Determination of loratadine and its active metabolite in plasma by LC/MS/MS: An adapted method for children

Qian Li[1,a] (BS), Hai-Yan Shi[2] (PhD), Kai Wang[3] (PhD), Min Kan[1] (BS), Yi Zheng[1] (PhD), Guo-Xiang Hao[1] (PhD), Xin-Mei Yang[2] (PhD), Yi-Lei Yang[2] (PhD), Le-Qun Su[2] (MS), Wei Zhao[1, 2*] (PharmD, PhD)

[1]Department of Clinical Pharmacy, School of Pharmaceutical Sciences, Shandong University, Jinan, China ; [2] Department of Pharmacy, Shandong Provincial Qianfoshan Hospital, Shandong University, Jinan, China ; [3] Department of Respiratory Disease, Shandong Provincial Qianfoshan Hospital, Shandong University, Jinan, China.

ABSTRACT: An adapted method of liquid chromatography-mass spectrometry (LC/MS/MS) was developed and validated to measure the concentrations of loratadine (LOR) and its active metabolite descarboethoxyloratadine (DCL) from pediatric plasma. After being mixed with the internal standard (IS, propranolol) and precipitated with methanol, samples were centrifuged and 20 μL of the supernatants were injected into the HPLC system. Separation was carried out on a reversed-phase C18 gradient column using a mobile phase consisting of water (containing 0.1% formic acid) and acetonitrile. The flow rate was 0.5 mL/min and the running time was 5.0 min for each sample. Quantitation of LOR, DCL and IS was performed using MRM mode and the transitions were: 383.1 → 337.1 for LOR, 311.1 → 259.0 for DCL and 260.2 → 116.0 for propranolol, respectively. The method was validated according to FDA guidelines, precisions and accuracies met the requirements in all cases. Calibration curves were 0.2–50.0 ng/mL for both LOR and DCL. This method was then applied for a pilot study examining the pharmacokinetics and therapeutic drug monitoring of LOR in children.

Key Words: loratadine; descarboethoxyloratadine; plasma; LC/MS/MS; children

*Address correspondence to this author at Department of Clinical Pharmacy, School of Pharmaceutical Sciences, Shandong University, Wen Hua West Road 44, Jinan, China; Tel/Fax: +86 531 8838 3308; E-mail: zhao4wei2@hotmail.com

1. INTRODUCTION

Loratadine (LOR) (Figure 1), an active H_1 receptor antagonist, is often used in the treatment of allergic disorders such as seasonal allergies and skin rash [1]. LOR was clinically approved for symptomatic relief of nasal and non-nasal symptoms of allergic rhinitis in children ≥2 years [2]. It has a good safety profile and is well tolerated making LOR a popular choice for anti-allergy therapy in pediatric clinical practice. Additionally, LOR is absorbed rapidly and undergoes extensive metabolism in vivo. One of the main metabolites, descarboethoxyloratadine (DCL) (Figure 1), is reported to have more pharmacological potencies than LOR [3-4], and the pharmacokinetics of LOR

manifest large inter-individual variability [5-6]. In Chinese subjects, a greater variability has been demonstrated in the metabolic ratios of DCL to LOR [7].

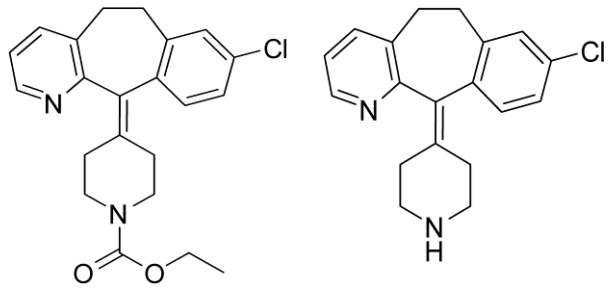

Figure 1. Chemical structures of loratadine (LOR), descarboethoxyloratadine (DCL) and propranolol (IS).

LOR is metabolized to DCL predominantly by CYP3A4 (70%) as well as CYP2D6 [8]. However, on study showed that the catalytic formation rate in CYP2D6 was greater than in CYP3A4 [9], and there is more polymorphic variability of CYP2D6 activity in vivo. In Chinese populations, there is a higher frequency of the CYP2D6*10 allele (C > 100T with decreased CYP2D6 activity) and the pharmacokinetic variability of LOR is significantly affected by CYP2D6 polymorphisms [10]. For these reasons, close monitoring of LOR and its active metabolite DCL concentrations in children is warranted.

