BJPS

Brazilian Journal of
Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902022e20767

Article

Application of capillary zone electrophoresis to determine second-generation H1 antihistaminic drugs, loratadine and rupatadine

Cintia Maria Alves Mothé[1]*, Aline de Souza[1]*, Anil Kumar Singh[1], Nádia Araci Bou-Chacra[1], María Segunda Aurora-Prado[1]

**These authors contributed equally to this study*

[1]University of São Paulo, Faculty of Pharmaceutical Sciences, Department of Pharmacy, São Paulo, SP, Brazil

The second generation of H1 antihistamines from the piperidine group are often used for treating allergic diseases due to their action on histaminic receptors, the primary mediator of allergy. Moreover, the antihistamines have anti-inflammatory action, mediated through platelet-activating factor blocking activity. A simple and rapid capillary zone electrophoresis method was developed and validated for the determination of loratadine (LOR) and rupatadine (RUP) in tablets. The analyses were carried out using a fused silica capillary of 50.2 cm (40 cm effective length), 75 µm i.d. The background electrolyte was composed of boric acid 35 mmol/L, pH 2.5. Voltage of 20 kV, hydrodynamic injection of 3447.3 Pa for 3s, temperature at 25 ºC, and UV detection at 205 nm were applied. Electrophoretic separation was achieved at 1.8 and 2.8 min for RUP and LOR, respectively. The method was linear for both drugs in a range of 50.0 to 400.0 µg/mL (r>0.99). The limits of detection and quantification were 46.37 and 140.52 µg/mL, for LOR and 29.60 and 89.69 µg/mL for RUP respectively. The precision was less than 5.0 % for both drugs. The average recovery was approximately 100 %. The proposed novel method can significantly contribute to the rapid detection of counterfeit products and in quality control of drug products containing antihistamines.

Keywords: Antihistamines. Quality control. Capillary electrophoresis. Analytical method development.

INTRODUCTION

According to the World Health Organization (WHO, 2020), 235 million people are affected by asthma with approximately 383 thousand deaths around the world in 2015. People with severe asthma may be at higher risk of becoming ill from COVID-19 (Shaker *et al.*, 2020). The prevalence of allergies has increased drastically in the recent past. Around 30 % of the world´s population has some form of allergic disease. Thus, it must be regarded as a significant healthcare problem (Pawankar, 2014).

Among the antihistamines available on the market, the second-generation H1 antihistamines have a high affinity for H1 receptors and are relatively less lipophilic than those belonging to the first-generation H1 antihistamines. Due to these features, they hardly cross the blood-brain barrier and do not cause drowsiness (Fein *et al.*, 2019). The second-generation H1 antihistamines are highly potent, with long half-life allowing daily dose posology (Yanai *et al.*, 2017). The basic structure of these antihistaminic drugs consists of a tertiary amine group, which is connected via a

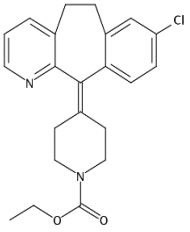
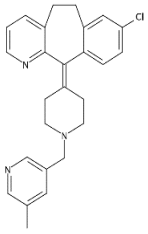
***Correspondence:** N. A. Bou-Chacra. Departamento de Farmácia. Faculdade de Ciências Farmacêuticas. Universidade de São Paulo. Av. Prof. Lineu Prestes, 580, Bl-15. 05508-900, São Paulo, SP, Brasil. Phone: +55 11 3091-3628. E-mail: chacra@usp.br. ORCID: https://orcid.org/0000-0002-5271-5468. M. S. Aurora-Prado. Departamento de Farmácia. Faculdade de Ciências Farmacêuticas. Universidade de São Paulo. Av. Prof. Lineu Prestes, 580, Bl-15. 05508-900, São Paulo – SP, Brasil. Phone: +55 11 3091-3655. E-mail: msaprad06@usp.br. ORCID: https://orcid.org/0000-0002-9956-9410. C. M. A. Mothé: cintia_mothe@hotmail.com, ORCID: https://orcid.org/0000-0002-4683-2676. A. de Souza: alinesza.sp@gmail.com, ORCID: https://orcid.org/0000-0003-4307-6497. A. K. Singh: anil@usp.br

chain of two or three atoms, to two or more aromatic substituents. Structurally, Loratadine (LOR), and rupatadine (RUP), have conjugated cyclohepta attached to phenyl and a pyridine ring, responsible for basicity. RUP has an additional pyridine ring (Yanai *et al.*, 2017) (Table I).

