Liquid Chromatographic Method for Simultaneous Determination of Citalopram with NSAIDs in Bulk Drug, Pharmaceutical Formulation and Human Serum

Saeeda Nadir Ali, Nida Naz, Amtul Qayoom* and Shumaila Akram
Department of Chemistry, NED University of Engineering and Technology, Karachi.
amtulq@neduet.edu.pk*

(Received on 19th January 2017, accepted in revised form 30th May 2017)

Summary: A high performance liquid chromatographic method was developed and validated to simultaneously quantify citalopram with piroxicam, celecoxib and diclofenac sodium. Chromatographic analysis was performed at ambient temperature using Shimadzu Shim-pack CLC-ODS (M) 25M column linked to a UV-visible detector adjusted at 230 nm, employing 80:20 (v/v) methanol: water (pH 3.5) as mobile phase with flow rate 1.0 mL min^{-1}. Validation was performed in the ranges 0.6-20, 0.9-28, 0.6-20 and 1.0-32 µg mL^{-1} with lowest level corresponding to detection limit 16.45, 23.33, 27.66 and 14.44 ng mL^{-1} respectively. With-in the day precision ranged from 0.14-1.67% and between-day precision from 0.40-1.50%, accuracies were 99.61-100.86%. The analytes were successfully detected without any observable interference in pharmaceutical formulation and human serum samples demonstrating effectiveness of method.

Keywords: Citalopram, Piroxicam, Celecoxib, Diclofenac sodium, HPLC-UV.

Introduction

Multiple medical problems are often managed together e.g. antibiotics are prescribed to heal infection; however Non-steroidal anti-inflammatory drugs (NSAIDs) are also given to get pain relief [1]. Similarly patients suffering from tuberculosis and diabetes are cured at the same time [2]. Since a patient simultaneously takes medicines for multiple medical reasons, this is interesting to develop a method for simultaneous determination of co-administered drugs in biological samples. There are several reported analytical methods for simultaneous determination of different drugs [3]–[6]. In past, we have also reported simultaneous determination of alprazolam with antihistamines [7] and ACE inhibitors [8].

Number of people including children and adults are mainly influenced by psychotic disorders which may lead to intensify suicidal risk, psychosocial impairment as well as depression, eating disorders, delinquency, chronicity and drug and alcohol abuse. Although, many clinical treatments for depression have been evidenced but Selective Serotonin Reuptake Inhibitor (SSRIs) by reason of fewer adverse side-effect profile is considered to be better tolerated and safer than classical tricyclic anti-depressants (TCA) [9]. SSRIs are effective against depression as well as anxiety [10]. Citalopram, a highly selective SSRI, offers a broad spectrum of therapeutic action against depression, anxiety, obsessional and impulse control syndromes [11]. Moreover, it acts by averting the endorsement of a neurotransmitter called serotonin via nerve cells [12]. Previously reported methods for determination of citalopram are potentiometric determination [13], adsorptive square wave voltammetry (ASWV) [14], chemiluminescence [15], spectrophotometry [16], spectrofluorometry [17], micellar electro kinetic chromatographic method [18], HPTLC [19], LC-MS/MS [20] and HPLC [21]. NSAIDs are clinically prescribed worldwide to provide relief against pain, fever, inflammation [22]. It shows efficacy by inhibiting the activity of cyclooxygenase-1(COX-1) and cyclooxygenase-2(COX-2) enzymes in the body and thus prevents synthesis of prostaglandins [23]. Moreover, risk of arising Alzheimer's disease can be diminished by consuming NSAIDs for extended period [24]. Piroxicam, celecoxib and diclofenac sodium are clinically prescribed NSAIDs for osteoarthritis and rheumatoid arthritis as well as to treat severe pain, ankylosing spondylitis and acute pain in musculoskeletal condition and serious gout [25-27]. Literature survey reveals several methods for determination of NSAIDs including GC-MS [28], RP-HPLC [29], GC [30], HPLC-DAD [31], capillary electrophoresis [32], HILIC-MS/MS [33] and spectrofluorometry [34].

