

Benefits of Using Mass Detection for Pharmaceutical Raw Materials Analysis of 1-(3-Dimethylaminopropyl)-3-Ethylcarbodiimide Hydrochloride

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GOAL

To replace titrimetric and flow injection analysis of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride raw material with an alternative approach using an ACQUITY UPLC® System with an ACQUITY® QDa® Detector.

BACKGROUND

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

1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) is a cross linking reagent used for peptide synthesis, protein crosslinking to nucleic acids, and in preparation of immunoconjugates. Methods for analysis of EDC·HCl found in the literature include a fluorometric method coupled with a titration technique² and spectrophotometric flow injection analysis.³ While effective, these methods are not ideal for QC laboratories. The titration technique lacks reproducibility required for routine testing, while the flow injection analysis depends on reaction efficiency and often lacks the sensitivity required for analysis of pharmaceutical samples.

The ACQUITY UPLC System coupled with an ACQUITY QDa Detector eliminates titrimetric and flow injection techniques and provides simplistic analysis of the EDC·HCl raw material.

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride

- Molecular formula: C₈H₁₈ClN₃
- Average mass: 191.70 Da
- Monoisotopic mass: 191.12 Da
- Free base monoisotopic mass: 155.24 Da
- Detected mass [M + H]⁺: 156.2 Da

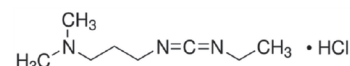


Figure 1. Chemical information and structure of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride.

Here we describe a robust and quick UPLC® method for analysis of EDC·HCl. This methodology was developed in partnership with a large multinational biopharmaceutical company. The UPLC method utilizes an ACQUITY QDa Detector for fast, information-rich, and accurate testing of raw materials.

THE SOLUTION

The sample used in this study was prepared by dissolving EDC·HCl material in water to make a stock solution at 0.5 mg/mL. The stock solution was then diluted with water to a working concentration of 2.0 µg/mL.

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

The chromatographic data for analysis of EDC·HCl is displayed in Figure 3. The UV trace at 215 nm (Figure 3A) shows that a small peak was detected, indicating that EDC·HCl exhibits poor UV absorbance. The ACQUITY QDa Detector data

collected across the entire mass range (50 – 250 Da, Figure 3B) is referred to as the total ion chromatogram (TIC). A specific mass of interest can be extracted from the scanning data (TIC) to generate an extracted ion chromatogram (XIC) as illustrated in Figure 3C. In this case we monitor the mass-to-ratio (m/z) of 156.2 Da corresponding to EDC•HCl. For targeted assay analyses, the data can be collected using single ion recording (SIR) mode, which records the signal intensity for a specific ion of interest and can simplify both the analysis and increase of S/N further, Figure 3D.

The performance of the UPLC method was verified by evaluating repeatability of six replicate injections of the 2.0 µg/mL standard according to the specifications defined in the USP General Chapter, <621>, Chromatography.⁴ The ACQUITY UPLC System suitability results, processed using SIR collected for a mass of 156.2 Da, are shown in Figure 4. The retention times and area repeatability were well within the USP specifications of less than 2% relative standard deviation (RSD).

Linearity

Linearity of the method for EDC•HCl with SIR at 156.2 Da was evaluated over 10 concentration levels ranging from 0.05 to 5.00 µg/mL. The method showed good linear behavior with a correlation coefficient (R^2) ≥ 0.997 , Figure 5.

SUMMARY

The ACQUITY UPLC System coupled with an ACQUITY QDa Detector eliminates the titrimetric and flow injection techniques and provides simplistic analysis of the EDC•HCl raw material. System suitability and linearity of the method calculated using mass data were excellent. The new method provides improved confidence associated with sample confirmation and increased productivity.

