Chapter 13

Cetirizine Quantification by High-Performance Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

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Abstract

A multiple reaction monitoring (MRM), positive ion electrospray ionization, LC/MS/MS method is described for the quantification of cetirizine. The compound was isolated from human plasma by protein precipitation using acetonitrile. Cetirizine d4 was used as an internal standard. Chromatographic conditions were achieved using a C18 column and a combination of ammonium acetate, water, and methanol as the mobile phase. MRMs were: cetirizine, 389.26 → 165.16, 201.09; cetirizine d4, 393.09 → 165.15, 201.10. Calibration curves were constructed by plotting the peak area ratios of the calibrators' target MRM transition area to labeled internal standard target MRM transition area versus concentration.

Key words Cetirizine, Antihistamine, H_1-receptor, Allergic rhinitis and chronic urticaria

1 Introduction

Levocetirizine (RS)-2-[2-[4-[(2-Chlorophenyl)phenylmethyl] piperazin-1-yl]ethoxy]acetic Acid Dihydrochloride is a second-generation antihistamine and *R*-enantiomer of the cetirizine an active metabolite of hydroxyzine (a first-generation H_1-receptor inverse agonist) [1–4]. Cetirizine is a highly selective H_1-receptor inverse agonist and a potent non-sedating antihistamine [1–3]. As compared to other commonly used antihistamines, cetirizine has less affinity for calcium channel, adrenergic α_1, dopamine D_2, serotonin 5-HT_2 receptors, and muscarinic receptors. Cetirizine is minimally metabolized with an elimination half-life of approximately 8 h. The drug is 91 % protein bound, and also has a small volume of distribution (Vd) of 0.4 L/kg [5, 6]. Due to these pharmacologic properties, cetirizine is commonly prescribed to patients with allergic disease (e.g. allergic rhinitis and chronic urticaria) and is approved for use in children (2 years of age and older) and adults.

Various methods for cetirizine measurement including gas chromatography, high-performance liquid chromatography (HPLC) with UV or mass spectrometry detection have been

Uttam Garg (ed.), *Clinical Applications of Mass Spectrometry in Drug Analysis: Methods and Protocols*, Methods in Molecular Biology, vol. 1383, DOI 10.1007/978-1-4939-3252-8_13, © Springer Science+Business Media New York 2016

described in the literature [7–13]. We developed a simple, rapid, and highly sensitive method utilizing HPLC-tandem mass spectrometry for the measurement of cetirizine.

2 Materials

2.1 Samples

Heparinized plasma.

2.2 Solvents and Reagents

1. 7.5 M Ammonium acetate. Purchased as solution (Sigma-Aldrich, St Louis, MO).
2. Precipitating reagent containing internal standard: Add 200 μL of 50 μg/mL of secondary internal standard to a 100 mL volumetric flask and bring to volume with acetonitrile. Stable for 1 year at −20 °C.
3. Mobile phase A (20 mM ammonium acetate in water): To 1 L water, add 2.7 mL of 7.5 mM ammonium acetate and 570 μL of formic acid. Mix and degas. Store at ambient temperature. Stable for 1 month.
4. Mobile phase B (20 mM ammonium acetate in methanol): To 1 L methanol, add 2.7 mL of 7.5 mM ammonium acetate and 570 μL of formic acid. Mix and degas. Store at ambient temperature. Stable for 1 month.
5. Fresh frozen plasma: Obtain outdated fresh frozen plasma from blood bank or commercial source. Centrifuge at 2000 × *g* for 10 min to remove particulates.

2.3 Internal Standards and Standards

1. Stock standard of Cetirizine (Sigma-Aldrich, St Louis, MO): Quantitatively prepare a 1 mg/mL stock standard of cetirizine, using cetirizine dihydrochloride, in methanol. Stable for 1 year when stored at −20 °C.
2. 100 μg/mL primary standard: Prepared by transferring 1 mL of stock standard to a 10 mL volumetric flask and diluting with methanol. Stable for 1 year when stored at −20 °C.
3. 10 μg/mL secondary standard: Prepared by transferring 1 mL of primary standard to a 10 mL volumetric flask and diluting with methanol. Stable for 1 year when stored at −20 °C.
4. 1 μg/mL tertiary standard: Prepare by transferring 1 mL of secondary standard to a 10 mL volumetric flask and diluting with methanol. Stable for 1 year when stored at −20 °C.
5. 1 mg/mL primary internal standard (Cetirizine-*d4*, C/D/N/Isotopes): Quantitatively prepare a 1 mg/mL primary standard of Cetirizine-$d4_1$ in methanol. Stable for 1 year when stored at −20 °C.

Table 1
Preparation of calibrators using drug-free plasma

Calibrator	Primary standard (μL)	Secondary standard (μL)	Tertiary standard (μL)	Final concentration (ng/mL)
Blank				
1			10	1
2		10		10
3		50		50
4		100		100
5	50			500

The final volume of each calibrator is 10 mL

Table 2
Preparation of quality controls using drug-free plasma

QC	Primary standard (μL)	Secondary standard (μL)	Tertiary standard (μL)	Final concentration (ng/mL)
1			30	3
2	25			250
3	40			400

The final volume of each control is 10 mL

6. 50 μg/mL secondary internal standard: Prepare by transferring 500 μL of primary standard to a 10 mL volumetric flask and diluting with methanol. Stable for 1 year when stored at −20 °C.

2.4 Calibrators and Controls

1. Calibrators: Prepare calibrators 1–5 according to Table 1.
2. Quality Controls: Prepare control 1–3 according to Table 2.