Several methods have been described for simultaneous determination of LOR and DCL in human plasma, such as high performance liquid chromatography (HPLC) [11], liquid chromatography with mass spectrometry (LC/MS/MS) and gas chromatography with mass spectrometry (GC/MS) [7, 12-15]. Among the published methods using serum or plasma, the volume of plasma used for such determinations is large, mostly 1 mL. These methods are not easy adaptable to children as the volume and the number of samples that can be taken at once are limited in this patient group. Besides, the sample preparation is complex, as most methods employ a liquid–liquid extraction procedure, with evaporation of the organic solvent and reconstitution with mobile phase, such a procedure is inconvenient. Others published methods of simultaneous determination of LOR and DCL have a relatively higher lower limit of quantification (LLOQ, 0.5 ng/mL).

We aimed to develop a sensitive, reliable, and clinically feasible LC/MS/MS method for simultaneous determination of LOR and DCL in plasma with a small sample volume. The method could be practically applied in LOR pharmacokinetic study of children as well as in the therapeutic drug monitoring of LOR in paediatric patients.

2. EXPERIMENTAL

2.1 Chemicals and reagents

The reference standards of loratadine (LOR), descarboethoxyloratadine (DCL) and propranolol (IS) (Figure 1) were obtained from National institute for Food and Drug Control (China). Formic acid was obtained from Sinopharm (Shanghai, China) and acetonitrile and methanol were purchased from Tedia (Fairfield, USA). Pure water was provided by Wahaha group Co. (Hangzhou, China) and drug-free human plasma was collected from healthy volunteers.

2.2 Chromatographic and mass system conditions

A Shimadzu LC-20AD pump (Kyoto, Japan) reversed-phase chromatographic system was used with a C18 column (2.1 mm×100 mm, 5 μm, Kyoto, Japan) analytical column. The mobile phase was composed of water containing 0.1% (v/v) formic acid and acetonitrile, and the gradient elution procedure was shown in Table 1. Chromatographic analysis was performed at a flow rate of 0.5 mL/min and chromatographic separation was performed at 40 °C. The injection volume was 20 μL and the running time for one sample was 5.0 min.

The MS/MS system consisted of an AB SCIEX TripleQuad 4500 MD mass spectrometer (CA, USA) equipped with a TurbolonSpray ionization source. Quantitation was performed in MRM mode and the transitions were monitored as follows: m/z 383.1 → m/z 336.8 for LOR, m/z 311.1 → m/z 259.0 for DCL, and m/z 260.2 → m/z 116.0 for IS, respectively. Turbo gas temperature was 500 °C and the ionspray voltage was 4500 V. The collision energy and the entrance potential were 30 and 10, respectively while the ion source and curtain gas were 50 and 40 psi, respectively. Declustering potential was 115, 100 and 70 for LOR, DCL and IS, respectively. A weighted linear regression, with a weighting factor of

1/concentration2, was used to generate calibration curves and calculate the sample concentrations.

Table 1 Gradient elution program for HPLC separation of loratadine (LOR), descarboethoxyloratadine (DCL) and propranolol (IS)

Time (min)	Solvent A (%)	Solvent B (%)
0	80	20
1.0	80	20
2.0	10	90
4.0	10	90
4.1	80	20
5.0	80	20

Solvent A, water containing 0.1% (v/v) formic acid; Solvent B, acetonitrile

2.3 Preparation of stock solutions, calibration standards and quality control (QC) samples

The stock solutions of LOR (1000 ng/mL), DCL (1000 ng/mL) and propranolol (100 ng/mL) were prepared in methanol, divided into 1.0 mL aliquots and stored at -80°C. Working solutions (2, 10, 25, 100, 250, 500 ng/mL) were prepared in methanol with serial dilution from the stock solution. Working solutions were freshly prepared on each experimental day.

Plasma calibration curves were obtained by adding suitable volumes of working solutions into blank plasma to yield the following concentrations: 0.2, 1.0, 2.5, 5.0, 10.0, 25.0, and 50.0 ng/mL. Quality control (QC) samples were prepared independently in the same way to obtain the concentrations of 1.0 (low), 15.0 (medium), and 37.5 ng/mL (high). All quality control samples were separated into aliquots of 200 µL and stored at −80 °C until analysis.

