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Low level quantitation of Loratadine from plasma using LC/MS/MS

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Shailesh Damale, Deepti Bhandarkar, Shruti Raju, Rashi Kochhar, Shailendra Rane, Ajit Datar, Pratap Rasam, Jitendra Kelkar
Shimadzu Analytical (India) Pvt. Ltd., 1 A/B Rushabh Chambers, Makwana Road, Marol, Andheri (E), Mumbai-400059, Maharashtra, India.

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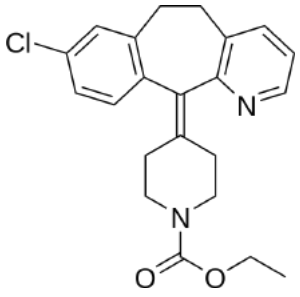
Introduction

Loratadine is a histamine antagonist drug used for the treatment of itching, runny nose, hay fever and such other allergies. Here, an LC/MS/MS method has been developed for high sensitive quantitation of this molecule from plasma using LCMS-8050, a triple quadrupole mass spectrometer from Shimadzu Corporation, Japan. Presence of heated Electro Spray Ionization (ESI) interface in LCMS-8050 ensured good quantitation and repeatability even in the presence of a complex matrix like plasma. Ultra high sensitivity of LCMS-8050 enabled development of a low ppt level quantitation method for Loratadine.

Loratadine



Ethyl 4- (8-chloro-5, 6-dihydro-11H-benzo [5, 6] cyclohepta [1, 2-b] pyridin-11-ylidene) -1-piperidinecarboxylate

Figure 1. Structure of Loratadine

Loratadine, a piperidine derivative, is a potent long-acting, non-sedating tricyclic antihistamine with selective peripheral H1-receptor antagonist activity. It is used for relief of nasal and non-nasal symptoms of seasonal allergies and skin rashes[1,2,3]. Due to partial distribution in central nervous system, it has less sedating power compared to traditional H1 blockers. Loratadine is given orally, is well absorbed from the gastrointestinal tract, and has rapid first-pass hepatic metabolism; it is metabolized by isoenzymes of the cytochrome P450 system, including CYP3A4, CYP2D6, and, to a lesser extent, several others. Loratadine is almost totally (97–99 %) bound to plasma proteins and reaches peak plasma concentration (T_{max}) in ~ 1–2 h[4,5].

Method of Analysis

This bioanalytical method was developed for measuring Loratadine in therapeutic concentration range for the analysis of routine samples. It was important to develop a simple and accurate method for estimation of Loratadine in human plasma.

Preparation of matrix matched plasma by protein precipitation method using cold acetonitrile

To 100 µL of plasma 500 µL cold acetonitrile was added for protein precipitation. It was placed in rotary shaker at 20 rpm for 15 minutes for uniform mixing. This solution was centrifuged at 12000 rpm for 15 minutes. Supernatant was taken and evaporated to dryness at 70 °C . The residue was reconstituted in 200 µL Methanol.

Preparation of calibration standards in matrix matched plasma

1 ppt, 5 ppt, 50 ppt, 100ppt, 500 ppt, 1 ppb, 5 ppb and 10 ppb of Loratadine calibration standards were prepared in cold acetonitrile treated matrix matched plasma.

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LC/MS/MS analysis

LCMS-8050 triple quadrupole mass spectrometer by Shimadzu Corporation, Japan (shown in Figure 2A), sets a new benchmark in triple quadrupole technology with an unsurpassed sensitivity (UFsensitivity) with Scanning speed of 30,000 u/sec (UFscanning) and polarity switching speed of 5 msecs (UFswitching). This system ensures highest quality of data, with very high degree of reliability.

In order to improve ionization efficiency, the newly developed heated ESI probe combines high-temperature gas with the nebulizer spray, assisting in the desolvation of large droplets and enhancing ionization. This development allows high-sensitivity analysis of a wide range of target compounds with considerable reduction in background.

Presence of heated Electro spray interface in LCMS-8050 (shown in Figure 2B) ensured good quantitative sensitivity even in presence of a complex matrix like plasma.

The parent m/z of 382.90 giving the daughter m/z of 337.10 in the positive mode was the MRM transition used for quantitation of Loratadine. MS voltages and collision energy were optimized to achieve maximum transmission of mentioned precursor and product ion. Gas flow rates, source temperature conditions and collision gas were optimized, and linearity graph was plotted for 4 orders of magnitude.