There are different methods to identify and quantify LOR and RUP in pharmaceutical preparations and biological tissue. LOR (El Ragehy, Badawey, Khateeb, 2002; Rupérez, Fernández, Barbas, 2002; Radhakrishna *et al.*, 2003; Ulavapalli *et al.*, 2011) and RUP (Nogueira *et al.*, 2008; Trivedi, Patel, 2012) were determined or quantified by HPLC. Capillary electrophoresis (CE) was also used as a method to determine LOR (Rambla-Alegre *et al.*, 2010; El-Awady, Belal, Pyell, 2013; Hancu *et al.*, 2014) and RUP (Nogueira *et al.*, 2008). To the best of our knowledge, no single analytical method using CE has been developed for both second-generation H1 antihistamines.

CE is a separation technique that uses an electric field to separate ionic or ionizable analytes. It is considered cheaper when compared to HPLC due to the price of columns and the use of a small volume of reagents (El Deeb *et al.*, 2016; Koenka, Hauser, 2017; Souza *et al.*, 2019). The goal of this study is to develop and validate a CE method for separation and determination of two second-generation H1 antihistamines, LOR and RUP, in tablets. This method can be useful in laboratories with a high demand in evaluating counterfeit products.

TABLE I - Structure, identification and predicted properties of antihistamines

Antihistamine	Chemical Name	Chemical Structure	Molar mass (g/mol)	pKa (strongest basic)	Log P
Loratadine (LOR) $C_{22}H_{23}ClN_2O_2$	Ethyl 4-(8-chloro-5,6-dihydro-11H-benzo[5,6] cyclohepta[1,2-b] pyridin-11-ylidene)-1-piperidinecarboxylate		382.88	4.33	4.55
Rupatadine (RUP) $C_{26}H_{26}N_3Cl$	8-Chloro-11-{1-[(5-methyl-3-pyridinyl) methyl]-4-piperidinylidene}-6,11-dihydro-5H-benzo[5,6] cyclohepta[1,2-b] pyridine		415.96	7.19	5.37

MATERIAL AND METHOD

Reagents and solvents

Methanol (MeOH), HPLC grade and orthophosphoric acid p.a. were purchased from J. T. BAKER® (United States). Purified water was obtained from MilliQ-Plus®. Sodium hydroxide was purchased from Labsynth (Brazil), boric acid was obtained from Sigma Aldrich (United States) and monobasic sodium phosphate salt (MSP) was from MERK® (Germany).

Instrumentation

The CE method was developed on a PACE/ MDQ (Beckman Coulter®, Fullerton, CA, U.S.A.) capillary

electrophoresis system, equipped with diode array detector (DAD) and thermostat sampler. Fused silica capillaries (Polymicro Technologies, Phoenix, AZ, U.S.A.) with a total length of 50.2 cm (40.0 cm effective length) x 75 µm i.d., 375 µm o.d. were used. All sample solutions were filtered through HV 0.45 µm membrane filters (Millipore®, Bedford, MA, USA). The new capillary was conditioned with 0.1 M NaOH solution for 30 min, followed by 15 min with deionized water and 20 min with the background electrolyte (BGE). At the beginning of each day a fused silica capillary was conditioned with a solution of 0.1 M NaOH for 20 min, followed by deionized water for 10 min and then by working BGE for 20 min. Between consecutive runs, the capillary was washed for 3 min with BGE. At the end of each day, the capillary was washed with NaOH solution 0.1 M, for 15 min, followed by deionized water for 15 min.

The separation was carried out by using BGE composed of boric acid 35 mmol/L, pH 2.5, adjusted with phosphoric acid, constant voltage of 20 kV, temperature at 25 ºC and UV detection at 205 nm. Sample injections were performed by an applied pressure of 3447.32 Pa for 3 s.

Standards and samples

LOR (99.5 %) and RUP (99.8 %) drug substances were obtained from commercial sources (Sigma-Aldrich, St. Louis, MO, USA). Drug products were purchased from a local market, with corresponding antihistaminic drugs. The placebos were prepared in the laboratory using corresponding excipients for each tablet formulation. The placebos (total of four) were stored protected from light and moisture, in hermetically sealed bottles and adequately identified.

