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Rapid Determination of Loratadine Level in Human Plasma by LCMS/MS Assay

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ABSTRACT

A rapid liquid chromatographic tandem mass spectrometry (LC-MS/MS) assay for the measurement of loratadine level in human plasma was developed and validated. One ml plasma samples containing loratadine and 0.18 µg of metoclopramide as (internal standard, IS) were extracted with 5 ml *tert*-butyl methyl ether and reconstituted with 80 µl of acetonitrile. Analysis was performed using a reversed phase Atlantis dC_{18} column and a mobile phase consisting of 0.4% formic acid and acetonitrile (20:80, v:v) and delivered at a flow rate of 0.25 ml/min. The eluents were monitored using electrospray ionization in the positive ion mode with transition mass to charge ratio (m/z) at 383.4→337.2 and 299.8→226.9 for loratadine and IS, respectively. The retention times of the IS and loratadine were around 1.53 and 2.33 min, respectively. Mean matrix effect was measured as -11.4% for loratadine and -14.4% for the IS. Detection limit of loratadine in plasma was 0.3 ng/ml. The relationship between loratadine concentration in plasma and the peak area ratio of loratadine / IS was linear ($R^2 \geq 0.9945$) in the range of 0.5–100 ng/ml, and the intra- and inter-day coefficient of variations (CV) were ≤ 11.3%. Mean extraction recoveries for loratadine and the IS were 87% and 91% respectively, whereas accuracy (relative recovery) ranged from 99% to 111% quality control samples and from 93% to 105% using back- calculated concentrations. The method was applied to assess the stability of loratadine under various conditions generally encountered in the clinical laboratory. Stability for processed samples (24 hours at room temperature, 48 hours -20 ºC) and unprocessed samples (24 hours at room temperature, 12 weeks -20 ºC) was ≥ 94%.

Key words: Loratadine, Metoclopramide, Human plasma, HPLC

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INTRODUCTION

Loratadine (CAS: 79794-75-5), Ethyl-4-(8-Chloro-5,6-dihydro-11H- benzo [5,6]cyclohepta[1,2-b]pyridin-11-ylidene)-1-piperidinecarboxylate, is a second-generation H1 histamine antagonist, closely related to tricyclic antidepressants. It is used in treating various kind of allergies and chronic idiopathic urticaria[1,2]. Plasma protein binding for loratadine and its active metabolite, desloratadine, are 98% and 75%, respectively. Following 10 mg oral dose, loratadine is rapidly absorbed with peak plasma concentrations (C_{max}) of 4.64 ng/ml for loratadine and 5.25 ng/ml for desloratadine, with time to maximum concentration (T_{max}) of 1.3 hrs and 2.5 hrs for loratadine and desloratadine, respectively[3].

Several high-performance liquid chromatography (HPLC) methods have been reported for determination of loratadine in biological samples[4-6]. HPLC-UV methods showed a limit of quantitation (LOQ) of ≥ 33 ng/ml[4]. An HPLC-fluorescence method showed lower limit of quantification of 0.5 ng/ml. However, sample preparation required extraction with organic solvent followed by back extraction with diluted phosphoric acid[5]. Finally, LCMS/MS methods have been used to improve the detection limits of loratadine in human plasma [7-13].

In this paper, we describe a precise and accurate LCMS/MS method for determination of loratadine level in human plasma. The method is based on liquid extraction and uses 1.0 ml plasma sample. The method was fully validated and successfully used to monitor stability of loratadine in human plasma for samples.

MATERIALS AND METHOD

Apparatus

Liquid chromatography was performed on tandem mass spectrometric (LC-MS/MS) system consisting of a Water Alliance 2695 separation module equipped with Micromass Quattro micro API bench-top triple quadruple mass spectrometer interfaced with a Z-spray electrospray ionization (ESI) source. Analysis was performed on reversed phase Atlantis dC_{18} column (2.1 x 100 mm, 3µm) protected by guard column Symmetry C_{18} (3.9 x 20 mm, 5 µm), Waters Corporation, Milford, MA, USA. MassLynx software (Ver 4.0) working under Microsoft Window XP professional environment was used to control the instrument parameters and for data acquisition.

