Developing a high-performance liquid chromatography method for simultaneous determination of loratadine and its metabolite desloratadine in human plasma

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Abstract

Background: Allergic diseases are considered among the major burdons of public health with increased prevalence globally. Histamine H_1-receptor antagonists are the foremost commonly used drugs in the treatment of allergic disorders. Our target drug is one of this class, loratadine and its biometabolite desloratadine which is also a non sedating H_1 receptor antagonist with anti-histaminic action of 2.5 to 4 times greater than loratadine.

Objective: To develop and validate a novel isocratic reversed-phase high performance liquid chromatography (RP-HPLC) method for rapid and simultaneous separation and determination of loratadine and its metabolite, desloratadine in human plasma.

Methods: The drug extraction method from plasma was based on protein precipitation technique. The separation was carried out on a Thermo Scientific BDS Hypersil C_{18} column (5µm, 250 x 4.60 mm) using a mobile phase of MeOH : 0.025M KH_2PO_4 adjusted to pH 3.50 using orthophosphoric acid (85 : 15, v/v) at ambient temperature. The flow rate was maintained at 1 mL/min and maximum absorption was measured using PDA detector at 248 nm.

Results: The retention times of loratadine and desloratadine in plasma samples were recorded to be 4.10 and 5.08 minutes respectively, indicating a short analysis time. Limits of detection were found to be 1.80 and 1.97 ng/mL for loratadine and desloratadine, respectively, showing a high degree of method sensitivity. The method was then validated according to FDA guidelines for the determination of the two analytes in human plasma.

Conclusion: The results obtained indicate that the proposed method is rapid, sensitive in the nanogram range, accurate, selective, robust and reproducible compared to other reported methods.

Keywords: RP-HPLC; loratadine; desloratadine; protein preciptation; human plasma; FDA guidelines.

1. Introduction

Allergic diseases are considered among the the main four public health problems along with tumors, cardiovascular disease, and AIDS. The prevalence of allergic diseases have increased in recent decades in many countries.[1]. Histamine H_1-receptor antagonists are the foremost known therapeutic agents used to treat allergic disorders [2].

Loratadine (LOR) (Figure 1) is commonly used as a selective non sedating H_1 receptor antagonist mainly for allergic rhinitis, urticaria and, to a limited extent, in asthma [3]. Desloratadine (DES) (Figure1), the descarboethoxy form and the major active metabolite of loratadine, is also a non sedating H_1 receptor antagonist with anti-histaminic action 2.5 to 4 times greater than loratadine [4]. DES is also reported by the United States Pharmacopeia as a potential impurity in LOR bulk powder [5]. Both LOR and DES are weak bases where, the pKa of LOR is 5.25 while DES has two pKa's, 4.41 and 9.97 at room temperature [6]. As such, the close similarities between LOR and DES in respect of structure and physicochemical properties make their simultaneous analysis a challenging task. .

Please insert Figure 1.

Our literature survey showed different analytical methods for the simultaneous determination of LOR and DES including UPLC [7], HPLC [8-21], HPTLC [22], TLC [23], GC [24] spectrophotometric [25] and capillary electrophoretic [26] methods. However, many drawbacks were reported for the use of some of these methods despite using more advanced techniques and detectors. Examples of these drawbacks include; low sensitivities in respect of limits of detection and quantification [2, 7, 10, 12, 14, 15, 20-22, 25, 26], long chromatographic run time (ranging from 8 minutes up to 50 minutes) [10, 13-17, 20, 21], expensive, long and tedious extraction procedure [8, 9, 12, 13, 17-19, 24], in addition to low percentage recoveries which are not adequate for bioequivalence and pharmacokinetic studies (73.60% for LOR and 40.60% for DES) [8, 9, 17-19]. Therefore, there is still a strong demand to develop a simple, rapid and sensitive analytical method that would be able to detect and quantify both analytes in biological fluids without the need for a time-consuming sample pretreatment procedure. As such, the current study describes a novel, simple, sensitive and environmental friendly HPLC method for simultaneous determination of LOR and DES in spiked human plasma with a run time of less than 6 minutes with percentage recovery of 97.57 and 86.87% for LOR and DES, respectively.