Sample solution

All sample solutions were prepared in methanol at 1000 µg/mL concentration. The solutions were submitted to an ultrasonic bath for 15 min and subsequently stored under refrigeration. The working sample solutions were prepared daily in methanol at 200 µg/mL concentration. The placebo was prepared by mixing common excipients found in the pharmaceutical formulations.

CE analysis

The selection of initial conditions was based on the characteristics of drug and trial-error with several BGE. Variable parameters of the equipment were adjusted to a method that could be used for the analysis of all samples. Furthermore, to obtain rapid separations, fused silica capillaries with an internal diameter equivalent to 75 µm were tested. In both cases, monobasic sodium phosphate buffer (MSPB), and boric acid were tested as BGE.

The BGE stock solutions were prepared daily (100 mmol/L). From these solutions, aliquots were taken for the preparation of the working BGE solutions and the pH adjusted with orthophosphoric acid. All the solutions were filtered (HV 0.45) and sonicated for 10 min. The equipment variables such as applied voltage, injection time, and detection wavelengths were explored to obtain suitable and stable conditions.

Linearity, detection and quantitation limits, accuracy and robustness

The proposed methods were fully validated according to ICH guidelines (ICH, 2005; USP 42, 2019). The developed methods were applied in the separation and determination of LOR and RUP in pharmaceutical drug products by CE. Both methods proposed were validated for specificity, linearity, limits of detection and quantitation, precision, accuracy, and robustness.

The linearity of the method was evaluated at five different concentrations of the drugs, ranging from 50.0 to 400.0 µg/mL (50.0 µg/mL, 100.0 µg/mL, 200.0 µg/mL, 300.0 µg/mL, and 400.0 µg/mL), concentrations used to build the linear curve. The standard solutions of LOR and RUP were prepared fresh in methanol by dilution of standard stock solution (1000 µg/mL). Peak area was plotted versus the respective drug concentration. Each solution was injected in triplicate. The following equation represents the linearity curve (Equation 1):

$$Y = aX + b \qquad \text{(Equation 1)}$$

Where: a = angular coefficient (slope of the line), b = Y-intercept (intersection of the curve).

Limits of detection and quantification were calculated from the residual standard deviation of the regression line (σ) of the analytical curve and its angular coefficient (a) in accordance with the equations: limit of detection (LOD) = 3.3 (σ/a) and limit of quantitation (LOQ) = 10 (σ/a) (ICH, 2005).

The precision (repeatability) was determined by analysis of sample solutions, at 200.0 µg/mL (n = 10). For intermediate precision three concentration levels (100.0 µg/mL, 200.0 µg/mL, and 300.0 µg/mL), in triplicate (n=3) for three consecutive days were analyzed. All analyses were performed by the same analyst using the same equipment.

The accuracy of the proposed method was evaluated by the standard recovery method, described in validation guidelines (ICH, 2005; USP 42, 2019). The placebo, as well as commercial sample solutions of LOR, and RUP, were spiked with the respective standard solutions at three concentration levels (50.0 µg/mL, 100.0 µg/mL, and 150.0 µg/mL). All solutions were spiked and injected into the system, in triplicate (n=3).

For robustness, the following factors were evaluated: applied voltage (± 2 kV), pH of the BGE (± 0.2), and temperature (± 3.0 °C) (Table II).

To assess the selectivity of the proposed methods, solutions containing standard, commercial samples, placebos, and diluents were injected under normal analytical conditions.

TABLE II - Variables applied for testing of robustness

Analyses	Voltage (kV)	Temperature (°C)	Electrolyte pH
1	18	22	2.3
2	22	22	2.3
3	18	28	2.3
4	22	28	2.3
5	18	22	2.7
6	22	22	2.7
7	18	28	2.7
8	22	28	2.7

RESULTS AND DISCUSSION

CE Analysis

CE has been used for quality control of the pharmaceutical formulations to simultaneously determine more than one drug in a single run. CE has advantages such as a high separation efficiency, short analysis time, high peak capacity, small substance amounts, and volumes of chemicals used for BGE preparation. The analytical separation in CE depends on several parameters, such as BGE ionic strength, applied voltage, and physicochemical properties of analytes (Štěpánová, Kašička, 2014; Zhu, Scriba, 2018).

