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Rapid and sensitive method for simultaneous determination of citalopram with antihistamines by liquid chromatography

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Abstract: A highly sensitive liquid chromatographic method with UV detection has been developed for simultaneous determination of citalopram, levocetirizine and loratadine in bulk drug, pharmaceutical formulation and human serum at 230nm employing 80:20 v/v methanol-water as mobile phase with pH3.5, adjusting flow rate of 1.0mL.min^{-1}. Separation was achieved on Shimadzu Shim-pack CLC-ODS (M) 25M column within the linear range of 0.4-12.5, 0.8-25 and 0.8-25µg.mL^{-1} with R^2 >0.998 and detection limit 7.75, 3.35 and 10.26ng.mL^{-1}respectively. ICH guidelines were followed for validation showing 0.22-1.76, 0.06-1.83 and 0.22-2.11% RSD. The recovery of analytes in tablets and serum was found to be in acceptable range. The method was fruitfully employed for the determination of studied analyte in pharmaceutical formulation and human serum.

Keywords: Citalopram, levocetirizine, loratadine, HPLC, validation, serum

INTRODUCTION

Variety of phobias, other depressive states such as panic, obsessive-compulsive disorder, anxiety are very commonly seen in the modern era (Raggi *et al*., 2003). Frequently recommended anti-depressants, selective serotonin reuptake inhibitors (SSRIs) exhibit equivalent clinical efficacy as tricyclic anti-depressants and ranked as safer and better tolerated against adverse effects (Eap *et al*., 1998). Citalopram{1-[3-(dimethylamino) propyl-]-1-(4-fluorophenyl)-l,3-dihydroisobenzofuran-5 carbonitrile} is one of the potent SSRIs widely used to treat depressive disorders (Haupt, 1996). It is available as racemic mixture of S(+) and R(-) enantiomers, only S(+)-enantiomer is pharmacologically effective (Macek *et al*., 2001, Rao *et al*., 2006). Citalopram has been determined by several methods including RP-HPLC (Rop *et al*., 1985), HPLC with UV and florescence detection (Kristoffersen *et al*., 1999), temperature-programmed packed capillary liquid chromatography with on column (Molander *et al*., 2002), LC separation on chiral AGP-column (Haupt, 1996), cyclic voltammetry (Norouzi *et al*., 2007), solid phase microextraction coupled with HPLC (Unceta *et al*., 2008), capillary isotachophoresis (Buzinkaiová and Polonsky, 2000), LC-MS/MS (Jiang *et al*., 2010), adsorptive stripping voltammetry (Nouws *et al*., 2006), densitometric HPTLC and video densitometric HPTLC (Skibiński and Misztal, 2005), capillary electrophoresis (Şatana *et al*., 2006), direct and derivative UV-spectrophotometry (SKIBI-SKI and MISZTAL, 2005), spectrofluorimetry (Satana *et al*., 2007), screen printed ion selective potentiometry (Ali *et al*., 2014) and HPLC-MS/ESI (Juan *et al*., 2005).

Antihistamines are used to cure of number of allergic disorders (Deruaz *et al*., 2004). Levocetirizine is the active metabolite of the drug, cetirizine; an H_1-receptor blocker effective for chronic idiopathic urticaria, allergy rhinitis and seasonal allergies (Rathore *et al*., 2012). It shows efficacy to relieve nasal symptoms (Arayne *et al*., 2008). Loratadine, a long acting histamine antagonist, selective for H_1-receptors (Rupérez *et al*., 2002), lack in substantial effect on autonomic and central nervous system (El-Sherbiny *et al*., 2007). Reported methods for antihistamine are micellar liquid chromatography (Martinez-Algaba *et al*., 2006), voltametry (Ghoneim *et al*., 2001, Beltagi *et al*., 2008), HPTLC (Muller and Sherma, 1999), LC-MS/MS (Morita *et al*., 2008), LC-ESI-MS (Ryu and Yoo, 2012), RP-HPLC (Arayne *et al*., 2008), spectrophotometry (Radhakrishna *et al*., 2003), GLC (Johnson *et al*., 1994), HPLC/UV detection (Kunicki, 2001) and capillary electrophoresis (Fernandez *et al*., 2003).

