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Application Note

Benefits of Using Mass Detection for Analysis of Cetrimonium Bromide Pharmaceutical Raw Material

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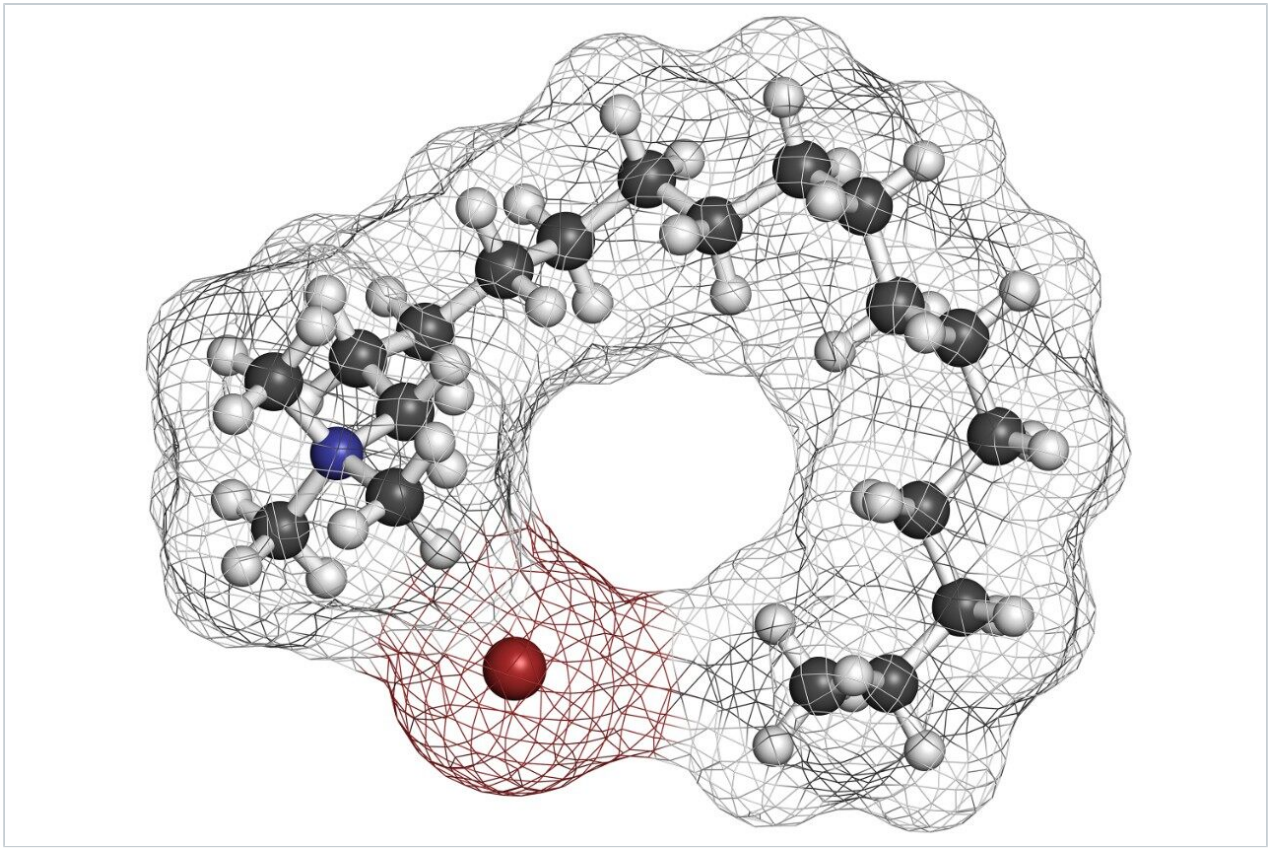


This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief demonstrates benefits of using ACQUITY UPLC combined with an ACQUITY QDa Detector for rapid raw material testing of cetrimonium bromide, instead of a multistep titrimetric assay.