The analytical development was conducted by using boric acid buffer pH 2.5, adjusted with orthophosphoric acid, with BGE MSPB also being tested. When MSPB was used, the migration time was longer than when using boric acid buffer, and this is due to the fact that the MSPB molar mass is greater, and consequently, the electrolyte viscosity is higher. Therefore, boric acid was chosen for the rest of analysis.

The BGE with acidic pH promotes protonation of amphoteric and/or basic antihistaminic drugs. LOR has protonated nitrogen in the cycloheptapyridine ring (at acid pH) while the carboxyl group connected to the piperidine ring prevents the protonation of its nitrogen. RUP has three nitrogen atoms and can accept three H^+, at low pH. The high ionization state contributes to the rapid migration of the microspecies.

LOR and RUP have a molar mass of 382.88 g/mol and 415.96 g/mol, respectively. As expected, RUP with the largest charge/mass ratio showed faster migration time (1.8 min), and LOR with the smallest charge/mass ratio presented the longest migration time (2.8 min) (Figure 1). Sebaiy and Ziedan, (2019) developed an HPLC method for LOR for the detection of this drug in human plasma with a retention time of 4.10 min, when compared with a CE method developed by Xia *et al.* (2010), Sebaiy and Ziedan (2019) showed better performance. Xia *et al.* (2010) developed their method using tubular capillary microextraction and sweeping for the LOR quantification in rabbit plasma with analysis time greater than 60 min; however, this method is long when compared to Hancu

et al. (2014) who developed a faster CE method for LOR (4.31 min).

RUP had a migration time of 1.8 min and showed to be faster than the MECK method developed by Nogueira *et al.* (2008) (migration time of 3.93 min). Figure 1 shows electropherograms of LOR and RUP. The result revealed attractive migration time, which achieved less than 3 min for both drugs.

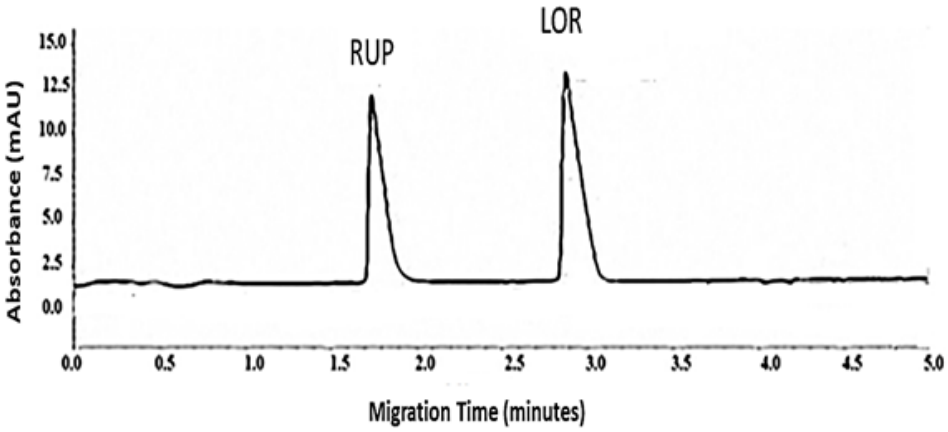


FIGURE 1 - Electropherograms of standard solutions. RUP – Rupatadine (400 µg/mL) migration time 1.8 min. LOR – Loratadine (200 µg/mL) migration time 2.8 min. Conditions: fused silica capillary 50.2 cm (40.0 cm effective length) x 75 µm i.d.; electrolyte: boric acid 35 mmol/L, pH 2.5, adjusted with orthophosphoric acid, applied voltage of 20 kV, hydrodynamic injection of 3447.32 Pa for 3 seconds, temperature of 25 ºC. UV detection at 205 nm.

Selectivity

To evaluate the selectivity of the CE methods, besides sample solutions, BGE solution, sample diluents (methanol), and placebo were injected (n=3)**.** The overlapping electropherograms indicate adequate selectivity of the proposed methods for the analysis of LOR and RUP (Figure 2).