Here we report the development of quick and sensitive RP-HPLC method for simultaneous determination of citalopram with levocetirizine and loratadine (fig. 1) by UV-detection using autosampler. Method has been validated following the ICH guidelines (Guideline, 2005). Moreover, developed chromatographic method is successfully applicable for determination of citalopram with levocetirizine and loratadine in active pharmaceutical ingredients, commercial formulation and human serum without interference of unwanted components.

MATERIALS AND METHODS

Reagents and chemicals

Standards citalopram, levocetirizine and loratadine were received from Eros Pharmaceutical Pvt. Ltd. As kind gift and pharmaceutical formulations Pramcit® (20mg), T-

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Day™ (5mg) and Jardin (10mg) were bought from local pharmacy of Karachi. Analytical grade acetonitrile, methanol and *o*-phosphoric acid were purchased from Merck (Darmstadt, Germany). Freshly prepared de-ionized water distilled twice was used for assay.

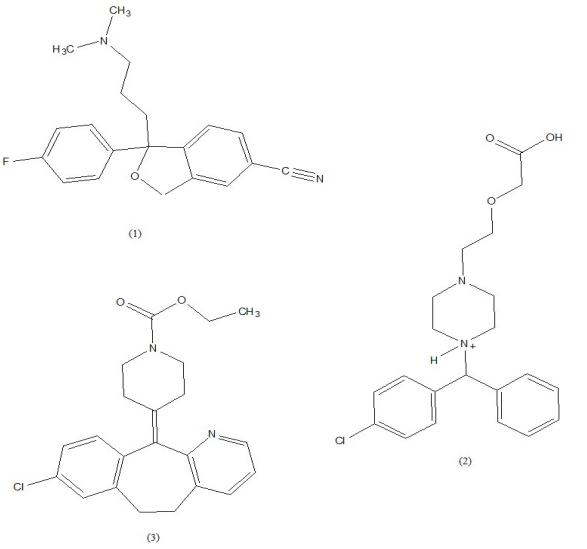

Fig. 1: Chemical structures of citalopram[1], levocetrizine[2] and loratidine[3]

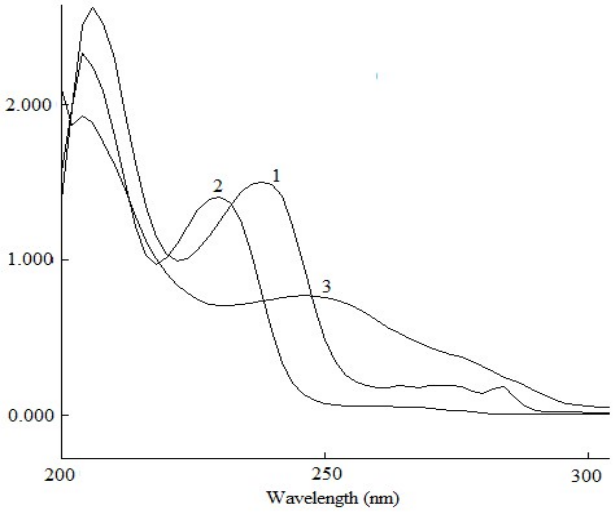

Fig. 2: UV-spectra of citalopram[1], levocetrizine[2] and loratidine[3]

Instrumentation

HPLC system (Shimadzu Corporation, Japan) equipped with two LC-20AT solvent delivery module, DGU-20A3/20A5 on-line degasser, SIL-20AHT/20ACHT UFLC auto sampler connected with SPD-20A/20AV UV/VIS detector and Shimadzu CBM-20A communication bus module. LC solution GPC Chromatographic software (version 1.25) was used for data acquisition.