Benefits

The UPLC method coupled with an ACQUITY QDa Detector eliminates titrimetric analysis with a complex sample preparation, resulting in time savings and increased productivity.

Introduction

Pharmaceutical raw materials are substrates or elements used for manufacturing of different drug products. Raw materials include active pharmaceutical ingredients (API), excipients, and other inactive ingredients. While they generally have no pharmacological effect, excipients are essential components that function as fillers, binders, disintegrants, lubricants, coloring agents, and preservatives.[1]

Cetrimonium bromide (CTAB) is an antiseptic agent used for prevention of bacteria and fungi growth. It can also be used as a cosmetic biocide, surfactant, and preservative.[2] The method listed in the United States Pharmacopeia (USP) Monograph for Cetrimonium Bromide[3] is based on a titrimetric analysis of a sample solution prepared using a multistep liquid-liquid extraction with chloroform. This is a complex and time-consuming procedure, not ideal for routine assay testing within a QC laboratory.

This method was developed for use by a large biopharmaceutical company to develop a robust and quick UPLC method for a cetrimonium bromide assay to replace a multistep titrimetric assay method. This application brief illustrates the use of UPLC coupled with an ACQUITY QDa – a small and robust mass detector for rapid raw material testing.

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

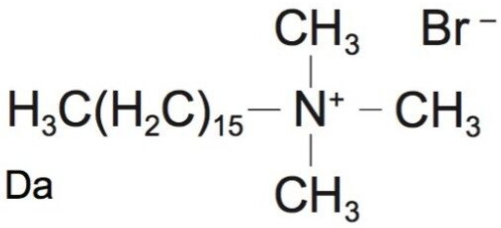


Figure 1. Structure and molecular information of cetrimonium bromide.

Experimental

Sample used in this study was prepared by dissolving cetrimonium bromide in water to make a stock concentration of 0.4 mg/mL. Stock solution was then diluted with water to the working concentration of 2.5 µg/mL.

The chromatographic separation was performed on an ACQUITY UPLC H-Class System using method conditions described in Figure 2.

Parameter	Description
System	ACQUITY UPLC H-Class with ACQUITY QDa and PDA
Column	ACQUITY UPLC CSH C_{18}, 2.1 x 50 mm, 1.7-µm
Column temp.	40 °C
Flow rate	0.6 mL/min
Injection vol.	1.0 µL
Mobile phase	Solvent A: 1% Formic acid in water (low pH) Solvent C: Water Solvent D1: Acetonitrile
Gradient	
Wash solvents	Purge: 50:50 water/acetonitrile Sample wash: 90:10 water/acetonitrile Seal wash: 90:10 water/acetonitrile
PDA detection	200 – 500 nm
Mass detection	**ACQUITY QDa (extended performance)** Ionization mode: ESI+, ESI- Acquisition range: 150 – 350 Da

	Time	Flow (mL/min)	%A	%B	%C	%D	Curve
1	Initial	0.600	10.0	0.0	40.0	50.0	Initial
2	2.00	0.600	10.0	0.0	0.0	90.0	6
3	2.50	0.600	10.0	0.0	0.0	90.0	6
4	2.60	0.600	10.0	0.0	40.0	50.0	6
5	4.50	0.600	10.0	0.0	40.0	50.0	6

Figure 2. Conditions of UPLC method for analysis of cetrimonium bromide.

Results and Discussion

Since cetrimonium bromide lacks chromophores required for UV detection, it cannot be directly detected by UV. However, it is readily ionizable, producing a robust MS signal on the ACQUITY QDa Detector. Figure 3 shows several traces for cetrimonium bromide. As expected, no peaks were detected on the UV trace at 215 nm (Figure 3a). The ACQUITY QDa data collected across the entire mass range (150 – 350 Da, Figure 3b) is referred as the total ion chromatogram (TIC). The TIC can be extracted at mass 284.3 Da to give an extracted ion chromatogram (XIC) specific to the target of interest as illustrated in Figure 3c. For targeted analyses and

a targeted assay desired, data can be collected simply using the single ion recording (SIR) scanning mode, which only scans for the peak of interest and can greatly simplify analysis as well as increase S/N and sensitivity further (Figure 3d.). The ACQUITY QDa Detector enabled direct detection of nonchromophoric cetrimonium bromide without the need for a complex sample preparation procedure.