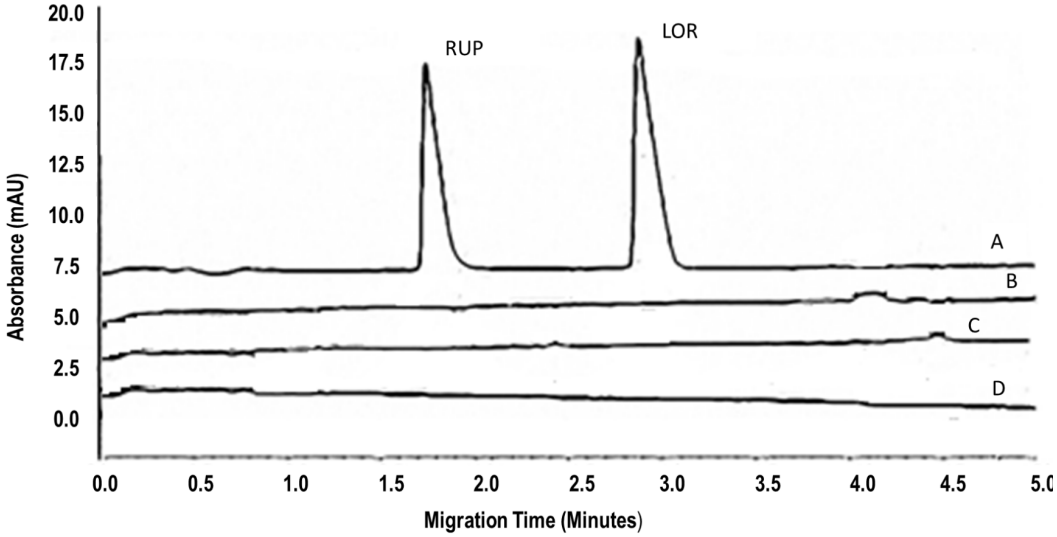


FIGURE 2 - Electropherograms of A) sample; B) placebo; C) BGE; D) sample diluent. Conditions: fused silica capillary 40.0 cm effective and 50.2 cm total x 75 µm i.d.; electrolyte: boric acid 35 mmol / L, pH 2.5, adjusted with orthophosphoric acid, applied voltage of 20 kV, hydrodynamic injection of 3447.32 Pa for 3 seconds, temperature of 25 ºC. UV detection at 205 nm. LOR: loratadine; RUP: rupatadine; BGE: background electrolyte.

Linearity, detection and quantitation limits and precision

According to the validation guidelines (ICH, 2005; USP 42, 2019) the coefficient of determination (r^2), calculated by least mean squares, must be above 0.99. The results (Table III) demonstrate the linearity between the studied concentrations of the samples and CE-UV responses. Experimental LOD and LOQ of the method were performed and the results are presented in Table III.

Table IV demonstrated the precision of the method and the results prove that the method has acceptable precision in the analysis for the two antihistamines analyzed. The relative standard deviation ranges from 1.4 to 5.1; this indicates the ability of the developed method to determine the drug substances in tablets.

TABLE III - The linear regression data, LOD, LOQ for LOR, and RUP

Parameter	LOR	RUP
Concentration range (µg/mL)	50-400	50-400
Slope	0.0065	0.0023
Intercept	-0.0263	-0.0063
Determination coefficient (R^2)	0.9903	0.9960
Standard error of intercept	0.0921	0.0206
Standard error of slope	0.0003	8.40E-05
LOD (µg/mL)*	1.11	1.05
LOQ (µg/mL)*	3.31	3.46
Migration time (min)	2.8	1.8

LOD: limit of detection; LOQ: limit of quantitation; LOR: loratadine; RUP: rupatadine. *experimental results.

TABLE IV - Precision (repeatability and intermediate precision) for LOR, and RUP

Drug	Concentration (µg/mL)	Repeatability (%RSD)[a]	Day 1 (%RSD)[b]	Day2 (%RSD)[b]	Day 3 (%RSD)[b]	Mean %RSD
	100	-	0.78	0.74	0.78	3.0
LOR	200	1.33	1.55	1.50	1.51	1.4
	300	-	2.09	2.27	2.15	4.2
%RSD		**3,3**				
	100	-	0.23	0.22	0.21	4.1
RUP	200	0.74	0.45	0.45	0.49	5.1
	300	-	0.65	0.66	0.64	1.5
%RSD		**1.6**				

LOR: loratadine; RUP: rupatadine; % RSD: relative standard deviation.
[a]: Mean of ten determinations; [b]: Mean of three determinations

Accuracy

The average recovery of LOR and RUP standards from spiked placebo and sample solutions was near 100%. Based on the results presented in Table V, the method can be considered accurate.