2. Experimental:

2.1. Apparatus:

Thermo scientific (Finnigan Surveyor)® HPLC instrument (USA) with a Thermo Scietific® BDS Hypersil C_{18} (5 µm, 250 x 4.60 mm), PDA plus absorbance detector, LC QUAT pumps and connected to PC loaded with chromQuest software v5. **Labomed®** UV-VIS Double Beam (UVD-2950) Spectrophotometer with matched 1 cm quartz cells and connected to windows compatible computer using UV Win 5 Software v6. **HANNA®**HI 8314 membrane pH-meter (Romania) for pH adjustment.

2.2. *Materials and reagents:*

All solvents and reagents were HPLC analytical grade. Methanol, potassium dihydrogen phosphate and ortho-phosphoric acid were purchased from Fisher Scientific, England. **LOR and DES** were supplied by EPICO®, EGYPT. Standard solutions of 200 µg/mL were prepared by dissolving 0.01 mg of each pure drug in 50 mL of the mobile phase. **The mobile phase** was a freshly prepared binary mixture of MeOH and 0.025 M potassium dihydrogen phosphate adjusted to pH 3.50 using ortho-phosphoric acid (85 : 15, v/v), filtered and degassed using 0.45 µm membrane filter. **The human plasma** was provided by Zagazig University Hospital and was tested to be drug and disease-free. Plasma was kept frozen before use, and was then stored either at -4º C between uses or at -20º C for freeze-thaw cycle stability studies.

2.3. Procedures:

2.3.1. Preparation of standard calibration curves:

Appropriate mixed dilutions of LOR and DES standard stock solutions were done in 10 mL volumetric flasks to get final concentrations of 47.50 up to 3125 ng/mL for both drugs. A 10 µL of each mixture was then injected into the column and the chromatogram was obtained at 248 nm. A graph was plotted as the concentration of drugs against response (peak area).

2.3.2. Human plasma samples preparation:

Calibration curve and validation QC samples at concentrations of 60, 125, 500, 1000, and 2500 ng/mL in plasma were prepared. Aliquots of 400 µL plasma samples and different drug mixture volumes ranging from 200 up to 400 µL were added into 10 mL centrifuge tubes and vortexed for 1 min. The mixture was then precipitated with methanol (total volume 4 mL). After vortexing for 1 min, the samples were centrifuged at 5000 rpm for 15 min. An aliquots of 10 µL of each supernatant was filtered through 0.45 µm PTFE syringe filters (Membrane solutions, USA) and injected directly into the HPLC system for analysis.

3. Results and discussion:

3.1. Optimization of Chromatographic Conditions:

All chromatographic conditions are illustrated in Table S1. Spectroscopic analysis of LOR and DES at the range of 200-400 nm showed that they have UV absorbance maxima (λ_{max}) at 248 and 246 nm, respectively (Figure 2). Therefore, the chromatographic detection was performed at 248 nm as the appropriate wavelength using a PDA detector. The method was performed on a Thermo Scientific® BDS Hypersil C_{18} column (5 µm, 250 x 4.60 mm).

Please insert Figure 2.

The mobile phase composition and pH were optimized by varying the composition ratio of methanol and potassium dihydrogen phosphate at different pH values. For best separation, the mobile phase was kept as a mixture of MeOH and 0.025 M potassium dihydrogen phosphate adjusted to pH 3.50 using ortho-phosphoric acid (85 : 15, v/v) at a flow rate of 1 mL/min. Under these conditions, LOR and DES were separated and eluted at 4.10 and 5.08 minutes, respectively (Figure 3A), indicating a much faster run time in comparison with the reported methods.. These run times make the method more feasible in comparison with other reported methods [10, 13-17, 20, 21] which were time consuming with long run times ranging from 8 to 50 minutes. In addition, the mixture determination in plasma didn't show the matrix interference effect as the human plasma was eluted separately at 2.92 minutes in correspondence with the migration times of the two drugs (Figure 3B).