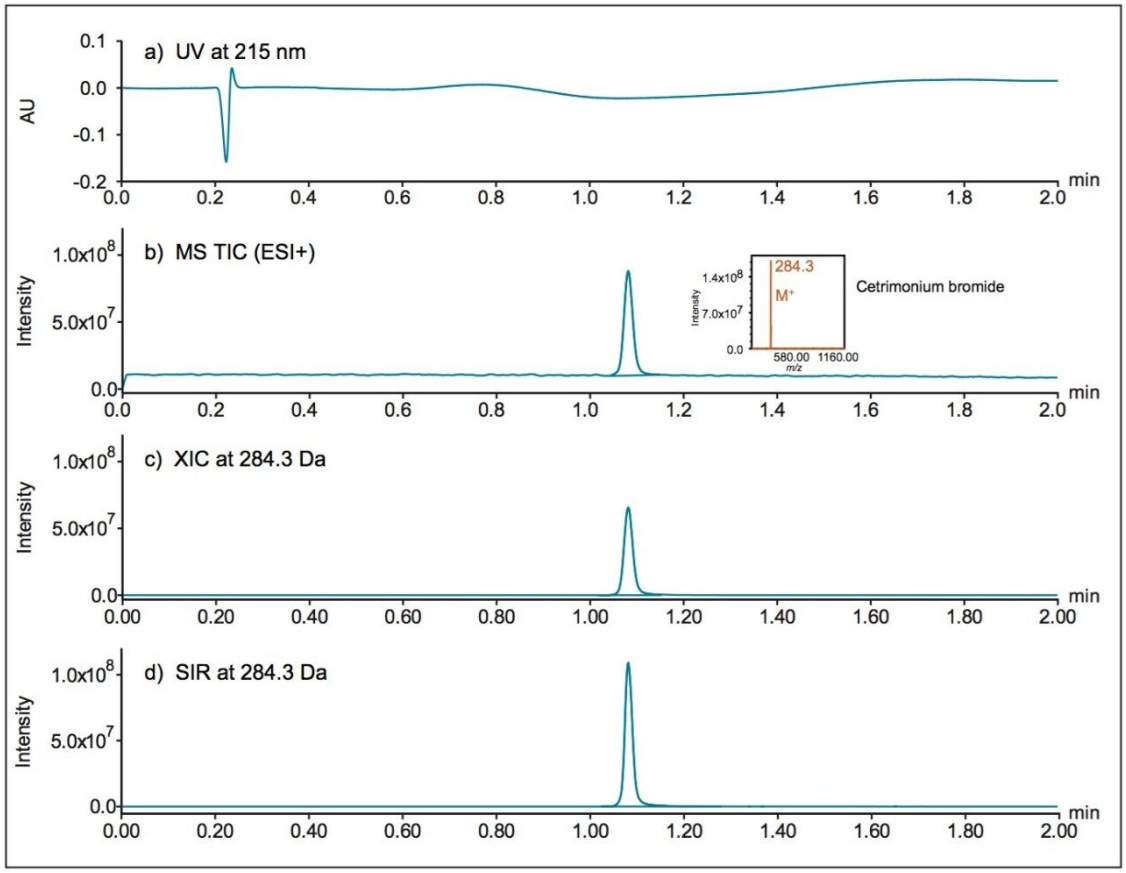
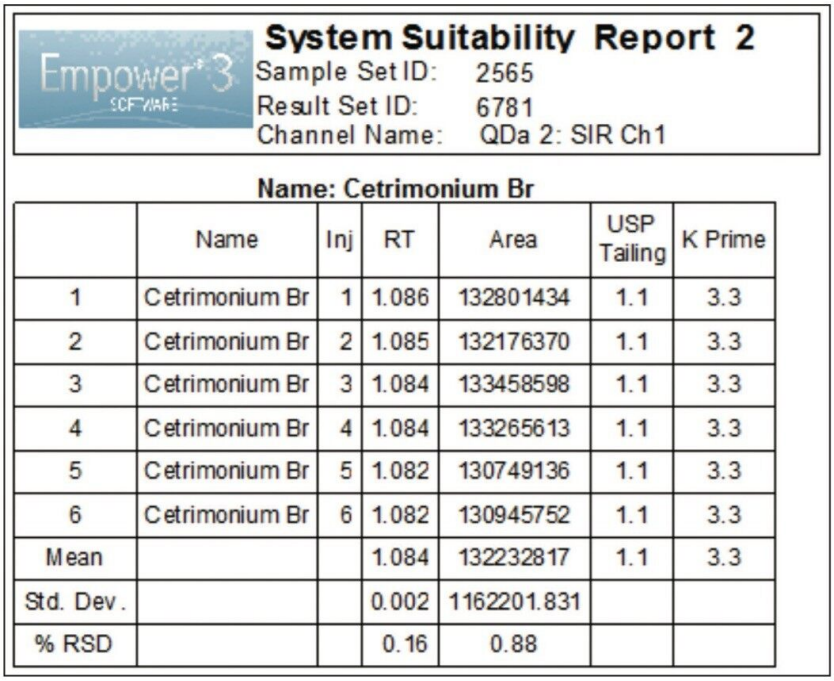