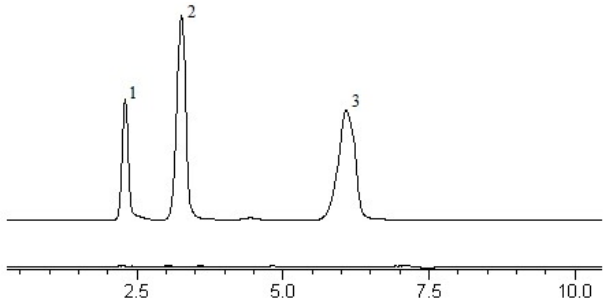

Fig. 3: Chromatogram representing citalopram[1], levocetrizine[2] and loratidine[3] in bulk drug

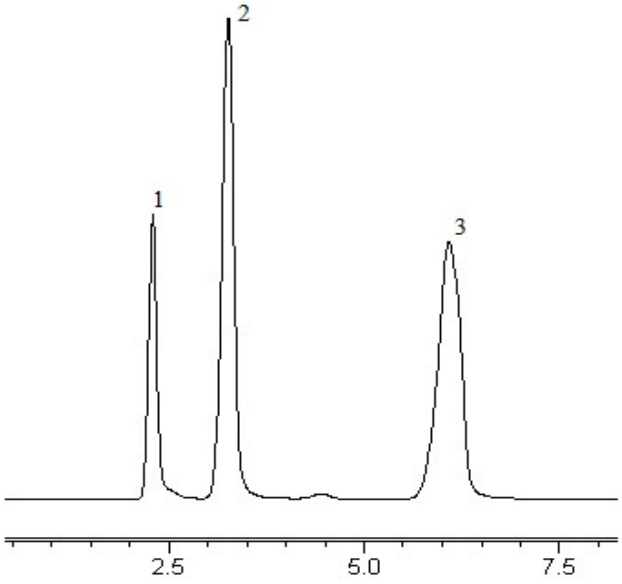

Fig. 4: Chromatogram representing citalopram[1], levoce trizine[2] and loratidine[3] in pharmaceutical formulation

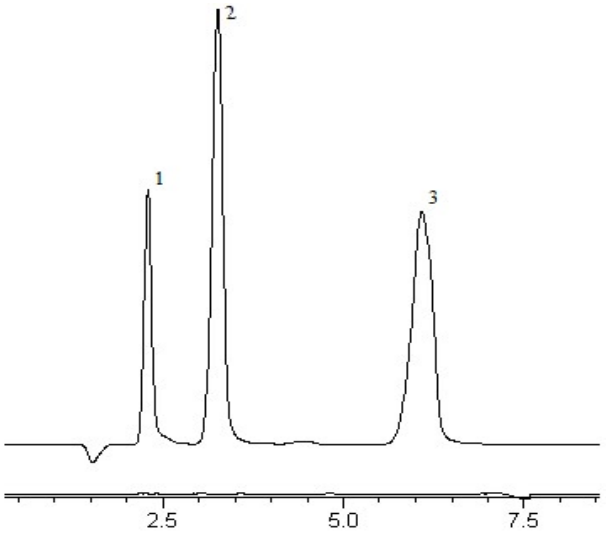

Fig. 5: Chromatogram representing citalopram[1], levocetrizine[2] and loratidine[3] in human serum

Table 1: System suitability

Drugs	t_R[a]	N[b]	T[c]	Res[d]
Citalopram	2.3	2054	1.118	….
Levocetirizine	3.1	2258	0.996	4.031
Loratadine	6.1	2463	0.932	3.442

[a]Retention time, [b]Theoritical plates, [c]Tailing factor, [d]Resolution

Table 2: Regression characteristics

Parameters	Citalopram	Levocetirizine	Loratadine
Slope	12653	15303	15461
Intercept	10398	10952	7422.4
R^{2a}	0.9988	0.9993	0.9999
SE[b]	0.2824	0.0999	0.2323
SEE[c]	0.4530	0.1653	0.1342
LOD (ng mL^{-1})	7.75	3.35	10.26
LOQ (µg mL^{-1})	0.023	0.010	0.031