Figure 2A. LCMS-8050 triple quadrupole mass spectrometer by Shimadzu

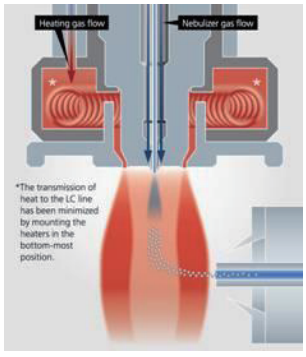

Figure 2B. Heated ESI probe

Table 1. LC conditions

Column	Shim-pack XR-ODS (100 mm L x 2.0 mm ID ; 2.2 µm)
Mobile Phase	A : 0.1% formic acid in water B : acetonitrile
Gradient Program	Time (min) / A conc. (%) / B conc. (%) 0.01 / 40 / 60 1.50 / 0 / 100 4.00 / 0 / 100 4.10 / 40 / 60 13.00 / Stop
Flow Rate	0.15 mL/min
Oven Temperature	40 °C
Injection Volume	20 µL

Table 2. LCMS conditions

MS Interface	ESI
Polarity	Positive
Nebulizing Gas Flow	2.0 L / min (nitrogen)
Drying Gas Flow	10.0 L / min (nitrogen)
Heating Gas Flow	15.0 L / min (zero air)
Interface Temp.	300 °C
Desolvation Line Temp.	250 °C
Heater Block Temp.	400 °C
MRM Transition	382.90 > 337.10

Results

LC/MS/MS Analysis

LC/MS/MS method for Loratadine was developed on ESI +ve ionization mode and 382.90>337.10 MRM transition was optimized for Loratadine. Checked matrix matched plasma standards for highest (10 ppb) as well as lowest (0.001 ppb) concentrations as seen in Figures 4A and 4B respectively. Optimized MS method to ensure no plasma interference at the retention time of Loratadine (Figure 5).

Calibration curve was plotted for Loratadine concentration range. Also as seen in Table 3, % Accuracy was studied to confirm the reliability of method.
Linear calibration curves were obtained with regression coefficients $R^2 > 0.998$. % RSD of area was within 15 % and accuracy was within 80-120 % for all calibration levels.

