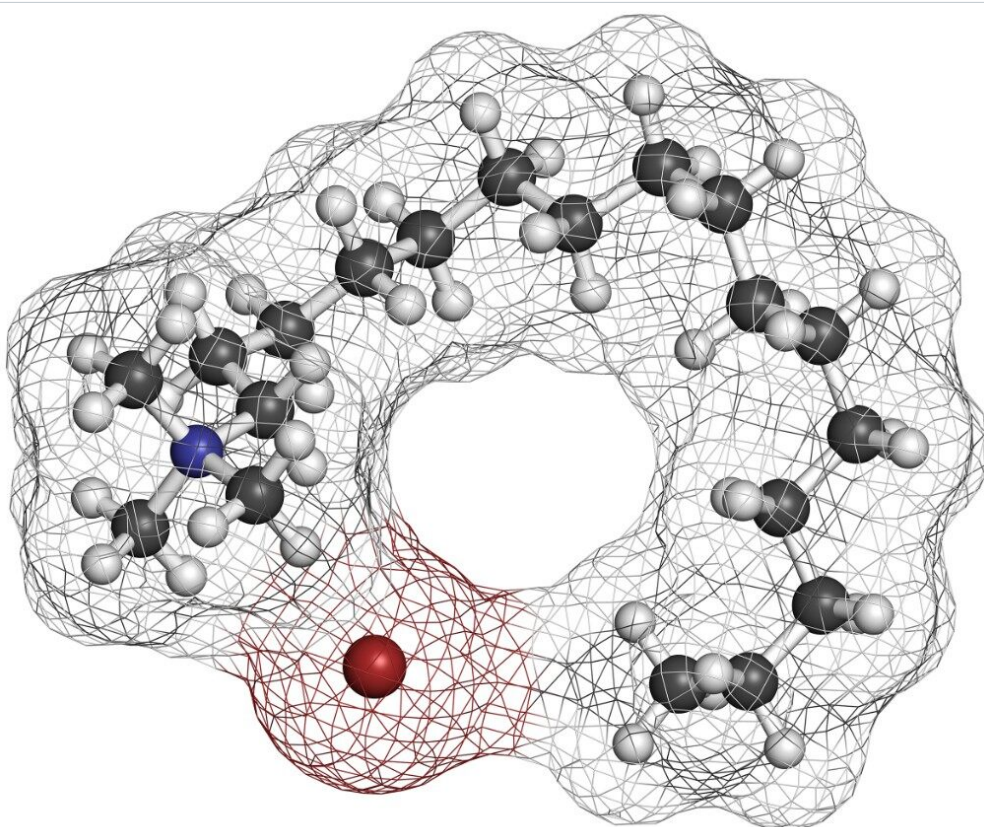


# Benefits of Using Mass Detection for Assessing Quality and Purity of Cetrimonium Bromide Pharmaceutical Raw Material

---

Margaret Maziarz, Mark Wrona, Dominic Moore, Chengxia O'Shea

日本ウォーターズ株式会社, Genzyme A Sanofi Company



---

## Abstract

In this application note, we present a robust and quick UPLC method for analysis of cetrimonium bromide raw material. This methodology was developed in partnership with Genzyme A Sanofi Company. The UPLC method utilizes an ACQUITY QDa Detector for fast, information-rich, and accurate assessment of quality and purity of raw materials. We demonstrate the system suitability and linearity of the method achievable with mass detection. This method was then used to confirm identity of, assess purity of, and assay cetrimonium bromide raw material purchased from three different suppliers.

Overall, the ACQUITY QDa Detector is a robust and simple-to-use mass detector that can be added as an orthogonal detection technique to the UV detection. It provides accurate and reliable results, making this technology ideal for routine testing of pharmaceutical raw materials in the QC laboratory.