[a]Correlation coefficient, [b]Standard error, [c]Standard error estimate

Table 3: Recovery in pharmaceutical formulation and human serum

Pharmaceutical Formulation								
Pramcit®(20 mg)			T-Day™ (5 mg)			Jardin (10 mg)		
Conc µg mL^{-1}	%Recovery	%Error	Conc µg mL^{-1}	%Recovery	%Error	Conc µg mL^{-1}	%Recovery	%Error
2	100.32	0.32	3.2	99.95	-0.05	3.2	100.58	0.57
1	98.90	-1.11	1.6	101.40	1.38	1.6	99.95	-0.05
0.5	99.40	-0.61	0.8	98.11	-1.92	0.8	100.91	0.90
Human serum								
Citalopram			Levocetirizine			Loratadine		
Conc µg mL^{-1}	%Recovery	%Error	Conc µg mL^{-1}	%Recovery	%Error	Conc µg mL^{-1}	%Recovery	%Error
6.0	99.74	-0.260	12.5	99.85	-0.148	12.5	98.90	-1.113
3.0	100.40	0.399	6.0	99.91	-0.092	6.0	100.01	0.013
1.5	99.94	-0.063	3.0	100.01	0.009	3.0	100.11	0.109

Table 4: Precision of the proposed method

Citalopram		Levocetirizine				Loratadine		
Conc µg mL^{-1}	%RSD[a]	%RSD[b]	Conc µgmL^{-1}	%RSD[a]	%RSD[b]	Conc µg mL^{-1}	%RSD[a]	%RSD[b]
12.5	0.86	0.45	25	0.38	0.41	25	0.61	0.36
6.0	1.52	1.76	12.5	1.59	1.83	12.5	0.36	2.11
3.0	1.22	1.61	6.0	1.43	1.20	6.0	0.70	0.91
1.5	0.54	0.22	3.0	1.64	0.26	3.0	0.03	0.56
0.8	1.34	0.88	1.5	0.50	0.32	1.5	1.91	0.51
0.4	0.91	1.04	0.8	1.03	0.06	0.8	0.46	0.22
Human serum								
Citalopram		Levocetirizine				Loratadine		
Conc µg mL^{-1}	%RSD	Conc µg mL^{-1}	%RSD			Conc µg mL^{-1}	%RSD	
6.0	0.45	12.5	0.18			12.5	1.77	
3.0	0.66	6.0	0.07			6.0	0.21	
1.5	0.15	3.0	0.09			3.0	0.64	

[a]Inter-day precision and [b]Intra-day precision

Chromatographic parameters

Separation was achieved using Shimadzu Shim-pack CLC-ODS (M) 25M column (4.6mm i.d. x 0.25mm), employing mobile phase 80:20 v/v methanol: water (pH 3.5 adjusted by *o*-phosphoric acid) at flow rate 1.0 $mL.min^{-1}$, filtered using 0.45µm pore size filter. Detection wavelength was attuned at 230nm, injection volume was 1.0µL. All the analyses were carried out at gradient condition.

Standard solution preparation

Standard solutions of 1000$\mu g.mL^{-1}$ of each drug were prepared by dissolving weighed amounts of citalopram, levocetirizine and loratadine equivalent to 100mg in 100 mL volumetric flask then volume were made up by diluent (80:20 v/v methanol-water), finally sonicated for 15min in an ultra-sonic bath.

Preparation of calibration standards

Appropriate volume of standard solutions were diluted using 80:20v/v methanol-water diluent to obtain seven concentration levels in the range 0.4-12.5, 0.8-25 and 0.8-25$\mu g.mL^{-1}$ for citalopram, levocetirizine and loratadine respectively.