For calibrators and controls add appropriate amount of standards to a 10 mL volumetric flask and qs to 10 mL with plasma (*see* **Note 1**).

2.5 Analytical Equipment and Supplies

1. AB Sciex LC-MS/MS 4000Q TRAP (Foster City, CA).
2. Shimadzu Prominence HPLC system with autosampler, two pumps and degasser (Lenexa, KS).
3. Autosampler vials with caps.
4. Analytical column: Supelcosil LC-18, 5 cm × 4.6 mm × 3 μm (Sigma-Aldrich, St Louis, MO).
5. Guard column: Pinnacle, C18, 10 mm × 4 mm × 5 μm (Restek, Belfonte, PA).

3 Methods

3.1 Stepwise Procedure

1. Pipette 100 μL of well mixed calibrators, patient plasma and controls to the appropriately labeled microcentrifuge tubes.
2. Add 200 μL of precipitating reagent containing internal standards to each tube.
3. Immediately cap the samples and vortex for ~20 s.
4. Rock the tubes for 10 min.
5. Centrifuge the tubes at 10,000 × *g* for 5 min.
6. Using disposable tips transfer 200 μL of supernatant into autosampler (*see* **Note 2**).
7. Inject 10 μL into liquid chromatography-tandem mass spectrometry (LC-MSMS) for analysis.

3.2 Instrument's Operating Conditions

Instrument's operating conditions are given in Table 3.

3.3 Data Analysis

1. Data are collected and analyzed using Analyst 1.5.1 software (AB Sciex, Foster City, CA).
2. Calibration curves are constructed from peak area ratios of MRM of calibrators and internal standards versus concentration.

Table 3
Instrument's operating conditions

A. HPLC		
Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.5	50	50
1.5	0	100
3	0	100
3.1	50	50
B. MS/MS parameters		
Source (electrospray ionization, positive mode)		
Curtain gas		25 psi
Source temperature		375 °C
Collision gas (CAD)		High
Ion source gas 1 (GS1)		50 psi
Ion source gas 1 (GS2)		60 psi

Column temperature—65 °C, Flow rate—1.0 mL/min

Table 4
Multiple reaction monitoring transitions

Analyte	Q1 mass (amu)	Q3 mass (amu)	Qualifier ion
Cetirizine	389.26	165.16	201.09
Cetirizine-d4	393.09	165.15	201.10

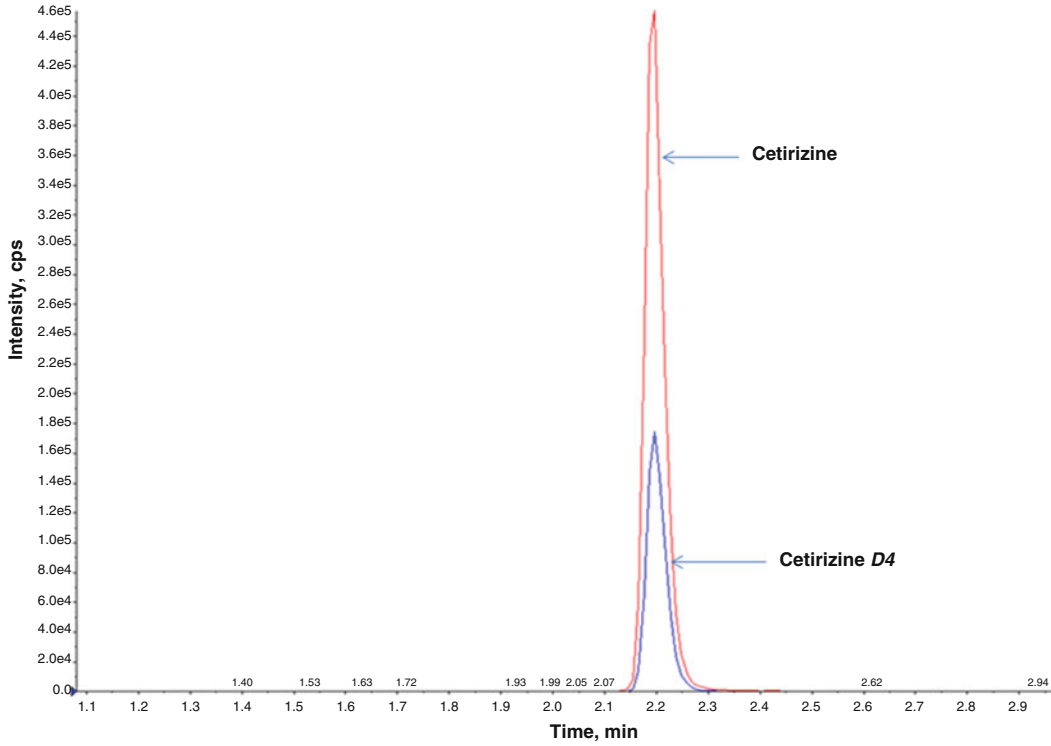

Fig. 1 A representative HPLC/MS/MS chromatogram of cetirizine and cetirizine-d4

3. A typical calibration curve has a correlation (r^2) >0.99.
4. Multiple reaction monitoring transitions for each analyte are given in Table 4.
5. A typical HPLC/MS/MS chromatogram of cetirizine is shown in Fig. 1.
6. Quality control samples are evaluated with each run. The run is considered acceptable if calculated concentrations of controls are within the ±20 % of target values.
7. Samples with results greater than upper limit of linearity should be diluted with blank.

4 Notes

1. When possible, calibrators and controls should be prepared from different lot of stock solution on separate days.
2. Be sure not to disturb the pellet.

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