2.4 Sample preparation

Plasma samples were defrosted before analysis at room temperature. Specifically, 50 µL of plasma was mixed with 10 µL of the IS stock solution (100 ng/mL), 150 µL of methanol was added to the sample. The tube was then vortex mixed for 30 s and centrifuged for 10 min at 12,000 rpm. Then the supernatant was transferred into a chromatographic vial and 20 µL was injected into the HPLC system.

2.5 Method validation

Method validations and documentations were done according to FDA guidelines for validation of bioanalytical methods [16]. The method was validated in terms of selectivity, linearity, lower limit of quantification, accuracy and precision, recovery and stability.

2.5.1 Selectivity

Selectivity means no interference of components from the blank human plasma on the retention time of LOR, DCL and IS. Six different blank plasmas were used to evaluate method selectivity.

2.5.2 Linearity

Freshly prepared solutions were used to determine linearity on three different days. Calibration curves were obtained by spiking the samples of matrix over concentrations ranging from 0.2–50.0 ng/mL for both LOR and DCL. The various regression parameters of slopes, intercepts and correlation coefficients (R) were obtained by using weighting factor (1/x2) linear regress. To validate the calibration curve, the following criteria was used: (1) the accuracy of LOR or DCL concentration deviation must be under ± 15% of the corresponding theoretical value, while at the lower limit of quantification (LLOQ) it should not be above ± 20%; (2) precision must not be above 15% (coefficient of variation) of the mean value, while for LLOQ, it should be under 20%.

2.5.3 Lower Limit of Quantification (LLOQ)

The lower limit of quantification (LLOQ) was determined as the signal to noise ratio of at least 10. LLOQ acceptance criteria were as follows: precision within 20% and accuracy within 100± 20%.

2.5.4 Accuracy and Precision

Analytical method, accuracy and precision were determined by injecting quality control samples into the HPLC instrument for intra-day evaluation of precision. For inter-day evaluation, the same process was repeated for three consecutive days to assess accuracy and precision. Inter- and intra-day assay precision were evaluated using the coefficient of variation (%) and were calculated as the percent (%) difference of the nominal and measured concentrations: [(measured concentration/nominal concentration) ×100]. For every sample, the concentration was assessed using calibration curves. To calculate the intra-day assay precisions and accuracies, five concentrations from three QC tests (0.5, 15.0 and 37.5 ng/mL) were used in a single analytical performance. To calculate the inter-day assay precision and accuracy, the three once-a-day QCs were analyzed.

Precisions must have been under 15%, while the accuracies must have been in a range 85 to 115%.

2.5.5 Recovery

Recovery analysis was conducted by comparing the peak areas resulting from extracted samples spiked with the specific amounts of analytes (low, middle and high) with the results from the pure compounds of the equal amount concentrations in water. The samples were analyzed at three different concentrations (0.5, 15.0 and 37.5 ng/mL) to estimate the recovery. Each concentration level was analyzed after extraction and results were compared with the non-extracted standards (with 100% recovery).

2.5.6 Stability

The stability evaluation was performed to determine analytes stability during collecting and handling of the samples. For short-term stability, the QC samples were assayed in triplicate after defrosting for six hours at room temperature and measuring the bias by comparing to the reference concentrations. For freeze-thaw stability, the QC samples were evaluated in triplicate over three freeze-thaw cycles. After that QC samples were kept at a temperature of -80°C for 24 hours, followed by unassisted thawing (at room temperature). After thawing, the plasma samples were refrozen again under similar conditions. The freeze-thawing cycles were repeated two times and the samples were analyzed after three freeze-thawing cycles. Then measured concentrations were compared to the reference concentration. For autosampler stability, three QC samples were assayed with the autosampler at 4°C by injecting the prepared extracts instantly, and re-injecting after 24 hours. For long-term storage stability, the QC samples were assayed at -80°C and the duration of study was 3 months.

2.6 Loratadine administration and samples collection

A total of 15 pediatric patients receiving LOR (Loratadine 10 mg; schering plough, Shanghai, China) as part of routine anti-allergy therapy were enrolled in this study. The drug was administered as follows: 1 tablet (10 mg) once daily > 30 kg and half a tablet (5 mg) once daily ≤ 30 kg. Trough plasma concentrations were taken from patients undergoing therapy. Samples were centrifuged at 4000 rpm for 10 minutes and stored at −80°C until analysis.