Assay for pharmaceutical formulation

1000$\mu g.mL^{-1}$ formulation solutions for each sample were prepared by grinding ten tablets finely. The amount equivalent to 100mg of each drug was dissolved in 100mL volumetric flask separately with 80:20 v/v methanol-water diluent, followed by sonication for 15min in an ultra-sonic bath and filtration through 0.45µm pore size filter.

Drug serum solution

Blood specimen, accumulated from healthy donor at Fatmid Foundation Karachi was transferred in a sterile EDTA glass tube followed by centrifugation at 1600xg for 10min at 4ºC to separate plasma. Into 1mL plasma, 9.0mL acetonitrile was added, vortexed for 1min and centrifuged at 10,000rpm for 10min. The obtained clear serum solution was spiked with respective analytes to get required concentrations of citalopram, levocetirizine and loratadine in human serum for assay at isosbestic point.

Method validation

Method validation was performed in accordance with ICH Q2 (R1) guidelines including linearity, accuracy, precision, system suitability, robustness, quantitation and detection limits. The System suitability tests were evaluated and column efficiency was investigated in terms of the parameters retention time (t_R), tailing factor (T), theoretical plates (N) and resolution (Res). Calibration curves were plotted between the peak responses and six different concentration levels of each drug for evaluating linearity and regression characteristics using slope, intercept, correlation coefficient, standard error and standard error estimate. Percentage recovery of standard drugs in commercial formulations was calculated to determine accuracy. Inter-day and intra-day precision were reported in terms of %RSD by preparing solution at six concentrations of each reference drug. LOD and LOQ were recorded where signal to noise ratio were three times and ten times of baseline of peak response. Minor changes were deliberately made in parameters like mobile phase composition, flow rate, pH and wavelength to check the feasibility of proposed method.

RESULTS

Allergic diseases are very common in the developing world. Moreover, a serious medical condition called depression, associated with high risk of health status exists commonly and causes severe effects in daily life functioning. It is a serious illness of modern era and is increasing day by day. It is a state of mental disorder, characterized by loss of interest and causes low mood. A newly developed class of antidepressants, SSRIs is considered more effective and well tolerated as compared to tricyclic antidepressants. Antihistamines are the class of drugs used for the allergic treatments. Furthermore, loratadine and levocetirizine are long acting peripheral selective H_1-receptor blocker having ant histaminic properties. Citalopram is one of the SSRIs, widely prescribed psychotic medication in Major Depressive Disorder (MDD) all over the world (Trkulja, 2010). Symptoms of allergy and depressions are need to be simultaneously treated (Marshall et al., 1993) and patient intake Selective Serotonin Reuptake Inhibitors along with antihistamines.

Present study describes a rapid and reliable HPLC method for determination of citalopram, levocetirizine and loratadine simultaneously in bulk drug and pharmaceutical formulation employing methanol/water (80:20% v/v) mobile phase at 1.0 $mL.min^{-1}$ flow rate with pH adjusted 3.5 by *o*-phosphoric acid at the detection wavelength of 230nm. Isosbestic point, determined on Shimadzu 1800 UV/Vis spectrophotometer was 230 nm for better separation and resolution. Different mobile phase proportion i.e. (60:40, 70:30, 80:20, 90:10) with various flow rates ranging from 0.7-1.2 $mL.min^{-1}$ were preliminary tried but best parameters were chosen as described previously. Method was fruitfully applied for studied analytes in commercial formulations. Figure 2 and 3 represent the UV spectra and respective chromatograms of citalopram, levocetirizine and loratadine.

Method validation

Developed method was validated following ICH guidelines (Guideline, 2005) for linearity, accuracy, precision, LOD and LOQ. In addition, robustness and system suitability tests were performed.

System suitability test

HPLC system was equilibrated for system suitability studies which were evaluated with initial concentration of mobile phase after introducing ten replicates of standard and observing the detector response. Parameters for system suitability including retention time (t_R), tailing factor (T), theoretical plates (N) and resolution (Res) were evaluated on each day of method validation in order to express column efficiency (table 1).