### Benefits

- Easy and direct technique for analysis of non-chromophoric raw materials with the ACQUITY QDa Detector with confirmation and quantitation
- Accurate identification of components by mass detection with the ACQUITY QDa Detector
- Eliminate complex and time-consuming sample preparation procedures
- Compatibility with existing UPLC systems and methodologies

---

## Introduction

Pharmaceutical raw materials are substrates or elements used for manufacturing different drug products. Raw materials include active pharmaceutical ingredients (API), excipients, and other inactive ingredients. Excipients and inactive ingredients generally have no pharmacological effect, yet they are essential components that function as fillers, binders, disintegrants, lubricants, coloring agents, and preservatives.<sup>1</sup> The quality and purity of excipients and inactive ingredients are critical to the safety of the final drug product and must be controlled.<sup>2</sup> Excipients with low purity or containing contaminants may often compromise the safety and efficacy of the end pharmaceutical product. It is therefore essential to have reliable methods for accurate assessment of quality and purity of the pharmaceutical raw materials.

Rapid and accurate analysis of components that lack UV chromophores or have low UV-extinction coefficient can be challenging. As these compounds cannot be directly detected by UV, alternative methods must be employed to accurately identify and measure them. In the case of cetrimonium bromide, a non-

chromophoric material, the assay method listed in the United States Pharmacopeia (USP) Monograph<sup>3</sup> is based on a titrimetric analysis using a multistep liquid-liquid extraction with chloroform. This is a complex and timeconsuming procedure, not ideal for routine testing within the QC laboratory. Mass detection enables quick, direct, and accurate analysis of non-chromophoric compounds, eliminating the need for complex sample preparation procedures.

In this application note, we present a robust and quick UPLC method for analysis of cetrimonium bromide raw material. This methodology was developed in partnership with Genzyme A Sanofi Company. The UPLC method utilizes an ACQUITY QDa Detector for fast, information-rich, and accurate assessment of quality and purity of raw materials. We demonstrate the system suitability and linearity of the method achievable with mass detection. This method was then used to confirm identity of, assess purity of, and assay cetrimonium bromide raw material purchased from three different suppliers.

Overall, mass detection enables quick identification and accurate analysis of pharmaceutical raw materials, which makes this technology suitable for routine testing in the QC laboratory.

---

## Experimental

### Solutions preparation

#### Standards

Cetrimonium bromide USP reference standard (USP p/n: 1102974) stock solution was prepared in water at a concentration of 0.5 mg/mL. The stock solution was then diluted with water (Fisher Scientific, Optima) to a working concentration of 20.0 µg/mL. This standard solution was used to prepare linearity standards by dilution with water. Linearity standards were prepared at the following concentrations: 0.10, 0.25, 0.50, 0.75, 1.0, 2.0, and 3.0 µg/mL.

#### Raw material samples

Cetrimonium bromide raw materials tested in this study were purchased from three different suppliers:

- Sigma-Aldrich, product number: H9151-25G
- Alfa Aesar, product number: A15235
- TCI, product number: H0081

Stock sample solutions were prepared at 0.5 mg/mL concentration and then diluted with water to a concentration of 1.0 µg/mL.

## UPLC conditions

LC system:	ACQUITY UPLC H-Class
Column:	ACQUITY UPLC CSH C <sub>18</sub> , 2.1 x 50 mm, 1.7 µm
Column temp.:	40 °C
Flow rate:	0.6 mL/min
Injection volume:	1.0 µL
Solvent A:	1% Formic acid in water
Solvent B:	Water
Solvent C:	Acetonitrile
Separation:	Gradient
Purge/sample wash:	50:50 water/acetonitrile
Seal wash:	90:10 water/acetonitrile
UV detector:	ACQUITY UPLC PDA: 200–500 nm

## Gradient

Step	Time(min)	Solvent A %	Solvent B %	Solvent C %	Curve
1	Initial	10.0	40.0	50.0	Initial
2	2.0	10.0	0.0	90.0	6
3	2.5	10.0	0.0	90.0	6
4	2.6	10.0	40.0	50.0	6

Step	Time(min)	Solvent A %	Solvent B %	Solvent C %	Curve
5	4.5	10.0	40.0	50.0	6

## MS conditions

Mass detector: ACQUITY QDa (performance option)