TABLE V - Accuracy of the proposed methods

Drug	Standard added to placebo (µg/mL)	Concentration (µg/mL)	Recovery (%)	Standard added to commercial sample (µg/mL)	Concentration (µg/mL)	Recovery (%)
	50	50.73	101.46	50	51.01	102.02
LOR	100	101.70	101.70	100	100.81	100.81
	150	149.94	99.96	150	151.73	101.15
%RSD			**0.93**			**0.61**
	50	50.07	100.14	50	50.39	100.78
RUP	100	100.23	100.23	100	100.17	100.17
	150	151.40	100.93	150	148.37	98.91
%RSD			**0.43**			**0.95**

LOR: loratadine; RUP: rupatadine.

Robustness

After deliberate variation in applied voltage (± 2.0 kV), BGE pH (± 0.2), and temperature (+ 2.0 °C) no significant impact was found on LOR and RUP electropherograms (Figures not shown). These results indicated that the methods could withstand deliberate changes in the analytical conditions.

The CE method for LOR and RUP has the potential to be a simple alternative method compared to HPLC. It was 21-fold faster than the CE method developed by Xia *et al.* (2010). This method can be used in quality control and for accurate detection of counterfeit products. H1 antihistamines have been illegally added to dietary supplements (Do *et al.*, 2017). According to the European Medicine Agency (EMA, 2020), counterfeit medicine neglects intellectual-right and regulatory laws. CE has shown to be an effective and low-cost method compared to HPLC in quality control to combat counterfeiting (Marini *et al.*, 2010; Höllein *et al.*, 2016).

CONCLUSION

The developed methods is simple, economical, rapid, and reliable for raid analysis of these antihistaminic drugs. Among the methods found in the literature, for high performance liquid chromatography, none presented such simple conditions in which little solvent was used and fast retention times were obtained, facilitating its application in drug quality control and for detection of counterfeit products. The major advantage of this method is that it consists of the same setup, BGE, and capillary. This method has the potential to be useful in laboratories with a high demand in evaluating counterfeit products.

ACKNOWLEDGMENTS

The authors thank the "Comissão de Aperfeiçoamento de Pessoal do Nível Superior do Brasil (CAPES)" for fellowship support and "Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, 2012/50595-8)" for financial support.

REFERENCES

Do JA, Noh E, Yoon SB, Lee JH, Park SK, Mandava S, et al. Collision-induced dissociation pathways of H1-antihistamines by electrospray ionization quadrupole time-of-flight mass spectrometry. Arch Pharm Res. 2017;40(6):736-745.

El Deeb S, Wätzig H, Abd El-Hady D, Sänger-van de Griend C, Scriba GKE. Recent advances in capillary electrophoretic migration techniques for pharmaceutical analysis (2013-2015). Electrophoresis. 2016;37(12):1591-1608.

El Ragehy NA, Badawey AM, Khateeb SZE. Stability indicating methods for the determination of loratadine in the presence of its degradation product. J Pharm Biomed Anal. 2002;28(6):1041-1053.

El-Awady M, Belal F, Pyell U. Robust analysis of the hydrophobic basic analytes loratadine and desloratadine in pharmaceutical preparations and biological fluids by sweeping-cyclodextrin-modified micellar electrokinetic chromatography. J Chromatogr A. 2013;1309:64-75.

European Medicines Agency. EMA. Falsified medicines: overview. Available from: https://www.ema.europa.eu/en/human-regulatory/overview/public-health-threats/falsified-medicines-overview, 2020.

Fein MN, Fischer DA, O'Keefe AW, Sussman GL. CSACI position statement: Newer generation H1-antihistamines are safer than first-generation H1-antihistamines and should be the first-line antihistamines for the treatment of allergic rhinitis and urticaria. Allergy Asthma Clin Immunol. 2019;15(1):61

Hancu G, Câmpian C, Rusu A, Mircia E, Kelemen H. Simultaneous determination of loratadine, desloratadine and cetirizine by capillary zone electrophoresis. Adv Pharm Bull. 2014;4(2):161-165.