Figure 3. UV and MS chromatographic data for cetrimonium bromide acquired using the ACQUITY UPLC H-Class System with PDA and ACQUITY QDa Detector.

System Suitability

The performance of the developed UPLC method was verified by evaluating repeatability of six replicate injections of the 2.5 µg/mL standard according to the specifications defined in the USP General Chapter, <621>, Chromatography.[4] The UPLC System suitability results were processed using SIR mass data at 284.3 Da (Figure 4). The retention times and area repeatability were well within the USP specifications of less than 2% RSD.



System Suitability Report 2

Sample Set ID: 2565
Result Set ID: 6781
Channel Name: QDa 2: SIR Ch1

Name: Cetrimonium Br

	Name	Inj	RT	Area	USP Tailing	K Prime
1	Cetrimonium Br	1	1.086	132801434	1.1	3.3
2	Cetrimonium Br	2	1.085	132176370	1.1	3.3
3	Cetrimonium Br	3	1.084	133458598	1.1	3.3
4	Cetrimonium Br	4	1.084	133265613	1.1	3.3
5	Cetrimonium Br	5	1.082	130749136	1.1	3.3
6	Cetrimonium Br	6	1.082	130945752	1.1	3.3
Mean			1.084	132232817	1.1	3.3
Std. Dev.			0.002	1162201.831		
% RSD			0.16	0.88		

Figure 4. System suitability results for six replicate injections of sample solution, SIR mass data at 284.3 Da.

Linearity

Linearity of the method for cetrimonium bromide with SIR mass detection at 284.3 Da was evaluated over 7 concentrations ranging from 0.1 µg/mL to 3 µg/mL. The method showed a good linear behavior between the peak areas and concentrations of centrimonium bromide with correlation coefficient (R^2) ≥0.999 (Figure 5)