System suitability parameters were chosen based on the guidance of the center for drug evaluation and research (DES) (See Table 1). The results showed that the optimum mobile phase led to symmetrical peaks ($1.13 < T < 1.20$), capacity factor ($k < 10$), resolution > 2 and theoretical plates > 2000 in plasma samples spiked with LOR and DES. [27].

Please insert Figure 3 and Table 1.

3.2. Method Validation:

The method validation was performed according to FDA guidelines. The method was validated for linearity, accuracy, precision, specificity, robustness, ruggedness, LODs and LOQs. Calibration curves in the spiked plasma were found to be linear over the clinical range of 60 -2500 ng/mL for both drugs. The mean

recoveries for LOR and DES in plasma were 97.57 and 86.87%, respectively, which make them lie within the acceptable range according to FDA guidelines [28, 29].

3.2.1. Linearity:

Eleven different concentrations in the range 47.50 - 3125 ng/mL of the drug mixture were used for linearity studies. Calibration curves were obtained by plotting peak area against each concentration (Table 2). Linear regression equations for LOR and DES were found to be y = 3267.5X +650.36, and y = 3360.2X + 57.704, respectively while the regression coefficient values (R^2) were 0.9999 for the both indicating a high degree of linearity (Figure S1).

3.2.2. Limits of detection and limits of quantification:

For determination of the limits of detection and quantitation, the adapted method was based on the signal-to-noise ratio (3:1 for LOD & 10:1 for LOQ). Limits of detection were found to be 1.28 and 1.31 ng/mL, while limits of quantification were calculated to be 3.89 and 3.98 ng/mL for LOR and DES, respectively (Table 2). As compared to referenced methods, Fathalla, B. *et al.* [2], for example, introduced a rapid micellar HPLC analysis for determination of LOR and DES in pharmaceutical and biological samples with LODs of 15 and 13 ng/mL, repectively. Other methods [7, 10, 12, 14, 15, 20-22, 25, 26] showed sensitivities in the range of 55-1000 ng/mL for LOR and DES. Our much lower LOD suggests that the proposed method is highly superior over these reported methods in terms of LOD and sensitivity. As such, the method would be more suitable for pharmacokinetic and bioequivalence studies, where the detection of small concentrations in plasma is required**.** According to Hilbert, J. *et. al.* [30] ,the plasma C_{max} was 30.5 ng/mL at 1.0 hour after oral dosing of 40 mg LOR while the plasma C_{max} for DES was 18.6 ng/mL at 2.2 hours after dosing. These concentrations are higher than our recorded LOQs confirming the applicability of the proposed method in biological studies.

Please insert Table 2.

3.2.3. Accuracy:

The accuracy of the method was determined by investigating the recoveries of LOR and DES at concentration levels covering the specified range (three replicates of each concentration) in spiked plasma samples. From the amount of the drug estimated, the percentage recovery was calculated and the results showed that LOR and DES had recovery ranges of 97.57 and 86.87% respectively in plasma samples (Table 3). The chosen analyte extraction method was simple and quick in comparison with other tedious reported

ones [8, 9, 12, 13, 17-19, 24] and seemed to positively affect the recovery ranges. The recovery ranges obtained were within the acceptable ranges stated by the FDA guidelines (85-115%) while previously reported methods' recovery ranges [8, 9, 17-19] were as low as 73.60% for LOR and 40.60% for DES.

Please insert Table 3.

