

Figure 8. Typical electropherogram of the Cation Test Mix. The concentration of each ion in the test mix is approximately 20 ppm.



Integration Parameters

The integration parameters in the analysis method should be optimized for each sample. As a starting point, use the integration values shown in Figure 9. These values will successfully integrate the Cation Test Mix.

Figure 9. Recommended integration parameters and corresponding initial values

#		Event	Start Time	Stop Time	Value
1	<input checked="" type="checkbox"/>	Integration Off	0.000	2.200	0
2	<input checked="" type="checkbox"/>	Width	0.000	0.000	0.1
3	<input checked="" type="checkbox"/>	Threshold	0.000	0.000	500
4	<input checked="" type="checkbox"/>	Shoulder Sensitivity	0.000	0.000	9999
5	<input checked="" type="checkbox"/>	<input type="text"/>			

- **Integration off** sets time intervals in the electropherogram that are not integrated.
- **Width** sets the sensitivity of the peak detection regarding changes in the baseline.
- **Threshold** determines how high a peak must rise above the baseline noise before it is recognized as a peak.
- **Shoulder sensitivity** enables the detection of shoulders in large peaks. Its value specifies the slope value for splitting a peak.

The integration parameter, known as **Minimum Cluster Distance**, can be used to split peaks when shoulder sensitivity does not provide proper integration. Its value specifies the distance between non-baseline separated peaks so that they are not identified as one peak.

Additional help is provided in the 32 Karat Help files.

Troubleshooting

Problem	Possible Cause	Corrective Action
Unstable current	Problem with capillary	Replace capillary with new one
No peaks	Wrong polarity in method	Use normal polarity in method
	No sample or sample at wrong location	Check sample vial position
No stable migration time	Buffer depletion	Replace all buffer vials after every 20 runs
Presence of ghost peaks	Contaminated buffers	Replace all buffer vials after every 20 runs
	Vial caps are wet	Replace caps with clean, dry caps
	Vial caps are dirty	Always use clean caps
Ammonium (NH_4^+) peak is missing or too small	Over time, ammonium converts into ammonia (NH_3) and evaporates	Replace samples with fresh ones and analyze immediately

Filter Installation

Installing the 200 nm Filter

Before installing the 200 nm filter, consult the **Installation UV detector wavelength filters** section in the *P/ACE MDQ Installation and Maintenance Guide* (A36419). In summary:

1. Set the buffer trays to the **load** position using Direct Control.
2. Lift the cartridge cover door and allow the coolant to drain from the capillary cartridge.
3. Turn off the P/ACE MDQ system.
4. Loosen the two thumb screws and lift the insertion bar.
5. Remove the capillary cartridge.
6. Loosen the thumb screws and remove the optics source assembly.
7. Wearing clean gloves, remove the filter wheel access cover and rotate the filter wheel to position 2.
8. Place the 200 nm filter at position 2 with the reflective side facing inward (toward the back of the instrument). Do not touch the filter with your hands.
9. Reinstall the filter wheel cover on the optics source assembly.
10. Replace the optics source assembly and tighten the two thumb screws.
11. Place the cartridge inside the instrument, lower the insertion bar, and tighten the two thumb screws.
12. Close the cartridge cover door.
13. Turn on the instrument.

Next, the 32 Karat software must be configured for performing cation analysis.

System Configuration

Configuring the P/ACE MDQ System

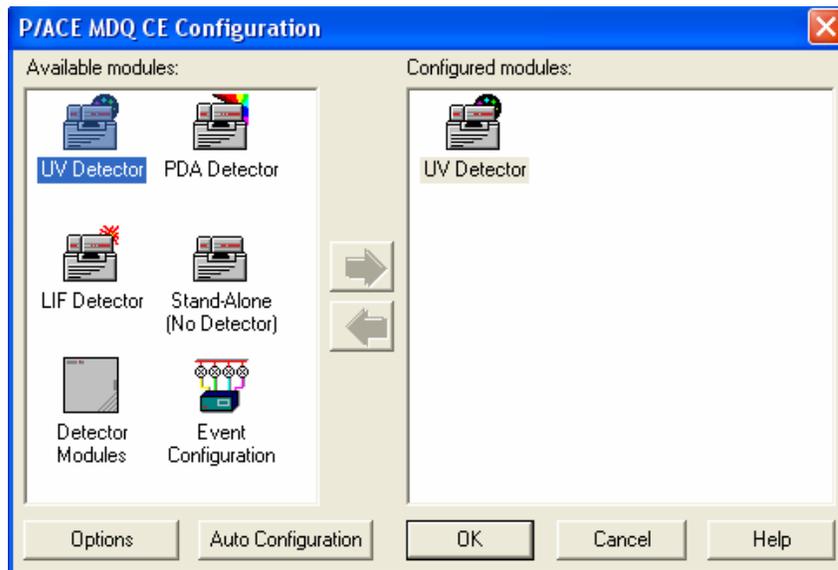
This procedure describes how to configure the P/ACE MDQ system to perform cation analysis.