Chemical and reagents

All reagents were of analytical grade unless stated otherwise. Loratadine and metoclopramide standards (purity ≥ 98 %) and *tert*-butyl methyl ether were obtained from Sigma-Aldrich, United

Kingdom. Formic acid and acetonitrile (HPLC grade) were purchased from Fisher Scientific, NJ. USA. HPLC grade water was prepared by reverse osmosis and was further purified by passing through Synergy (Millipore, Bedford, MA, USA). Drug-free human plasma was obtained from the blood bank of King Faisal Specialist Hospital & Research Centre (KFSHRC) Riyadh, Saudi Arabia.

Chromatographic conditions

The mobile phase consisted of 0.4% formic acid (pH = 3.0, adjusted with formic acid) and acetonitrile (20:80, v:v) and was delivered at a flow rate of 0.25 ml/min. The analysis was carried out at room temperature under isocratic condition. ESI was operated in the positive-ion mode at a capillary voltage of 4.0 kV and a cone voltage of 30 V. Nitrogen was used as nebulizing and desolvation gas at a flow rate of 50 and 600 L/hr, respectively. Argon was used as the collision gas at a pressure of 1.28 x 10^{-3} mbar. The optimum collision energy for loratadine and the IS was 25 eV. The ion source and the desolvation temperatures were maintained at 120 ºC and 350 ºC, respectively. Loratadine and IS were detected using positive ion multiple reaction monitoring (MRM) mode at the following transitions of mass to charge (m/z): 383.4→337.2 and 299.8→226.9 for loratadine and IS, respectively.

Preparation of standard and quality control samples

Stock solutions (1 mg/ml) of loratadine and metoclopramide (internal standard, IS) were prepared in acetonitrile. They were diluted to produce working solutions of 100 ng/ml of loratadine in plasma and 1.0 µg/ml of IS in acetonitrile. Calibration curve standards (nine concentrations) in the range of 0.5–100 ng/ml were prepared in human plasma. Four quality control (QC) samples (0.5, 1.0, 50, and 90 ng/ml) were also prepared in human plasma. 1.0 ml aliquots from each flask were transferred into teflon-lined, screw-capped, borosilicate glass 12 x 100 mm culture tubes (Fisher Scientific Co., Fairlawn, NJ, USA), stored at -20 ºC, and used for assay validation purposes.

Sample preparation

Aliquots of 1.0 ml of blank plasma, calibration curves, or QC samples culture tubes were allowed to equilibrate to room temperature (RT). To each tube, 180 µl of the IS working solution was added and the mixture was vortexed for 20 seconds. After the addition of 5 ml of *tert*-butyl methyl ether, the mixture was vortexed again for 2 min and then centrifuged at room temperature for 20 min at 4700 rpm. The clear supernatant organic layer was carefully transferred into clean tube and dried under gentle stream of nitrogen at 40 °C, and the residue was reconstituted with 80 µl of acetonitrile and transferred to an auto-sampler vial. Ten µl were injected into the system with a run time of 3 min.

Stability studies

Three QC samples (concentration 1.5, 50, and 90 µg/ml) were used for stability studies: five aliquots of each QC sample were extracted and immediately analyzed (baseline), five aliquots were allowed to stand on the bench-top for 24 hours at room temperature before being processed and analyzed, five aliquots were stored at -20 ºC for 12 weeks before being processed and analyzed, and five aliquots were processed and stored at room temperature for 24 hours or at -20 ºC for 48 hours before analysis. Fifteen aliquots of each QC sample were stored at -20 ºC for 24 hours. They were then taken out of freezer and left to completely thaw unassisted at room temperature. Five aliquots of each sample were extracted and analyzed and the rest were returned to -20 ºC for another 24 hours. The cycle was repeated three times.