Ionization mode: ESI+

MS acquisition range: 150–350 Da

SIR: 284.3 Da

Sampling rate: 10 pts/sec

Capillary voltage: 0.8 kV

Cone voltage: 15 V

Probe temperature: 600 °C

Data format: Centroid

Data Management

Empower 3 FR2 Chromatography Data System  
(CDS) Software

---

## Results and Discussion

Cetrimonium bromide is a quaternary ammonium salt that lacks chromophores required for UV detection (Figure 1). Thus, it cannot be directly detected by UV. However, it is readily ionizable, producing a robust MS signal on the ACQUITY QDa Detector. Chromatographic data for analysis of cetrimonium bromide is

displayed in Figure 2. The UV trace at 215 nm (Figure 2a) shows that no peaks were detected, as expected. The ACQUITY QDa data collected across the mass range (150–350 Da, Figure 2b) is referred as the total ion chromatogram (TIC) and shows that one major peak is present with a mass of 284.3 Da, corresponding to cetrimonium bromide. A specific mass of interest can be extracted from the TIC data to generate an extracted ion chromatogram (XIC) as illustrated in Figure 2c. For targeted assay testing, the data can be collected using single ion recording (SIR) scanning mode, which records the signal intensity for a specific ion of interest. SIR can simplify the analysis and quantitation, further increase signal-to-noise ratio (S/N), and is suitable for targeted assays (Figure 2d).

### **Cetrimonium bromide**

- Molecular formula:  $C_{19}H_{42}BrN$
- Average mass: 364.45 Da
- Monoisotopic mass: 363.25 Da
- Free base monoisotopic mass: 284.33 Da
- Detected mass:  $M^+ = 284.3$  Da

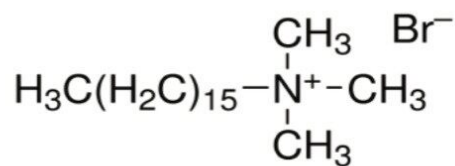


Figure 1. Structure and molecular information of cetrimonium bromide.