Risk of upper gastrointestinal bleeding has been associated with SSRIs [35] and it can be increased when SSRIs are taken with NSAIDs [36]. Main objective of this study is the advancement of validated HPLC method for simultaneous determination of citalopram with reported NSAIDs (Fig. 1). ICH guidelines have been followed for validation [37]. Furthermore, reported method is applicable successfully for quantification of

*To whom all correspondence should be addressed.

citalopram and NSAIDs in active pharmaceutical ingredient, dosage formulation and human serum without showing any interference of excipients or unwanted serum components.

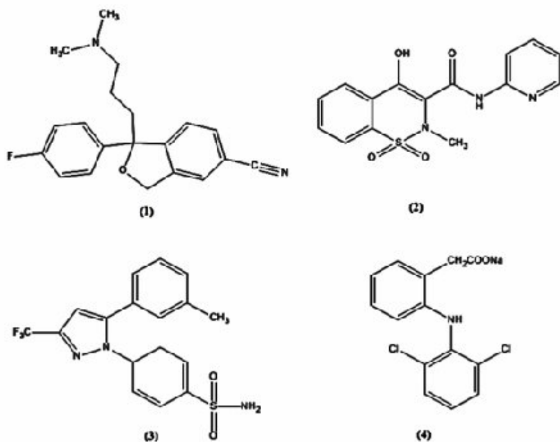

Fig. 1: Structures of citalopram[1], piroxicam[2], celecoxib [3] and diclofenac sodium[4]

Experimental

Reagents and Chemicals

Citalopram (> 98% purity) was acquired from Eros Pharmaceutical Pvt. Ltd. Pakistan. Standards of piroxicam, celecoxib and diclofenac sodium were obtained from Pfizer Pakistan Ltd, Getz Pharma Pakistan (Pvt.) Ltd. and Sami pharmaceuticals (Pvt.) Ltd respectively. Pakistan with purity over 99.1%. Pharmaceuticals formulation Pramcit (20 mg), Feleden flesh (20 mg), Celbex (100 mg) and Dicloran (50 mg) were purchased from local pharmacy of Karachi in the form of tablets or capsules. High performance liquid chromatographic grade methanol, acetonitrile and *o*-phosphoric acid purchased from Merck (Darmstadt, Germany). Ultra-pure water was used during the course of entire analysis.

Instrumentation

Liquid chromatographic analysis was performed on Shimadzu Corporation (Japan) chromatograph equipped with online degasser (DGU-$20A_3/20A_5$), two solvent delivery modules (LC-20AT), a SIL-$20A_{HT}/20AC_{HT}$ UFLC auto sampler coupled with UV/VIS detector (SPD-20A/20AV). All the data was acquired with Shimadzu communication bus module (CBM-20A) and processed with LC solution GPC software version 1.25.

Chromatographic Conditions

Chromatographic separation was achieved on Shimadzu Shim-pack CLC-ODS (M) 25M (4.6 mm *i.d* x 25 cm) column. Gradient elution was performed with (A) 80% methanol and (B) 20% water (pH 3.5 modified by *o*-phosphoric acid) at flow rate of 1.0 mL min^{-1} after filtration through 0.45 µm pore size. All the studied analytes were simultaneously determined at isosbestic point at 230 nm.

Preparation of stock and calibration standards solutions

1000 µg mL^{-1} stock standard solutions of citalopram, piroxicam, celecoxib and diclofenac sodium were prepared by dissolving 100 mg of individual drug in 100 mL diluent i.e. 80:20 v/v methanol-water. These solutions were kept at -20°C until analysis. Each stock standard solution was then diluted with diluent in 25 mL volumetric flask to prepare working standard solutions. Working standards for liquid chromatographic assay were prepared by serial dilution of stock standard solutions with 80:20 v/v methanol-water diluent to level up six concentrations in the range 0.6-20, 0.9-28, 0.6-20 and 1.0-32 µg mL^{-1} for citalopram, piroxicam, celecoxib and diclofenac sodium respectively. The solutions were introduced to auto sampler after micro filtration through 0.45 µm millipore filter paper. All the solutions were prepared once and analyzed daily for with-in day and in-between day precision.

