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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.1 The quality and purity of excipients and inactive ingredients are critical to the safety of the final drug product and must be controlled.2 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 Pharmacopoeia (USP) Monograph3 is based on a titrimetric analysis using a multistep liquid-liquid extraction with chloroform. This is a complex and time-consuming 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.
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™ C18, 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

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Purge/sample wash: 50:50 water/acetonitrile
Seal wash: 90:10 water/acetonitrile
UV detector: ACQUITY UPLC PDA: 200–500 nm

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).

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. 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 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.

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

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).

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

**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 µg/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 (±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.

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](image)

**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.

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

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.