Method validation

The LCMS/MS method was validated according to standard procedures described in the US Food and Drug Administration (FDA) bioanalytical method validation guidance[14]. The validation parameter included specificity, linearity, accuracy, precision, recovery, and stability.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

Optimal experimental conditions consisted of a mobile phase composed of 0.4% formic acid (pH=3, adjusted with formic acid) and acetonitrile (20:80, v:v) and delivered at a flow rate of 0.25 ml/min. Under these conditions loratadine and IS were well separated and detected within a 3 minute run. The retention times of the IS and the loratadine were around 1.53 and 2.33 min, respectively. **Figure 1** depicts total ion current (TIC) and multiple reaction monitoring (MRM) chromatograms of loratadine and metoclopramide (IS) mixture extracted from plasma.

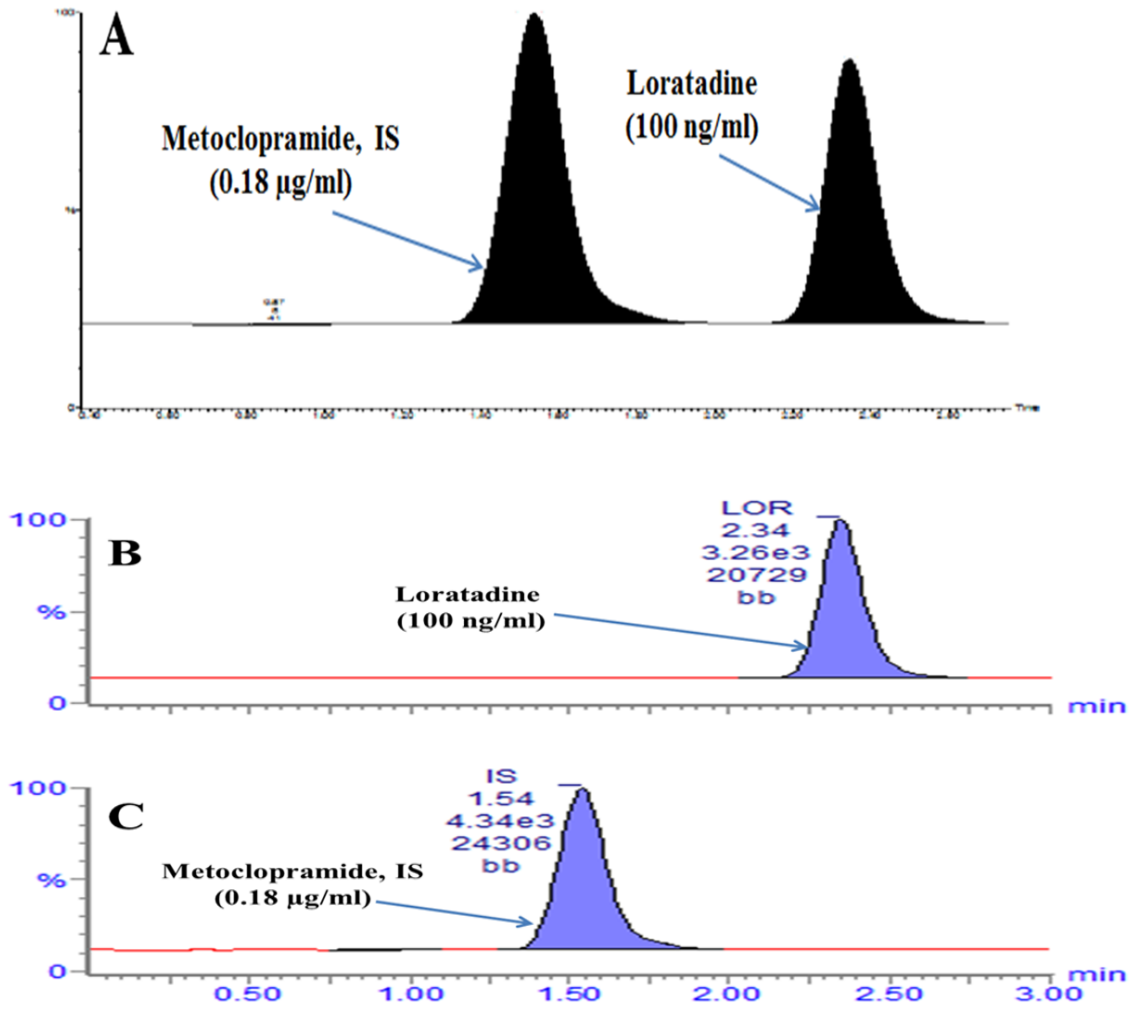

Figure 1: Caption: TIC and MRM chromatograms of loratadine and metoclopramide (IS) extracted from plasma.

Legend: (A) Total Ion Current (TIC) chromatogram standard mixture containing loratadine and metoclopramide. (B) Multiple reaction monitoring (MRM) chromatogram of loratadine (100 ng/ml), and (C) MRM chromatogram of metoclopramide (IS) 0.18 µg/ml.

The ESI source was operated at optimum capillary voltage of 4.0 kV and cone voltage of 30 V. The optimum collision energy for loratadine and the IS was 25 eV. The ion source and the desolvation temperatures were maintained at 120 ºC and 350 ºC, respectively

Specificity

We screened six batches of blank human plasma and nine frequently used medications (acetaminophen, ascorbic acid, aspirin, caffeine, diclofenac, ibuprofen, nicotinic acid, omeprazole, and ranitidine) for potential interference. No interference was found in plasma and none of the drugs co-eluted with loratadine or the IS. **Figure 2** depicts a representative MRM chromatogram of blank plasma used in the preparation of standard and QC samples.