Assay for Commercial Formulation

For the assay of drugs in dosage form, ten tablets of each drug were accurately weighed and crushed into fine powder. The amount of powder equivalent to 100 mg of citalopram and 100 mg of NSAIDs (piroxicam, celecoxib and diclofenac sodium) were transferred to 100 mL volumetric flask separately and dissolved fully in diluent by sonication in an ultra-sonic bath for 15 min and volumes were made up to mark to bring final concentration 1000 µg mL^{-1}. The resulting solutions were filtered through 0.45 µm pore size filter to remove undissolved excipients and finally diluted to the desired concentration for analysis of drug content.

Assay for Drug Serum Solution

Sample of blood, collected from a healthy donor at Fatmid Foundation Karachi was transferred to a sterile EDTA glass tubes followed by centrifugation at 1600 x g for 10 min at 4ºC to separate plasma. Into 1 mL portion of plasma, 9.0 mL

acetonitrile was mixed, vortexed for 1 min and centrifuged at 10,000 rpm for 10 min. Clear serum solution obtained was fortified with standard solutions of citalopram, piroxicam, celecoxib and diclofenac sodium in required concentrations for their assay.

Method validation

Validation procedure was followed as stated by guidelines of ICH Q2 (R1) [37] comprising system suitability, linearity, precision, robustness, accuracy, limit of quantitation and detection limit. Column efficiency was investigated by performing system suitability test including parameters like retention time (t_R), tailing factor (T), theoretical plates (N) and resolution (Res). Calibration curves were obtained to evaluate linearity and regression characteristics, which involves correlation coefficient, slope, standard error, intercept and standard error estimate. Accuracy of method was estimated from recovery of pure drug in dosage formulation. Precision in terms of inter-day and intra-day was evaluated as %RSD at six different concentrations of each standard analyte. Values of LOD and LOQ were reported as 3 and 10 times signal/noise ratio to baseline of peak response. Flow rate, pH, wavelength and mobile phase composition were altered deliberately to verify method feasibility.

Result and Discussion

Method Development and Optimization

Method development has great importance for advancement of different industrial products; it has also been widely used in pharmaceutical industry to monitor the drug at every step of quality control and quality assurance. This work describes a new method for simultaneous determination of citalopram with NSAIDs (piroxicam, celecoxib and diclofenac sodium), its validation and application in pharmaceutical formulation and human serum.

For optimization, a variety of operating conditions were rationally evaluated. Different mobile phase compositions were tested at first, including (1) acetonitrile and water and (2) methanol and water. Comparison data confirmed that the best separation with finest peak shape was achieved with methanol and water. Both mobile phases were tried in various ratios in the gradient elution increasing the organic solvent content from 50% to 90%. It has been observed that in reversed-phase chromatography, organic solvent content lessens the static retention and reduce the retention time of analyte. Acetonitrile-water system eluted all targets in short analysis time, however resolution was not appropriate. Thus the condition of 80% methanol and 20% water was maintained throughout the analysis at flow rate 1.0 mL min^{-1}. In addition, to stabilize the retention and selectivity, it is imperative to regulate pH in reversed phase liquid chromatographic system. For this purpose, pH of water (used for gradient elution) was adjusted with *o*-phosphoric acid and scanned between pH 2 to 4. Well separated, narrow peaks with symmetry suggested pH 3.5 ± 0.2 is appropriate for the proposed method. Furthermore, to best select the detector wavelength, each drug was scanned in the region 200-800 nm on Shimadzu-1800 UV-visible detector and compared. The isosbestic point 230 nm was found to be satisfactory (Fig. 2), permitting the detection of all the studied analytes with adequate sensitivity confirming the applicability of the developed method on commercial formulation (Fig. 3) and human serum (Fig. 4).