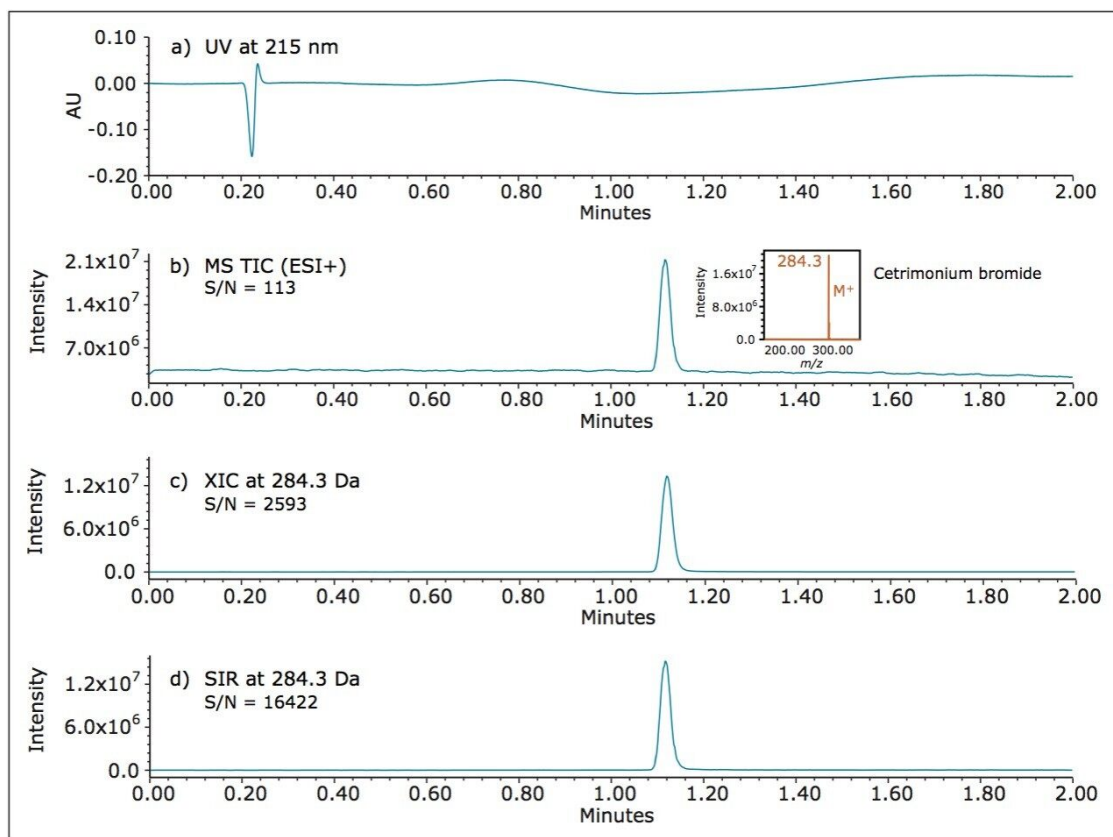


Figure 2. UV and MS chromatographic data for cetrimonium bromide acquired using the ACQUITY UPLC H-Class System with ACQUITY PDA and ACQUITY QDa Detector.

## System suitability

The performance of the developed UPLC method was verified by evaluating repeatability of six replicate injections of the USP reference standard at 2.0 µg/mL (Figure 3) according to the specifications defined in the USP General Chapter, <621>, Chromatography.<sup>4</sup> The UPLC system suitability results were processed using SIR scanning mode at 284.3 Da (Figure 4). The retention times and area repeatability were well within the USP specifications of less than 2.0% RSD.

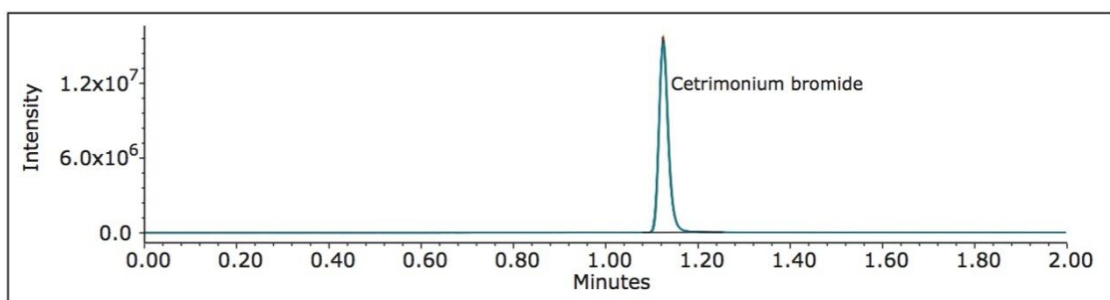


Figure 3. Overlay of six replicate injections of cetrimonium bromide USP reference standard, SIR at 284.3 Da.

<div> <div>Empower<sup>3</sup> SCFWARE</div> <div> <b>System Suitability_Report</b>  Sample Set ID: 10071  Result Set ID: 13076  Channel Name: QDa 2: SIR Ch1 </div> </div>						
Name: Cetrimonium Br						
	Name	Inj	RT	Area	USP Tailing	K Prime
1	Cetrimonium Br	1	1.124	21573313	1.2	3.5
2	Cetrimonium Br	2	1.124	21848031	1.2	3.5
3	Cetrimonium Br	3	1.125	21937619	1.2	3.5
4	Cetrimonium Br	4	1.125	21780348	1.2	3.5
5	Cetrimonium Br	5	1.126	21776335	1.2	3.5
6	Cetrimonium Br	6	1.125	21495826	1.2	3.5
Mean			1.125	21735245	1.2	3.5
Std. Dev.			0.001	167894.075		
% RSD			0.06	0.77		

Figure 4. System suitability results for six replicate injections of cetrimonium bromide USP reference standard, SIR at 284.3 Da.

## Linearity

Linearity of the method for cetrimonium bromide with SIR at 284.3 Da was evaluated over 7 concentrations ranging from 0.1 to 3 µg/mL. The method showed acceptable linearity with a correlation coefficient ( $R^2$ )  $\geq 0.9969$  (Figure 5). In addition, the percent deviation of the calculated X values or concentrations were less than 10% except for the 0.2585 µg/mL standard, which was less than 18.0% (Figure 6).