Parameter	Description																																																
System	ACQUITY UPLC H-Class with ACQUITY QDa and PDA																																																
Column	ACQUITY UPLC BEH C ₁₈ , 1.7 µm, 2.1 x 50 mm																																																
Column temp.	40 °C																																																
Flow rate	0.6 mL/min																																																
Injection vol.	1.0 µL																																																
Mobile phase	Solvent B: 1% Ammonium hydroxide in water (high pH) Solvent C: Water Solvent D1: Acetonitrile																																																
Gradient	<table border="1"> <thead> <tr> <th></th> <th>Time</th> <th>Flow (mL/min)</th> <th>%A</th> <th>%B</th> <th>%C</th> <th>%D</th> <th>Curve</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>Initial</td> <td>0.600</td> <td>0.0</td> <td>10.0</td> <td>85.0</td> <td>5.0</td> <td>Initial</td> </tr> <tr> <td>2</td> <td>3.00</td> <td>0.600</td> <td>0.0</td> <td>10.0</td> <td>0.0</td> <td>90.0</td> <td>6</td> </tr> <tr> <td>3</td> <td>3.50</td> <td>0.600</td> <td>0.0</td> <td>10.0</td> <td>0.0</td> <td>90.0</td> <td>6</td> </tr> <tr> <td>4</td> <td>3.60</td> <td>0.600</td> <td>0.0</td> <td>10.0</td> <td>85.0</td> <td>5.0</td> <td>6</td> </tr> <tr> <td>5</td> <td>5.50</td> <td>0.600</td> <td>0.0</td> <td>10.0</td> <td>85.0</td> <td>5.0</td> <td>6</td> </tr> </tbody> </table>		Time	Flow (mL/min)	%A	%B	%C	%D	Curve	1	Initial	0.600	0.0	10.0	85.0	5.0	Initial	2	3.00	0.600	0.0	10.0	0.0	90.0	6	3	3.50	0.600	0.0	10.0	0.0	90.0	6	4	3.60	0.600	0.0	10.0	85.0	5.0	6	5	5.50	0.600	0.0	10.0	85.0	5.0	6
	Time	Flow (mL/min)	%A	%B	%C	%D	Curve																																										
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5	5.50	0.600	0.0	10.0	85.0	5.0	6																																										
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: 50 – 250 Da																																																

Figure 2. Conditions of UPLC method for analysis of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride.

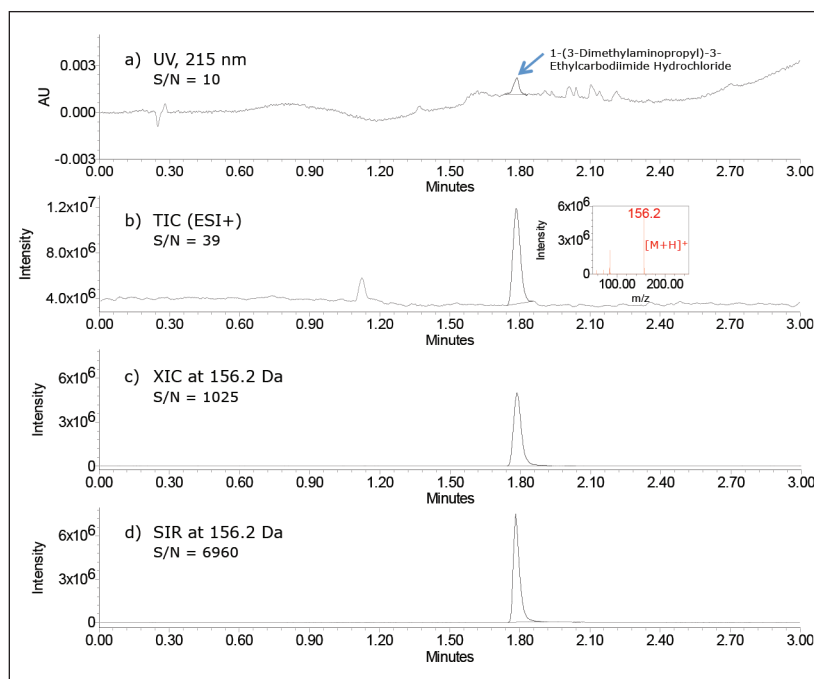


Figure 3. UV and MS chromatographic data acquired using an ACQUITY UPLC H-Class System with ACQUITY UPLC PDA and ACQUITY QDa Detector.