3. RESULTS

3.1 Selectivity

Typical blank plasma and LLOQ chromatograms are shown in the Figure 2. No interference was observed at the retention times of LOR (2.83 min), DCL (2.29 min) and IS (2.46 min).

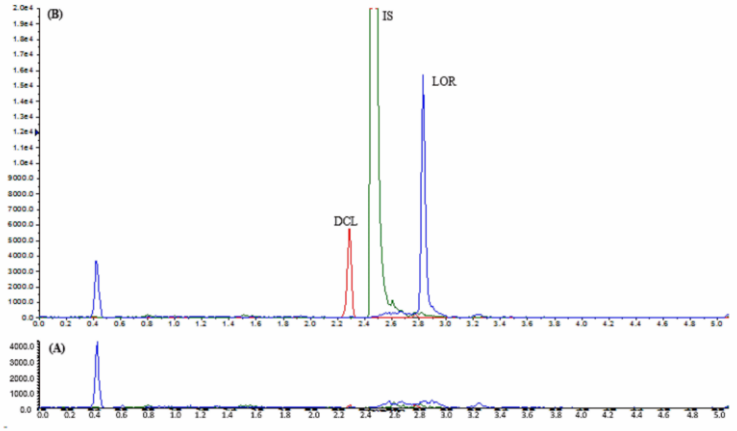

Figure 2. (A): Chromatogram of blank plasma; (B): Chromatograms of LLOQ plasma standard with 0.2 ng/mL loratadine (LOR) and 0.2 ng/mL descarboethoxyloratadine (DCL) and 100 ng/mL propranolol (IS).

3.2 Linearity

Linear calibration curves parameters, obtained from statistical analysis of two independently set calibration curves in plasma, indicating a good linearity for standards in these matrices. Calibration curves for LOR and DCL showed an excellent linearity within the range of 0.2–50.0 ng/mL. The linear regression equations were Y = 0.010 + 0.3274 X (r = 0.999) for LOR and Y = 0.003 + 0.1205 X (r = 0.999) for DCL, respectively.

3.3 Lower limit of quantification (LLOQ)

The LLOQ of the assay was 0.2 ng/mL for both LOR and DCL. Signal-to-noise ratio at LLOQ was greater than 10. The precision and accuracy of the assay were 6.7% and 102.5% for LOR and 7.7% and 105.5% for DCL, respectively.

3.4 Accuracy and Precision

Intra-day and inter-day precision of LOR and DCL were both below 9.2%. The accuracy of LOR and DCL were 102.5−109.2% and 101.8−108.8%, respectively. The detailed results of precision and accuracy are shown in Table 2.

3.5 Recovery

Recovery was in the range of 87.7−90.3% for LOR and 88.5−90.6% for DCL. The recoveries of LOR, DCL and IS at three various concentrations (n = 3 for each concentration) are given in Table 3.

3.6 Stability

The results of short-term, freeze-thaw, autosampler and long-term stability showed no significant decline (<15%) in concentrations of LOR and DCL (Table 4). There were no stability-related problems were identified during routine analysis of the samples.

Table 2 Precision and accuracy of loratadine (LOR) and descarboethoxyloratadine (DCL) (n = 5, Intra-day; n = 15, inter-day, 3 days)

Analyte	Nominal conc. (ng/mL)	Intra-day (n=5) Mean ± SD (ng/mL)	Accuracy (%)	CV (%)	Inter-day (n=15) Mean ± SD (ng/mL)	Accuracy (%)	CV (%)
LOR	0.2 (LLOQ)	0.21±0.02	102.5	6.7			
	0.5	0.55±0.01	109.2	9.2	0.54±0.03	107.6	7.6
	15	16.33±0.97	108.9	8.9	16.37±0.65	109.2	9.2
	37.5	39.78±2.48	106.1	6.1	40.37±2.35	107.6	7.7
DCL	0.2 (LLOQ)	0.21±0.01	105.5	7.7			
	0.5	0.52±0.03	104.0	4.0	0.51±0.05	101.8	1.8
	15	15.48±0.92	103.2	3.2	15.34±0.95	102.2	2.2
	37.5	40.80±1.69	108.8	8.8	40.57±1.84	108.2	8.2

Table 3 Recoveries of loratadine (LOR), descarboethoxyloratadine (DCL) and propranolol (IS)