3.2.4. Precision:

The precision of the method was determined for both intra-day and inter-day precision using validation QC samples at concentrations of 95, 625 and 100 µg/mL. In term of intra-day precision, both standard deviation (SD) and coefficient of variation (CV%) were calculated for three replicate analyses using the same pure drug solution at the first day of analysis. The SD and CV% values (varied from 0.001 to 1.59) revealed the high precision of the method. For inter-day reproducibility, the day-to-day SD and CV% values were also in the acceptable range of 0.002 - 2.63 (Table 4). These results showed that the proposed method has an adequate precision in the simultaneous determination of LOR and DES in pure samples.

Please insert Table 4.

3.2.5. Selectivity and Specificity:

The selectivity of the method was checked by injecting the solutions of LOR and DES into the column separately where two sharp peaks were obtained at retention times of 4.10 and 5.08 minutes, respectively, and these peaks were not obtained for the blank solution. The specificity of the method was demonstrated through the complete separation of peaks of analyzed drugs from the peak of the used sample matrix (human plasma).Sharp and well-resolved peaks were obtained for all the three entities. (Figure 3B).

3.2.6. Stability of human plasma samples:

Stability studies were conducted by storage of QC plasma samples at 4 ^{o}C and applying freeze-thaw cycles at -80 ^{o}C (over three days) and results are summarized in Table 4. The SD and CV% values (varied from 0.001 to 10.29) show that the spiked drug plasma samples are highly stable indicating the high suitability of the method in pharmacokinetic studies regarding LOR and DES mixture in biological samples.

4. Conclusion:

In this study, a novel RP-HPLC method was developed and validated for rapid and simultaneous estimation of loratadine (LOR) and desloratadine (DES) in biological fluids within 6 minutes. The results obtained indicated that the proposed method is rapid, sensitive in the nanogram range, accurate, selective, robust and reproducible. This analytical method is adequate and useful for the clinical estimation of LOR and DES in human plasma samples which can be applied for therapeutic drug monitoring according to FDA guidelines for pharmacokinetic and bioequivalence studies

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

Present in manuscript and supplementary file.

Ethics approval and consent to participate

Not applicable.

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Figure Captions

Figure 1: Chemical structures of loratadine (LOR) and desloratadine (DES).

Figure 2: Overlain spectra of 1000 ng/mL loratadine (LOR.....) and desloratadine (DES___) at maximum wavelengths of 248 and 246 nm, respectively.

Figure 3: HPLC Chromatogram of (A) authentic mixture of loratadine (LOR) and desloratadine (DES) and (B) spiked mixture of loratadine (LOR) and desloratadine (DES) in human plasma using Thermo Scientific® BDS Hypersil C_{18} column (5 µm, 250 x 4.60 mm) column and a mobile phase of MeOH : 0.025 M KH2PO4 adjusted to pH 3.50 using ortho - phosphoric acid (85 : 15, v/v). Other chromatographic conditions are stated in Table S1.

Table 1. System suitability parameters for LOR and DES in plasma samples.

Parameters	LOR	DES	Reference values [27]
Retention time, tr	4.10	5.08	
Capacity factor, k'	1.73	2.38	Accepted k' value (1-10)
Peak asymmetry (Tailing factor, T)	1.13	1.20	Accepted T value $\leq$ 2
Therotical plates, N	2151	3329	Accepted N value > 2000
Resolution, Rs	---	2.20	Accepted value > 2
Selectivity (Separation factor, α)	---	1.37	

Table 2. Results of analysis for LOR and DES using the proposed method.