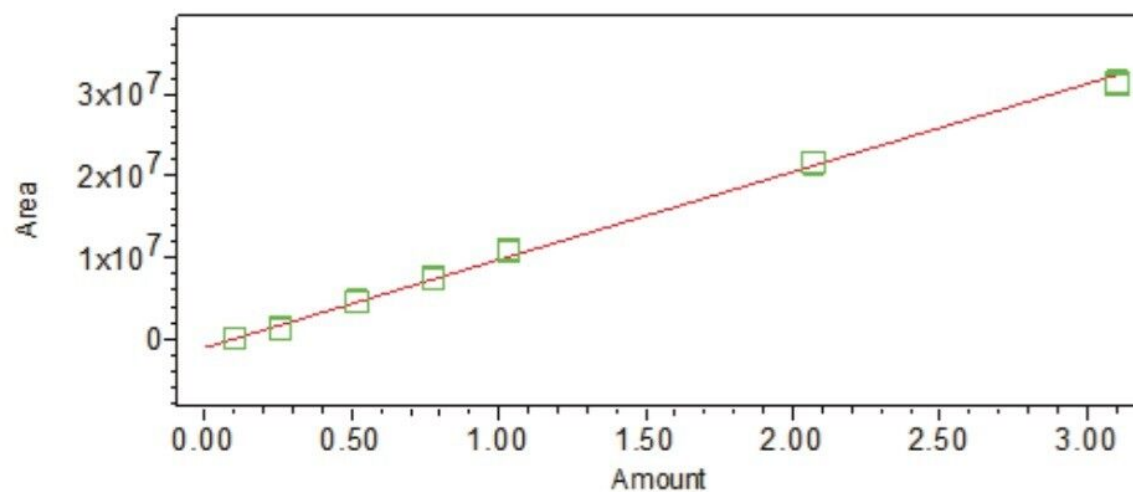


Empower 3  
SOFTWARE

## Calibration Curve

Sample Set ID: 10071  
Process Channel: QDa 2: SIR Ch1

Result Set ID: 13076  
Calibration Id: 13127



Name: Cetrimonium Br; Equation:  $Y = 1.08e+007 X - 1.09e+006$ ; A: -1.088515e+006; B: 1.080975e+007; R<sup>2</sup>: 0.996964; R: 0.998481

Figure 5. Linearity of the method for cetrimonium bromide. Data processed using SIR at 284.3 Da.

<div> <div>Empower<sup>3</sup> SOFTWARE</div> <div> <b>Cal Curve % Deviation_Accuracy</b>            Sample Set ID: 10071      Result Set ID: 13076            Channel Name: QDa 2: SIR Ch1         </div> </div>					
<b>Peak: Cetrimonium BrX Value (ug/mL): 0.10340</b>					
	Injection	Response	X Value (ug/mL)	Calc. Value (ug/mL)	% Deviation
1	1	103451	0.10340	0.11027	6.642
2	2	110301	0.10340	0.11090	7.255
3	3	94141	0.10340	0.10941	5.809
<b>Peak: Cetrimonium BrX Value (ug/mL): 1.03400</b>					
	Injection	Response	X Value (ug/mL)	Calc. Value (ug/mL)	% Deviation
1	1	10724680	1.03400	1.09283	5.689
2	2	10704598	1.03400	1.09097	5.510
3	3	11081568	1.03400	1.12584	8.882
<b>Peak: Cetrimonium BrX Value (ug/mL): 0.25850</b>					
	Injection	Response	X Value (ug/mL)	Calc. Value (ug/mL)	% Deviation
1	1	1266680	0.25850	0.21788	-15.715
2	2	1220622	0.25850	0.21362	-17.363
3	3	1292652	0.25850	0.22028	-14.785
<b>Peak: Cetrimonium BrX Value (ug/mL): 2.06800</b>					
	Injection	Response	X Value (ug/mL)	Calc. Value (ug/mL)	% Deviation
1	1	21569343	2.06800	2.09606	1.357
2	2	21569445	2.06800	2.09607	1.357
3	3	21684750	2.06800	2.10673	1.873
<b>Peak: Cetrimonium BrX Value (ug/mL): 0.51700</b>					
	Injection	Response	X Value (ug/mL)	Calc. Value (ug/mL)	% Deviation
1	1	4658207	0.51700	0.53162	2.829
2	2	4578027	0.51700	0.52421	1.394
3	3	4662449	0.51700	0.53202	2.905
<b>Peak: Cetrimonium BrX Value (ug/mL): 3.10200</b>					
	Injection	Response	X Value (ug/mL)	Calc. Value (ug/mL)	% Deviation
1	1	31475067	3.10200	3.01243	-2.888
2	2	31625936	3.10200	3.02638	-2.438
3	3	31234155	3.10200	2.99014	-3.606
<b>Peak: Cetrimonium BrX Value (ug/mL): 0.77550</b>					
	Injection	Response	X Value (ug/mL)	Calc. Value (ug/mL)	% Deviation
1	1	7480280	0.77550	0.79269	2.217
2	2	7513669	0.77550	0.79578	2.615
3	3	7333195	0.77550	0.77908	0.462