Höllein L, Kaale E, Mwalwisi YH, Schulze MH, Holzgrabe U. Routine quality control of medicines in developing countries: Analytical challenges, regulatory infrastructures and the prevalence of counterfeit medicines in Tanzania. TrAC - Trends in Analytical Chemistry. Elsevier B.V.; 2016;76:60-70.

International Conference of Harmonization. ICH. Validation of Analytical Procedures: Text and Methodology Q2 (R1). Int Conf Harmon. 2005;(November 1996):17.

Koenka IJ, Hauser PC. Background conductivity independent counter flow preconcentration method for capillary electrophoresis. Electrophoresis. 2017;38(21):2721-2724.

Marini RD, Rozet E, Montes MLA, Rohrbasser C, Roht S, Rhème D, et al. Reliable low-cost capillary electrophoresis device for drug quality control and counterfeit medicines. J Pharm Biomed Anal. 2010;53(5):1278-1287.

Nogueira DR, da Silva Sangol M, da Silva LM, Dalmora SL. Determination of rupatadine in pharmaceutical formulations by a validated stability-indicating MEKC method. J Sep Sci. 2008;31(16-17):3098-3105.

Pawankar R. Allergic diseases and asthma: A global public health concern and a call to action. World Allergy Organ J. 2014;7(1):12.

Radhakrishna T, Narasaraju A, Ramakrishna M, Satyanarayana A. Simultaneous determination of montelukast and loratadine by HPLC and derivative spectrophotometric methods. J Pharm Biomed Anal. 2003;31(2):359-368.

Rambla-Alegre M, Peris-Vicente J, Esteve-Romero J, Capella-Peiró MEE, Bose D. Capillary electrophoresis determination of antihistamines in serum and pharmaceuticals. Anal Chim Acta. 2010;666(1-2):102-109.

Rupérez FJ, Fernández H, Barbas C. LC determination of loratadine and related impurities. J Pharm Biomed Anal. 2002;29(1-2):35-41.

Sebaiy MM, Ziedan NI. Developing A High-performance Liquid Chromatography Method for Simultaneous Determination of Loratadine and its Metabolite Desloratadine in Human Plasma. Curr Drug Metab. 2019;20(13):1053-1059.

Shaker MS, Oppenheimer J, Grayson M, Stukus D, Hartog N, Hsieh EWY, et al. COVID-19: Pandemic Contingency Planning for the Allergy and Immunology Clinic. J Allergy Clin Immunol Pract. 2020;8(5):1477-1488.e5.

Souza A, Kedor-Hackmann ERM, Santoro MIRM, Aurora-Prado MS. Development of analytical method by free solution capillary electrophoresis for furosemide under stress degradation. Sep Sci PLUS. 2019;2(2):253-261.

Štěpánová S, Kašička V. Determination of impurities and counterions of pharmaceuticals by capillary electromigration methods. J Sep Sci. 2014;37(15):2039-2055.

Trivedi HK, Patel MC. Development of a stability-indicating RP-HPLC method for the determination of rupatadine and its degradation products in solid oral dosage form. Sci Pharm. 2012;80(4):889-902.

Ulavapalli KR, Sriramulu J, Mallu UR, Bobbarala V. Simultaneous determination of pseudoephedrine, fexofenadine and loratadine in pharmaceutical products using high resolution RP-HPLC method. J Pharm Res. 2011;4(4):1219-1221.

United States Pharmacopeia. USP 42. 42nd ed. USP United States Pharmacopeial Conv., editor. Rockville, MD: The United States Pharmacopeia. 2019.

World Health Organization. WHO. Asthma. Available from: https://www.who.int/news-room/fact-sheets/detail/asthma, 2020.

Xia Z, Gan T, Chen H, Lv R, Wei W, Yang F. A new open tubular capillary microextraction and sweeping for the analysis of super low concentration of hydrophobic compounds. J Sep Sci. 2010;33(20):3221-3230.

Yanai K, Yoshikawa T, Yanai A, Nakamura T, Lida T, Leurs R, et al. The clinical pharmacology of non-sedating antihistamines. Pharmacol Ther. 2017;178:148-156.

Zhu Q, Scriba GKE. Analysis of small molecule drugs, excipients and counter ions in pharmaceuticals by capillary electromigration methods – recent developments. J Pharm Biomed Anal. 2018;147:425-438.

Received for publication on 20th August 2020
Accepted for publication on 29th September 2021