Analy	Nominal conc. (ng/mL)	Mean (%)	CV (%)
LOR	0.5	90.3	2.5
	15	89.4	1.8
	37.5	87.7	2.7
DCL	0.5	91.8	1.6
	15	90.6	3.7
	37.5	88.5	1.5
IS	100	89.3	1.9

Table 4 Stability of loratadine (LOR) and descarboethoxyloratadine (DCL) (unit: % of theoretical value, mean±SD of 3 determinations)

Analyte	Nominal conc. (ng/mL)	Short Stability (25°C 6 h)	Freeze–thaw Stability (3 cycles)	Auto sampler Stability (4°C 24 h)	Long-term Stability (-80°C 3 months)
LOR	0.5	100.9±3.1	90.1±1.6	96.0±2.8	89.3±3.5
	15	101.3±5.5	93.2±2.1	98.9±4.2	87.9±2.2
	37.5	99.0±4.8	96.7±1.8	95.7±3.6	92.6±2.4
DCL	0.5	101.8±3.9	92.6±2.6	89.3±1.2	89.7±4.4
	15	98.2±1.9	102.9±1.5	92.6±3.0	91.6±1.8
	37.5	101.9±2.4	97.7±3.7	94.6±3.2	90.7±5.4

3.7 Therapeutic drug monitoring in children

The 15 enrolled pediatric patients had a median age of 4.9 years (range 2.1–9.0 years) and an average weight of 20.4 kg (range 14.0 to 42.0 kg). Their LOR concentrations ranged from 0.24 to 8.86 ng/mL with a median value of 1.24 ng/mL and the DCL concentrations ranged from 1.46 to 11.80 ng/mL with a median concentration of 4.28 ng/mL. The ratio of DCL concentration to LOR concentration ranged from 0.67 to 20.7 with a median ratio of 5.87.

4. DISCUSSION AND CONCLUSION

LOR pharmacokinetics has been demonstrated to have large inter-individual variability in Chinese adults [7, 10], and currently LOR pharmacokinetic data is limited in Chinese children. Obviously, lacking pharmacokinetic data may lead to improper treatment. A validated and reliable method is essential for the determination of LOR and DCL in children. The present method was sensitive (LLOQ of 0.2 ng/mL) with a small volume of sample (50 μL of plasma) and simple sample preparation.

In comparison to previously published methods, our method has some key unique features making it well adapted for pediatric research. First, only a small volume of plasma (50 μL) was required to determine LOR and DCL concentrations. This improvement to testing will decrease the technical barriers to perform pharmacokinetics in children. Secondly, we used a simple sample preparation of protein precipitation which is a convenient and simple extraction method without a significant matrix effect and could be easily applied in clinical practice. Finally, our method could achieve a relatively high sensitivity (LLOQ of 0.2 ng/mL), which is better than some HPLC and LC/MS/MS methods [11, 15]. It was found that the LLOQ of 0.2 ng/ml was sufficient for the determination of LOR and DCL in our pediatric research. These features would favor the measurement of pediatric plasma samples in clinical practice.

This method was further applied in a pediatric pharmacokinetics and TDM study. The trough concentrations of LOR and DCL and the ratios of DCL concentration to LOR concentration demonstrated had large inter-individual variability in children. This variability may be attributed to the first-pass metabolism of LOR in Chinese populations with a higher genotypic frequency of the CYP2D6*10 allele.

It should be noted that this high variability may result in different therapeutic effects of LOR, and some patients may have a risk of treatment failure. Therefore, therapeutic drug monitoring and suitable dose should be recommended to optimize treatment in these patients.

In conclusion, a reliable, sensitive and clinically feasible LC/MS/MS method for determination of LOR and DCL in human plasma was developed. Its small sample volume of plasma, simple protein precipitation procedure and relative lower limit of quantification made this method well-adapted to perform TDM of LOR in children.

5. ACKNOWLEDGEMENTS

This study is supported by the National Science and Technology Major Projects for Major New Drugs Innovation and Development (2017ZX09304029-002), and Young Taishan Scholars Program of Shandong Province and Qilu Outstanding Program of Shandong University.

REFERENCES

[1] Clissold, S. P.; Sorkin, E. M.; Goa, K. L., Loratadine. A preliminary review of its pharmacodynamic properties and therapeutic efficacy. Drugs 1989, 37 (1), 42-57.