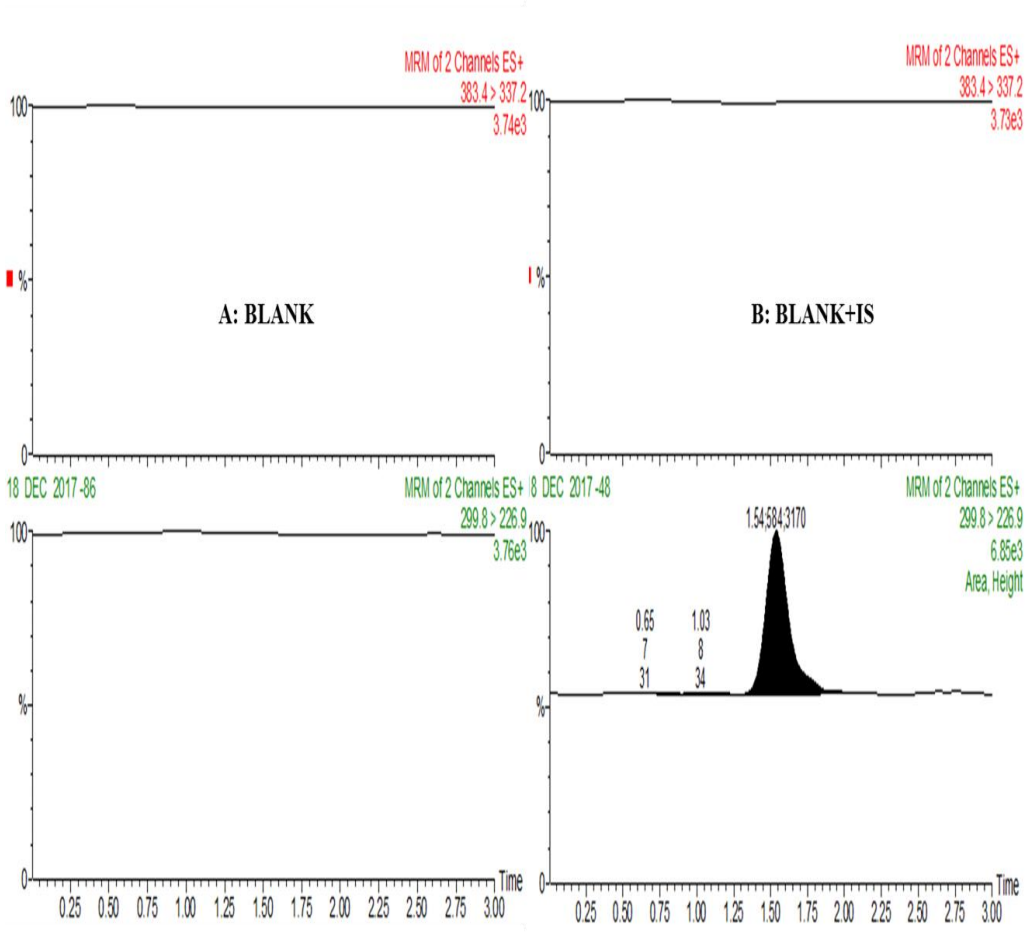

Figure 2: Caption: MRM chromatogram of blank and IS spiked plasma.

Limit of detection & quantification and linearity

The limit of quantification, defined as the lowest concentration on the calibration curve that can be determined with acceptable precision and accuracy (i.e., coefficient of variation and bias ≤ 20%), was 0.5 ng/ml. The limit of detection (signal to noise-ratio ≥ 3) was 0.3 ng/ml. Linearity of loratadine was evaluated by analyzing ten curves of nine (plus zero) standard concentrations prepared in human plasma. **Figure 3** depicts an MRM chromatogram of loratadine at lowest limit of quantification extracted from plasma. Mean (SD) of slope, intercept, and coefficient of determination (R^2) of the ten curves were 0.9980 (0.0006), - 0.0030 (0.0047), and 0.9960 (0.0015), respectively. The suitability of the calibration curves was confirmed by back-calculating the concentrations of loratadine (**Table 1)**. All back-calculated concentrations were well within the acceptable limits.