Parameters	LOR				DES			
	Taken ng/mL	Found ng/mL	Recovery %	Accuracy %	Taken ng/mL	Found ng/mL	Recovery %	Accuracy %
	3125	3129.12	100.13	0.13	**3125**	3144.13	100.61	0.61
	2500	2483.51	99.34	-0.65	**2500**	2484.33	99.37	-0.62
	1560	1544.26	98.99	-1.01	**1560**	1580.12	101.29	1.29
	1250	1242.06	99.36	-0.63	**1250**	1231.27	98.50	-1.49
	780	793.95	101.78	1.78	**780**	795.58	101.99	1.99
	625	626.06	100.17	0.17	**625**	625.48	100.07	0.07
	312	317.76	101.68	1.68	**312**	307.66	98.45	-1.54
	190	186.20	98.00	-1.99	**190**	186.80	98.32	-1.67
	156	155.06	99.24	-0.75	**156**	153.98	98.54	-1.45
	95	93.40	98.32	-1.67	**95**	94.00	98.94	-1.05
	47.50	46.70	98.32	-1.67	**47.50**	46.55	98.00	-1.99
Mean			**99.57**	-0.42			**99.46**	-0.53
±SD			1.272				1.339	
±RSD			1.277				1.346	
±SE			0.383				0.403	
Variance			1.618				1.793	
LOD (ng/mL)			1.28				1.31	
LOQ (ng/mL)			3.89				3.98	

Table 3. Result of analysis of proposed method in human plasma.

Parameters	LOR				DES			
	Taken ng/mL	Found ng/mL	Recovery %	Accuracy %	Taken ng/mL	Found ng/mL	Recovery %	Accuracy %
	2500	2492.53	99.70	-0.29	**2500**	2137.27	85.49	-14.50
	1000	952.20	95.22	-4.77	**1000**	880.33	88.03	-11.96
	500	496.18	99.23	-0.76	**500**	429.72	85.94	-14.05
	125	121.14	96.91	-3.08	**125**	109.22	87.37	-12.62
	60	58.08	96.80	-3.19	**60**	52.52	87.53	-12.46
Mean			**97.57**	-2.42			**86.87**	-13.12
±SD			1.861				1.096	
±RSD			1.907				1.262	
±SE			0.832				0.490	
Variance			3.464				1.203	

Table 4. Intra- & inter-day precision and stability results using 3 quality control samples of loratadine (LOR) and desloratadine (DES) in pure and plasma samples.

	Drugs	Concentrations (ng/mL)	Found concentrations (ng/mL)	Mean ± SD	CV (%)	Accuracy %
Intra-day runs (n=3)	**LOR**	2500 625 95	2446 620 93	98.05 ± 0.03 99.20 ± 0.01 98.11 ± 0.001	1.24 1.28 1.07	-2.04 -0.81 -1.89
	DES	2500 625 95	2465 633 95.66	98.65 ± 0.03 101.36 ± 0.01 100.61 ± 0.001	1.31 1.19 1.59	-1.34 1.36 0.62
Inter-day runs (n=3)	**LOR**	2500 625 95	2531 636 95.70	100.68 ± 0.03 101.90 ± 0.01 100.97 ± 0.002	1.37 1.46 2.63	0.67 1.87 0.99
	DES	2500 625 95	2526 636 96.66	101.15 ± 0.04 101.83 ± 0.01 101.68 ± 0.002	1.61 1.91 2.60	1.12 1.82 1.67
4°C temperature (n plasma =3)	**LOR**	2500 500 60	2400 505 57	96.16 ± 0.08 101.01 ± 0.01 95.51 ± 0.001	3.56 2.43 1.75	-3.84 1.01 -4.48
	DES	2500 500 60	2127 439 51	85.08 ± 0.01 87. 93 ± 0.04 85.21 ± 0.002	0.53 10.29 4.05	-14.90 -12.07 -14.80
−80°C temperature (n plasma =3)	**LOR**	2500 500 60	2433 511 58	97.49 ± 0.05 102.34 ± 0.01 96.80 ± 0.002	2.10 2.60 3.44	-2.51 2.33 -3.19
	DES	2500 500 60	2139 464 52	85.56 ± 0.01 92. 82 ± 0.03 86.21 ± 0.001	0.47 6.57 3.33	-14.43 -7.18 -13.80