Linearity

Linearity was obtained at seven different concentration levels for each standard drug within the range of 0.4-12.5 $\mu g.mL^{-1}$ for citalopram and 0.8-25 $\mu g.mL^{-1}$ for levocetirizine and loratadine respectively. Calibration curve showed correlation coefficient > 0.998 for all the studied analyte. Slope, intercept, standard error and standard error estimate are given in table 2.

Accuracy

For accuracy, recovery tests of standard drug and pharmaceutical formulation were carried out by analyzing six concentration levels of each bulk drug ranging from 0.4-12.5 $\mu g.mL^{-1}$ for citalopram and 0.8-25 $\mu g.mL^{-1}$ for levocetirizine and loratadine respectively. The percent recovery values obtained were within the range 98.90-100.32, 98.11-101.4 and 99.95-100.9 for citalopram, levocetirizine and loratadine respectively (table 3).

Precision

Analysis of seven concentration levels in linearity range of 0.4-12.5 $\mu g.mL^{-1}$ for citalopram and 0.8-25 $\mu g.mL^{-1}$ for levocetirizine and loratadine were analyzed within the day and till two days of method validation to evaluate inter and intraday precision of developed method. The RSD values found to be 0.22-1.76, 0.06-1.83, 0.03-2.11% for citalopram, levocetirizine and loratadine respectively which are within the acceptable range (table 4).

Detection and quantitation limits

LOD and LOQ values were obtained by finding standard deviation and slope from calibration curve. LOD is 3.3 times and LOQ is 10 times signal to noise ratio of the baseline. LOD of the proposed method obtained was 7.75, 3.35 and 10.26 $ng.mL^{-1}$ and LOQ was 0.023, 0.010 and 0.031 $\mu g.mL^{-1}$ for citalopram, levocetirizine and loratadine respectively.

Robustness

Robustness study was examined by making small changes in operating and chromatographic parameters like pH ranging (3.4-3.6), wavelength (228-232 nm), flow rate (0.9-1.1 mL) and mobile phase composition (78:22-82:18) MeOH:H_2O and observing the effect on instrumental response. The method withstood with the minor changes which ensured the reliability of proposed method. Robustness data is represented in table 5.

DISCUSSION

Proposed method was successfully applied for the simultaneous determination of citalopram with levocetirizine and loratadine. The developed method accomplished good separation with no interference of excipients in dosage forms (Figure 4). Results obtained from accuracy and precision calculation proved good percent recovery in the range 98.90-100.32%, 98.11-101.4% and 99.95-100.91% for citalopram, levocetirizine and loratadine respectively (Table 3 and 4). Detection, quantitation limits and correlation coefficient showed high sensitivity for developed method. It is established that the proposed study is appropriate and applicable to determine the citalopram with levocetirizine and loratadine in pharmaceutical formulations.

Applicability of method was ascertained by evaluating the recovery of citalopram with simultaneous determination of levocetirizine and loratadine in human serum. Serum was spiked with analytes in the concentration ranges 1.5-6.0 $\mu g.mL^{-1}$ for citalopram and 3.0-12.5 $\mu g.mL^{-1}$ for levocetirizine and loratadine respectively. Endogenous serum components did not show interference at the same retention time where the studied analytes were eluted (Figure 5). Recovery was in the range 99.94-100.40%, 99.85-100.01% and 98.90-100.11% for citalopram, levocetirizine and loratadine respectively (Table 3 and 4). It was confirmed that the method is suitable for analyzing citalopram with levocetirizine and loratadine in human serum with good percent recovery.

CONCLUSION

A highly sensitive, rapid and robust HPLC method has been reported in this paper for simultaneous determination of citalopram with levocetirizine and loratadine in commercial formulations and human serum. This method shows good separation with high resolution, obeying Beer's law for all the analytes. Calibration curves showed linearity with correlation coefficient greater than 0.998. The developed method shows high accuracy and precision with all analytical results in acceptable range. The developed and validated HPLC method is efficient and suitable for application of routine analysis in quality control laboratories.

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