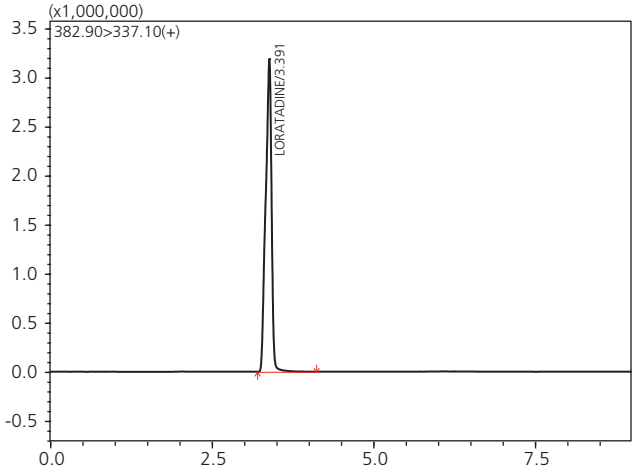

Figure 4A. Mass chromatogram 10 ppb

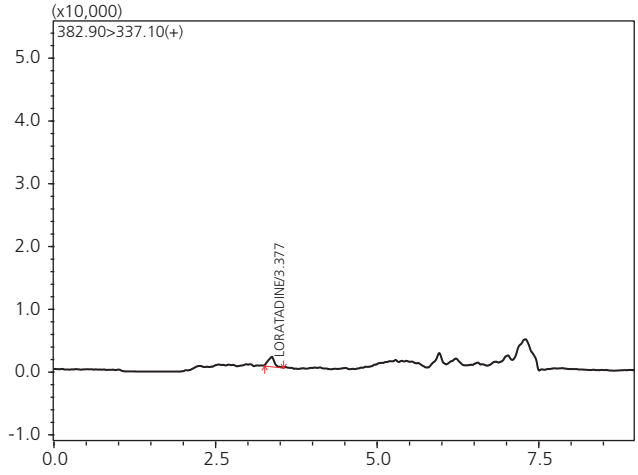

Figure 4B. Mass chromatogram 0.001 ppb

Specificity and interference

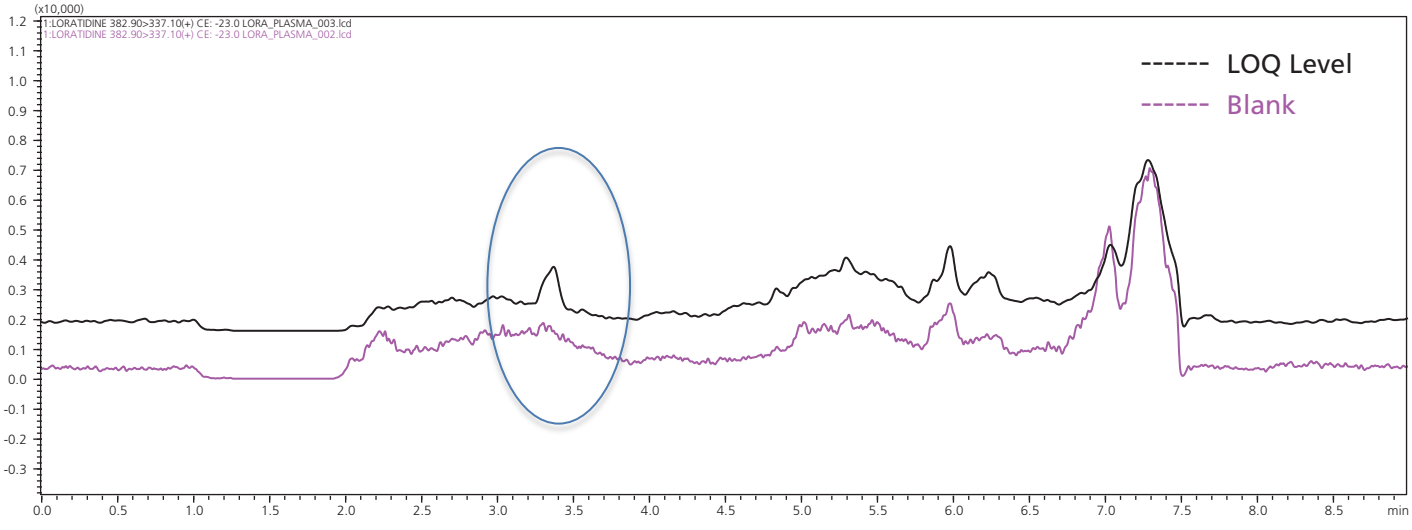

Figure 5. Overlay chromatogram

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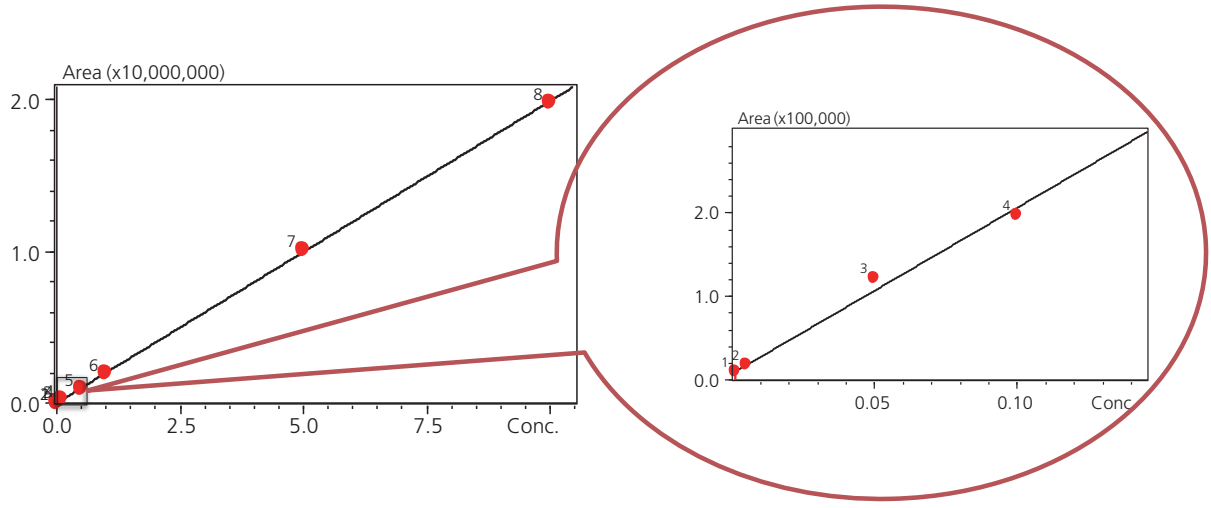

Figure 6. Loratadine calibration curve

Result Table

Table 3. Results of Loratadine calibration curve

Sr. No.	Standard	Nominal Concentration (ppb)	Measured Concentration (ppb)	% RSD for area counts (n=3)	% Accuracy (n=3)
1	STD-01	0.001	0.00096	0.62	95.83
2	STD-02	0.005	0.0050	5.24	100.73
3	STD-03	0.05	0.057	0.98	114.83
4	STD-04	0.1	0.095	1.81	95.40
5	STD-05	0.5	0.048	1.40	95.70
6	STD-06	1.0	0.986	0.11	98.53
7	STD-07	5.0	5.077	1.07	101.53
8	STD-08	10.0	9.983	1.96	99.37

Conclusion

- Highly sensitive LC/MS/MS method for Loaratadine was developed on LCMS-8050 system.
- Calibration was plotted from 10 ppb to 0.001 ppb, and LOQ was computed as 0.001 ppb.

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