Figure 6. Calibration curve data processed using SIR at 284.3 Da. The percent deviation of the calculated x values or concentrations were less than 10% except for the 0.2585  $\mu\text{g/mL}$  standard, which was less than 18.0%.

## Sample analysis

Cetrimonium bromide purchased from three different suppliers was analyzed using UPLC-MS. The ACQUITY QDa Detector enabled quick confirmation of peak identity by mass detection. For peak purity assessment, we used ACQUITY QDa TIC data collected across a mass range (150–350 Da). We evaluated the MS TIC data for presence of any peaks with mass different than 284.3 ( $\pm 0.2$ ) Da, which corresponds to the mass of cetrimonium bromide. As shown in TIC traces (Figure 7), only one major peak was detected in the sample injections with a mass specific for cetrimonium bromide. In addition, the MS spectral data at the leading, apex, and tailing edge of the peak shows presence of one mass which corresponds to cetrimonium bromide. This demonstrates that cetrimonium bromide is not coeluting with other peaks. Overall, the TIC mass data demonstrated that no contaminants were present in the cetrimonium bromide pharmaceutical

raw materials tested in this study.

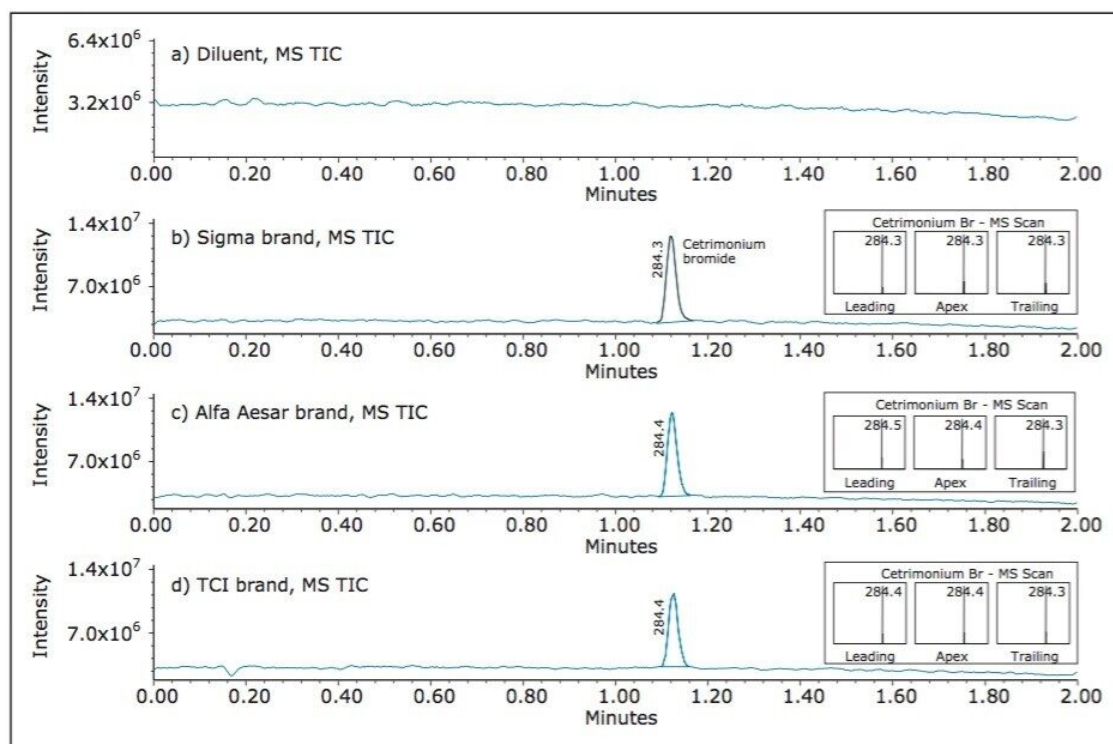



Figure 7. Cetrimonium bromide raw material purchased from three different suppliers. TIC MS and mass spectral data at the leading, apex, and tailing edge of the peak.

For assay testing, we compared cetrimonium bromide materials purchased from three different suppliers against the USP reference standard. The assay results generated using SIR at 284.3 Da (Figure 8) ranged from 98.4 to 100.4 % for all raw materials, which meet the USP acceptance criteria of 96.0 to 101.0 % defined in the USP Monograph for cetrimonium bromide.



**Sample\_Analysis\_Report**  
Sample Set ID: 9461  
Result Set ID: 13201  
Channel Name: QDa 2: SIR Ch1

SampleName: Sigma brand

	SampleName	Name	RT (min.)	Area	Calibration Id	Calculated Amount (ug/mL)	Target Amount (ug/mL)	Assay (%)
1	Sigma brand	Cetrimonium Br	1.124	10684061	13127	1.0891	1.0850	100.38

SampleName: Alfa Aesar brand

	SampleName	Name	RT (min.)	Area	Calibration Id	Calculated Amount (ug/mL)	Target Amount (ug/mL)	Assay (%)