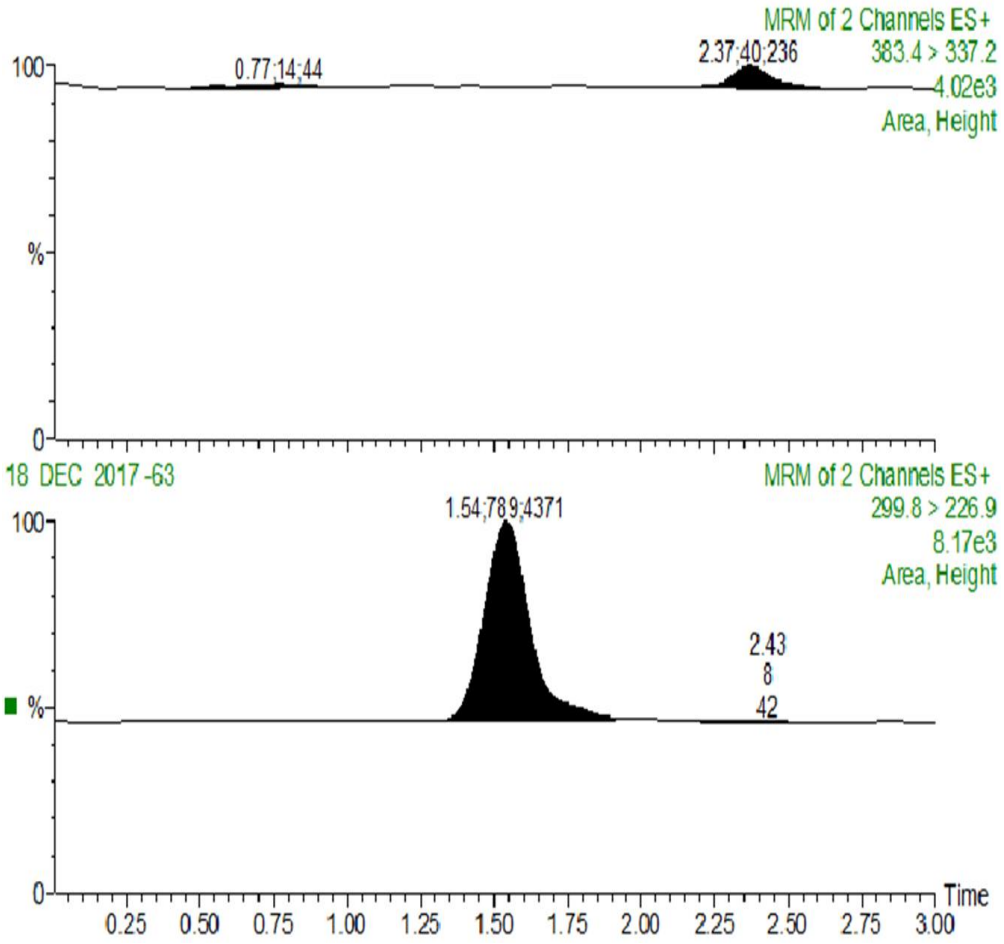

Figure 3: MRM chromatogram of loratadine at lowest limit of quantification extracted from plasma.

Table 1: Back-calculated loratadine concentrations from ten calibration curves

Nominal level (ng/ml)	Measured level (ng/ml)	CV (%)	Bias (%)
	Mean (SD)		
0.5	0.53 (0.07)	14.0	6.0
1.0	1.05 (0.08)	7.4	5.0
2.0	2.34 (0.22)	9.2	17
5.0	5.24 (0.44)	8.5	4.8
10	9.32 (0.43)	4.6	-6.8
20	18.84 (0.86)	4.6	-5.8
40	39.96 (3.55)	8.9	-4.0
80	78.87 (4.43)	5.6	1.4
100	100.89 (3.25)	3.2	0.9

SD, standard deviation. CV, standard deviation divided by mean measured concentration x100.

Bias = (mean measured concentration – nominal concentration divided by nominal concentration) × 100.

Precision and bias (inaccuracy)

The intra-day and inter-day precision and bias of the method were evaluated by analyzing four QC concentrations (0.5, 1.5, 50, and 90 ng/ml). Intra-day precision and bias (n = 10) ranged from 7.5% to 11.3% and from 2.0% to 10.5%, respectively. Inter-day precision and bias were determined over three different days (n = 20) and ranged from 8.1% to 10.3% and from –2.0 % to 7.3%, respectively. The results are summarized in **Table 2**. **Figure 4** depicts MRM chromatograms of loratadine quality control samples.