[2] Salmun, L. M.; Herron, J. M.; Banfield, C.; Padhi, D.; Lorber, R.; Affrime, M. B., The pharmacokinetics, electrocardiographic effects, and tolerability of loratadine syrup in children aged 2 to 5 years. Clin Ther 2000, 22 (5), 613-21.

[3] Geha, R. S.; Meltzer, E. O., Desloratadine: A new, nonsedating, oral antihistamine. J Allergy Clin Immunol 2001, 107 (4), 751-62.

[4] Ramanathan, R.; Reyderman, L.; Kulmatycki, K.; Su, A. D.; Alvarez, N.; Chowdhury, S. K.; Alton, K. B.; Wirth, M. A.; Clement, R. P.; Statkevich, P.; Patrick, J. E., Disposition of loratadine in healthy volunteers. Xenobiotica 2007, 37 (7), 753-69.

[5] Matzke, G. R.; Halstenson, C. E.; Opsahl, J. A.; Hilbert, J.; Perentesis, G.; Radwanski, E.; Zampaglione, N., Pharmacokinetics of loratadine in patients with renal insufficiency. J Clin Pharmacol 1990, 30 (4), 364-71.

[6] Radwanski, E.; Hilbert, J.; Symchowicz, S.; Zampaglione, N., Loratadine: multiple-dose pharmacokinetics. J Clin Pharmacol 1987, 27 (7), 530-3.

[7] Zhang, Y. F.; Chen, X. Y.; Zhong, D. F.; Dong, Y. M., Pharmacokinetics of loratadine and its active metabolite descarboethoxyloratadine in healthy Chinese subjects. Acta Pharmacol Sin 2003, 24 (7), 715-8.

[8] Ghosal, A.; Gupta, S.; Ramanathan, R.; Yuan, Y.; Lu, X.; Su, A. D.; Alvarez, N.; Zbaida, S.; Chowdhury, S. K.; Alton, K. B., Metabolism of loratadine and further characterization of its in vitro metabolites. Drug Metab Lett 2009, 3 (3), 162-70.

[9] Yumibe, N.; Huie, K.; Chen, K. J.; Snow, M.; Clement, R. P.; Cayen, M. N., Identification of human liver cytochrome P450 enzymes that metabolize the nonsedating antihistamine loratadine. Formation of descarboethoxyloratadine by CYP3A4 and CYP2D6. Biochem Pharmacol 1996, 51 (2), 165-72.

[10] Yin, O. Q.; Shi, X. J.; Tomlinson, B.; Chow, M. S., Effect of cyp2d6*10 allele on the pharmacokinetics of loratadine in chinese subjects. Drug Metab Dispos **2005,** 33 (9), 1283-7.

[11] Yin, O. Q.; Shi, X.; Chow, M. S., Reliable and specific high-performance liquid chromatographic method for simultaneous determination of loratadine and its metabolite in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci **2003,** 796 (1), 165-72.

[12] Johnson, R.; Christensen, J.; Lin, C. C., Sensitive gas-liquid chromatographic method for the determination of loratadine and its major active metabolite, descarboethoxyloratadine, in human plasma using a nitrogen-phosphorus detector. J Chromatogr B Biomed Appl **1994,** 657 (1), 125-31

[13] Naidong, W.; Addison, T.; Schneider, T.; Jiang, X.; Halls, T. D., A sensitive LC/MS/MS method using silica column and aqueous-organic mobile phase for the analysis of loratadine and descarboethoxy-loratadine in human plasma. J Pharm Biomed Anal **2003,** 32 (4-5), 609-17.

[14] Sutherland, F. C.; de Jager, A. D.; Badenhorst, D.; Scanes, T.; Hundt, H. K.; Swart, K. J.; Hundt, A. F., Sensitive liquid chromatography-tandem mass spectrometry method for the determination of loratadine and its major active metabolite descarboethoxyloratadine in human plasma. J Chromatogr A **2001,** 914 (1-2), 37-43.

[15] Vlase, L.; Imre, S.; Muntean, D.; Leucuta, S. E., Determination of loratadine and its active metabolite in human plasma by high-performance liquid chromatography with mass spectrometry detection. J Pharm Biomed Anal **2007,** 44 (3), 652-7.

[16] U.S Food & Drug Administration. Bioanalytical Method Validation Guidance for Industry. May **2018.**