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

References

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2. Chen S-H. Fluorometric Determination of Carbodiimides with trans-Aconitic Acid. *Anal Biochem*. 1983 Jul 15;132(2):272-5.
3. Seno K, Matsumura K, Ohita K, Ohima M, Motomizu S. Development of Sequential Injection Analysis for Determination of 1-(3-Dimethylaminopropyl)-3-Ethylcarbodiimide Hydrochloride (EDC-HCl) Based on Condensation Reaction between Malonic Acid and Ethylenediamine. *J Flow Injection Anal*. 2009;26(1): 27-30.
4. USP General Chapter, <621>, Chromatography, USP37-NF32, The United States Pharmacopeia Convention, official August 2, 2014.

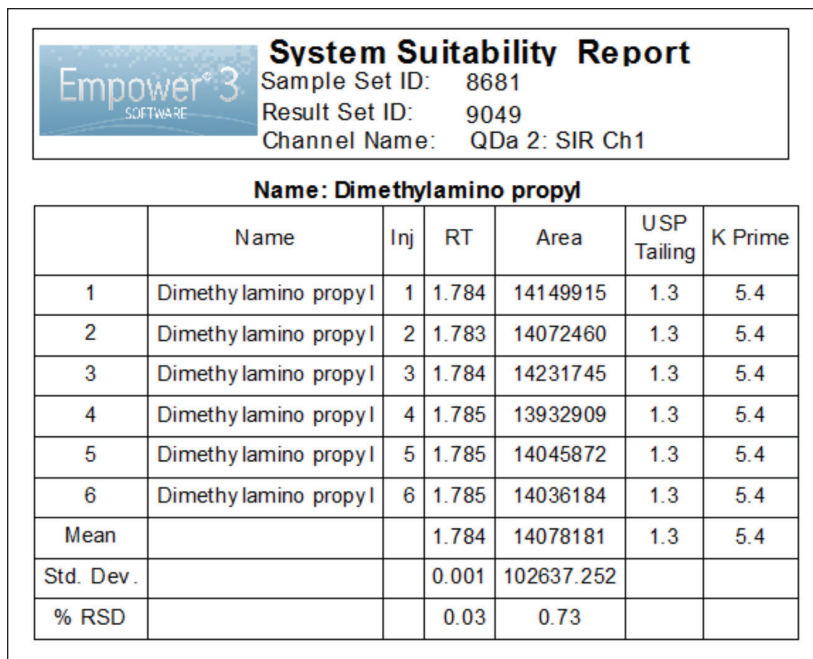


Figure 4. System suitability results for six replicate injections of sample solution, SIR at 156.2 Da.

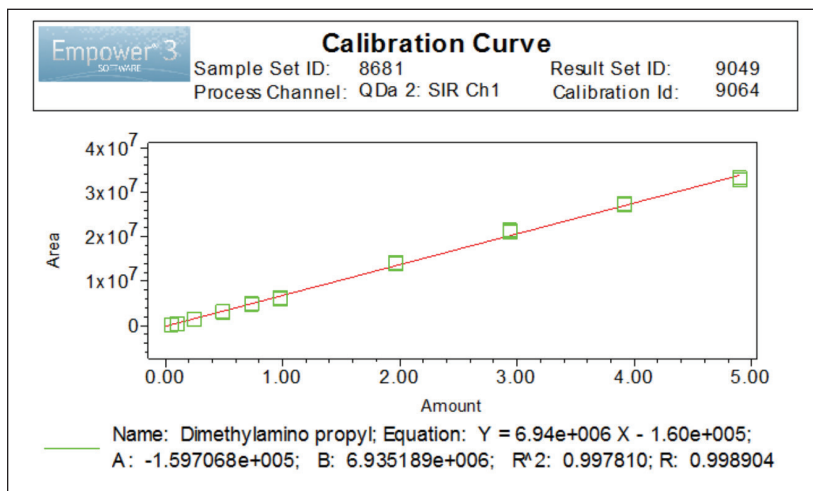


Figure 5. Linearity of the method for 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride. Data processed using SIR at 156.2 Da.

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