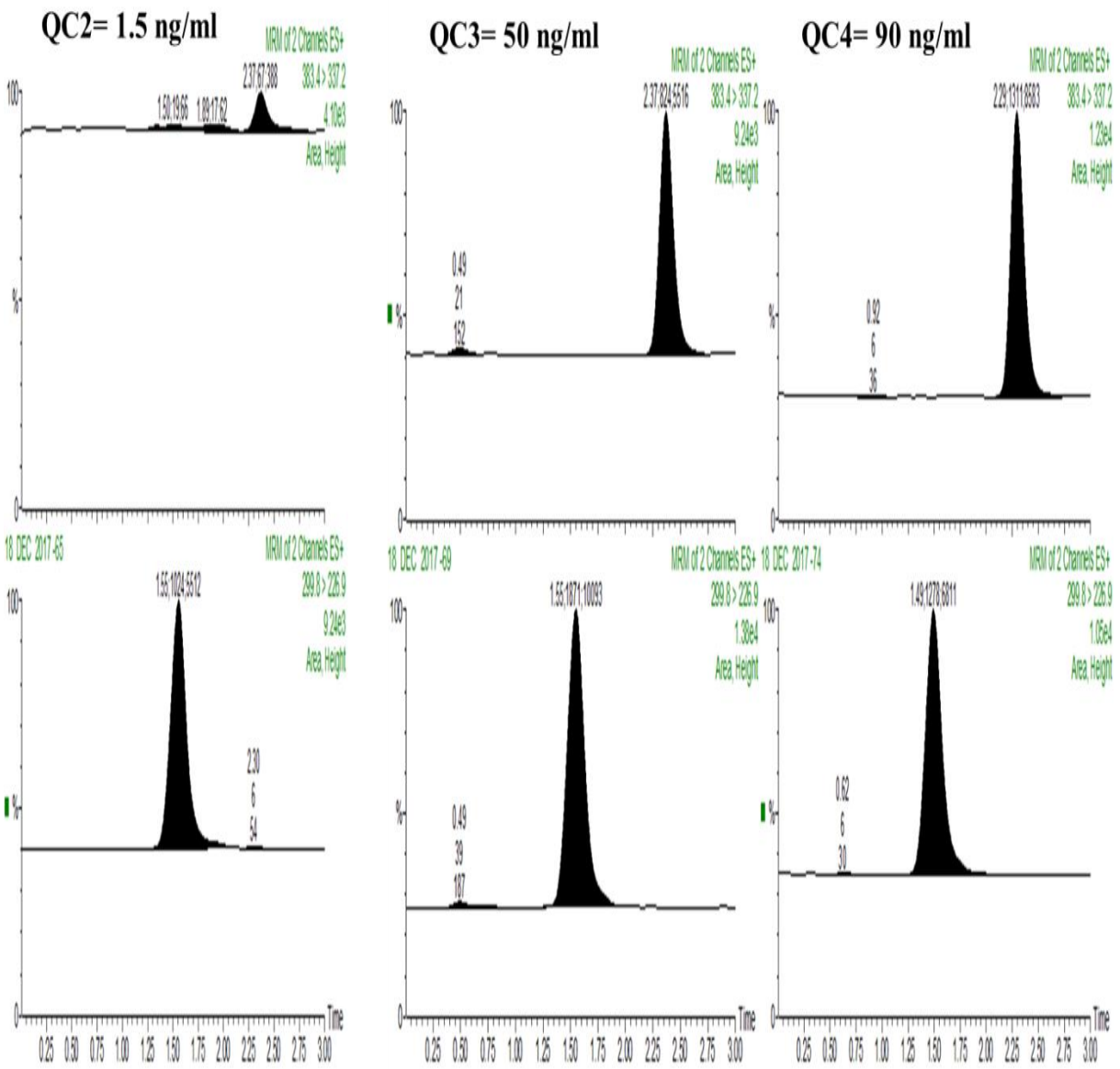

Figure 4: MRM chromatograms of loratadine quality control samples extracted from plasma

Table 2: Intra and inter-day precision and bias of loratadine assay

Nominal level (ng/ml)	Measured level (ng/ml) Mean (SD)	CV (%)	Bias (%)
Intra-day (n = 10)			
0.5	0.51 (0.06)	11.3	2.0
1.5	1.58 (0.17)	10.5	5.3
50	55.27 (4.17)	7.5	10.5
90	95.38 (8.17)	8.6	6.0
Inter-day (n = 20)			
0.5	0.49 (0.05)	9.3	- 2.0
1.5	1.51 (0.15)	10.3	0.7
50	53.66 (4.33)	8.1	7.3
90	92.09 (7.80)	8.5	2.3

SD, standard deviation. CV, standard deviation divided by mean measured concentration x100.

Bias = (mean measured concentration – nominal concentration divided by nominal concentration) × 100.

Recovery

Extraction recovery of loratadine was assessed by direct comparison of peak areas from plasma and mobile phase samples, using five replicates for each of four QCs (0.5, 1.5, 50, and 90 ng/ml). Similarly, the recovery of the IS was determined by comparing peak areas of the IS in five aliquots of human plasma spiked with 180 µl of 1.0 µg/ml in acetonitrile IS solution with the peak areas of equivalent samples prepared in the mobile phase. The results are presented in **Table 3**. Mean recovery of loratadine and the IS were 87% and 91%, respectively.