1	Alfa Aesar brand	Cetrimonium Br	1.122	9244808	13127	0.9559	0.9660	98.96

SampleName: TCI brand

	SampleName	Name	RT (min.)	Area	Calibration Id	Calculated Amount (ug/mL)	Target Amount (ug/mL)	Assay (%)
1	TCI brand	Cetrimonium Br	1.125	9496532	13127	0.9792	0.9950	98.41

Figure 8. Assay results for cetrimonium bromide raw materials purchased from three different suppliers, SIR at 284.3 Da.

## Conclusion

Mass detection using the ACQUITY QDa Detector coupled with UPLC enables quick and easy analysis of non-chromophoric cetrimonium bromide raw material. The new method eliminates the need for a complex titrimetric procedure, hence improving productivity. Furthermore, the LC-MS method provides improved confidence associated with sample confirmation and accurate assessment of purity and assay of cetrimonium bromide pharmaceutical raw material. The system suitability and linearity of the method calculated using mass data were excellent.

Overall, the ACQUITY QDa Detector is a robust and simple-to-use mass detector that can be added as an orthogonal detection technique to the UV detection. It provides accurate and reliable results, making this technology ideal for routine testing of pharmaceutical raw materials in the QC laboratory.

## References

1. Overview of pharmaceutical excipients used in tablets and capsules, October 24 2008.
2. The United States Pharmacopeia (USP) Monograph Excipients Introduction.

3. USP Monograph, Cetrimonium Bromide, USP37-NF32, The United States Pharmacopeia Convention, official August 1, 2014.
4. USP General Chapter, <621>, Chromatography, USP37-NF32, The United States Pharmacopeia Convention, official August 2, 2014.

---

## Featured Products

ACQUITY UPLC H-Class PLUS System <<https://www.waters.com/10138533>>

ACQUITY UPLC Columns <<https://www.waters.com/513206>>

ACQUITY QDa Mass Detector <<https://www.waters.com/134761404>>

Empower 3 Chromatography Data Software <<https://www.waters.com/513188>>

### Available for purchase online

ACQUITY UPLC CSH C18 Column, 130Å, 1.7 µm, 2.1 mm X 50 mm, 1/pkg <  
[https://www.waters.com/waters/partDetail.htm?locale=en\\_US&partNumber=186005296](https://www.waters.com/waters/partDetail.htm?locale=en_US&partNumber=186005296)>

720005324, March 2015