 **IMPORTANT**

Make sure that the P/ACE MDQ system is turned on, and that the UV Detector has been installed.

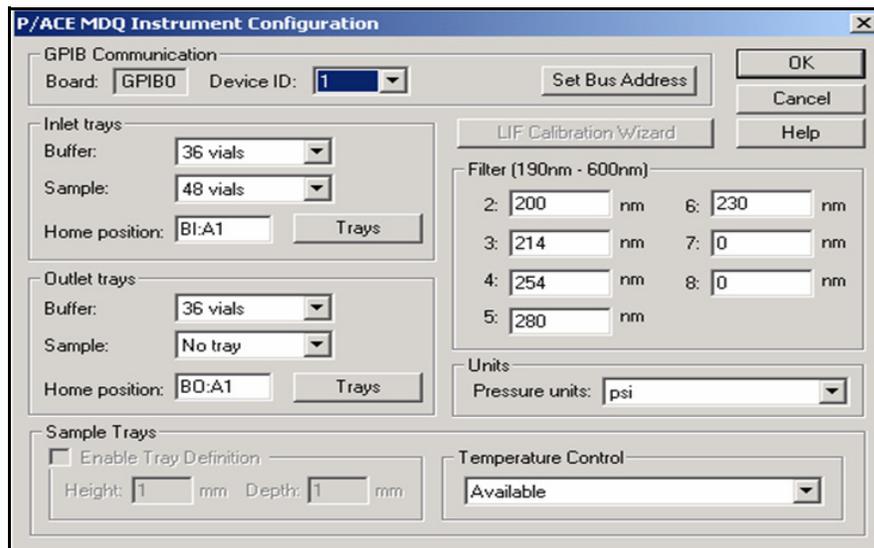
1. Open the 32 Karat software.
2. Right click in the right pane of the **Enterprise** window.
3. Select **New > Instrument**.
4. A new icon that looks like a question mark appears. Right click on this icon, and select **Rename**.
5. Rename this icon **Cation**.
6. Right click on the **Cation** icon and select **Configure**.
7. Select **P/ACE MDQ CE** as the instrument type and click **Configure**.
8. A new window opens. Click on the **UV detector** icon on the left.
9. Click on the **Green arrow**. The UV detector icon should now be on the right side under **Configured Modules**, as shown in Figure B.1.

Figure B.1 Selecting the UV detector



10. Double click the UV Detector icon to display the configuration settings.
11. The Configuration settings should match the ones shown in Figure B.2.
12. Click OK to accept the detector configuration.
13. Go to the next section to activate Caesar integration.

Figure B.2 P/ACE MDQ instrument configuration required for running cation analysis

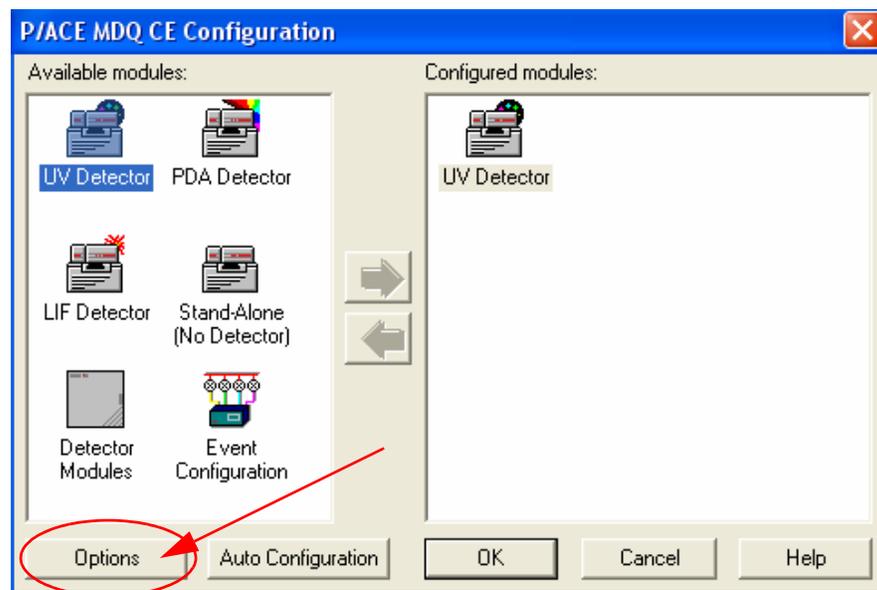


Activating Caesar Integration in 32 Karat

The Caesar Integration must be activated in the cation configuration to perform peak integration and quantitation.

1. In the **P/ACE MDQ System Configuration** window, click **Options**, as shown in Figure B.3.
2. Under **General**, make sure that only **Qualitative Analysis** and **Caesar Integration** have been selected.
3. Click **OK** in the next three windows to approve the changes.

Figure B.3 P/ACE MDQ CE configuration for cation analysis



System Configuration

Activating Caesar Integration in 32 Karat

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