Table 3: Recovery of loratadine and the internal standard from 1.0 ml human plasma

Concentration (ng/ml)	Human plasma *Mean (SD)	Mobile phase *Mean (SD)	Recovery (%)
Loratadine			
0.5	72 (7)	91 (7)	79
1.5	161 (17)	194 (14)	83
50	3675 (181)	4105 (89)	90
90	7347 (124)	7556 (251)	97
IS (0.18 µg/ml)	4413 (531)	4853 (221)	91

Mean peak area (standard deviation), n = 5. Recovery was calculated as mean peak area in human plasma divided by mean peak area in mobile phase x 100**.**

Matrix effect

Matrix effect was evaluated by comparing the peak area response of pre and post extracts of loratadine at four concentrations (0.5, 1.5, 50, and 90 ng/ml) and the IS at 0.18 µg/ml. Twenty five extracted blank plasma samples were reconstituted using 80 µl of acetonitrile containing equal

amount of loratadine and IS. Matrix effect was calculated as ion suppression **(Table 4)**. Mean ion suppressed effect was measured as -11.4% for loratadine and -14.4% for the IS.

Table: 4 Human plasma matrix effect on loratadine and the internal standard

Concentration (ng/ml)	Pre-treatment plasma Mean (SD)	Post-treatment plasma Mean (SD)	Matrix Effect (%)
Loratadine			-14.1
0.5	99 (10)	85 (11)	
1.5	218 (13)	184 (12)	-15.4
50	4345 (223)	4105 (196)	-5.6
90	9219 (497)	8243 (659)	-10.6
IS (180)	6154 (140)	5268 (27)	-14.4

Matrix Effect (%) = Mean peak area (post-treatment) – mean peak area (pre-treatment) divided by mean peak area (pre-treatment) x100. N = 5.

Robustness

The robustness of the current assay was evaluated by slightly altering proportions of acetonitrile (± 2.0%), and the pH of the mobile phase (± 0.2). No significant changes were observed.

Stability

Loratadine and IS stability in processed and unprocessed plasma samples was investigated using three QCs (1.5, 50, and 90 ng/ml). Loratadine in processed samples was found to be stable for 24 hours at room temperature (≥ 94%) and for 48 hours at −20 °C (≥ 94 %). Loratadine in unprocessed plasma samples was stable for at least 24 hours at room temperature (≥ 94%), 12 weeks at −20 °C (≥ 94%), and after three freeze-and thaw cycles (≥ 94%). **Table 5** summarizes the results of stability studies.

Table 5: Stability for loratadine in human plasma

Stability (%) Nominal level (ng/ml)	Unprocessed		Processed		Freeze-Thaw Cycle		
	24 hrs RT	12 wks 20 °C	24 hrs RT	48 hrs 20 °C	1	2	3
1.5	99	101	94	94	94	94	94
50	94	94	101	95	102	101	95
90	101	104	100	95	97	100	95

Stability (%) = mean measured concentration (n = 5) at the indicated time divided by mean measured concentration (n = 5) at baseline x 100. Spiked plasma samples were processed and analyzed immediately (baseline, data not shown), after 24 hours at room temperature (24 hrs RT), or after freezing at –20 ºC for 12 weeks (12 wks, –20 ºC), or processed and then analyzed after storing for 24 hours at room temperature (24 hrs, RT) or 48 hours at –20 ºC (48 hrs, -20 ºC). Freeze-thaw (FT), samples were frozen at -20 ºC and thaw at RT.

CONCLUSION

The described LC-MS/MS assay for the determination of loratadine in human plasma is simple sensitive, specific, precise and accurate; making it suitable for therapeutic drug monitoring and pharmacokinetic analysis. It requires 1.0 ml plasma and 3 minutes run time. It was successfully applied to monitor stability of loratadine under various conditions encountered in the clinical laboratories.

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