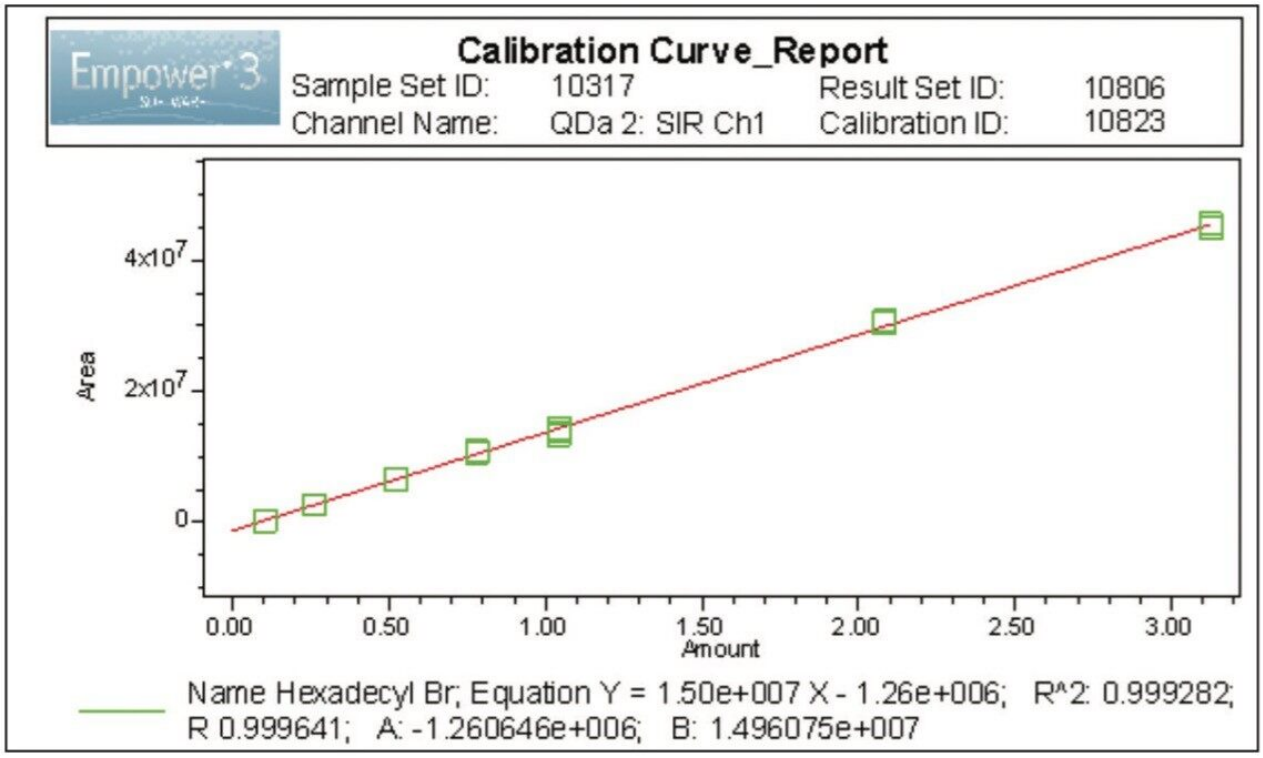


Figure 5. Linearity of the method for cetrimonium bromide. Data processed using SIR mass data at 284.3 Da.

Conclusion

The UPLC-MS method developed with ACQUITY UPLC and ACQUITY QDa eliminates a complex sample preparation procedure and enables a quick and easy assay of the raw material with confirmation and quantitation. This resulted in time savings and increased productivity. In addition, 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 easily added as an orthogonal detection technique. It provides accurate and reliable results, making this technology ideal for routine testing in the QC laboratory.

References

1. Overview of pharmaceutical excipients used in tablets and capsules, http://drugtopics.modernmedicine.com/drug-topics/news/modernmedicine/modern-medicine-news/overviewpharmaceutical- excipients-used-tablets, October 24 2008.
2. http://www.ewg.org/skindeep/ingredient/717624/CETRIMONIUM_BROMIDE/
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.

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ACQUITY UPLC M-Class System <https://www.waters.com/134776759>

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

Empower 3 Chromatography Data Software <https://www.waters.com/10190669>

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