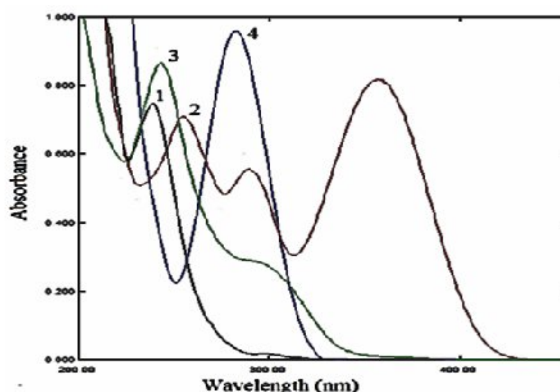

Fig 2: UV-spectra of citalopram[1], piroxicam[2], celecoxib[3] and diclofenac sodium[4].

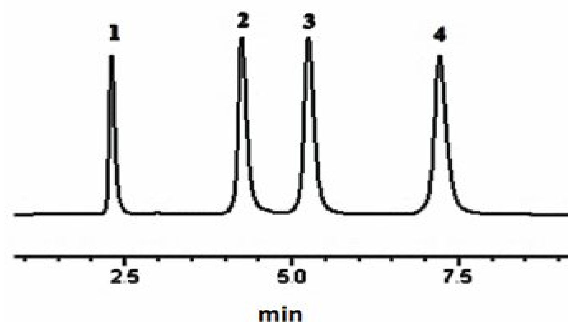

Fig 3: Chromatogram representing citalopram[1], piroxicam[2], celecoxib[3] and diclofenac sodium[4] in bulk drug. Three standard solutions in 80% (v/v) methanol in water contained 20, 30, 20 and 38 µg mL^{-1} of the analytes, respectively, maintaining pH 3.5 at λ_{max} 230 nm.

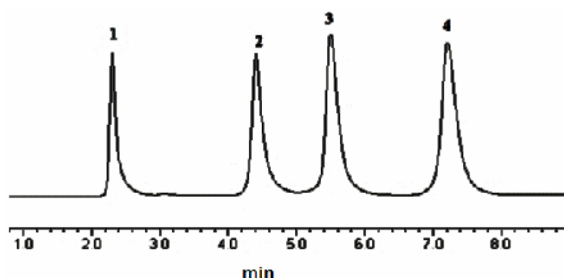

Fig 4a: Chromatogram representing citalopram[1], piroxicam[2], celecoxib[3] and diclofenac sodium[4] in pharmaceutical formulation. Three standard solutions in 80% (v/v) methanol in water contained 20, 30, 20 and 38 μg mL^{-1}of the analytes, respectively, maintaining pH 3.5 at λ_{max}230 nm.

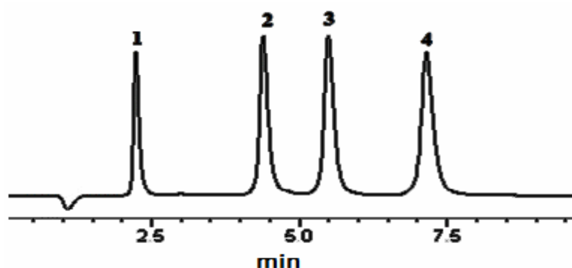

Fig 4b: Chromatogram representing citalopram[1], piroxicam[2], celecoxib[3] and diclofenac sodium[4] in human serum.

Method validation

The developed method was validated for system suitability, specificity, linearity, repeatability, reproducibility, recovery, limits of detection (LOD) and quantification (LOQ) and robustness following guidelines given by International Conference on Hormonization (ICH) [37].

System suitability test

System suitability tests were performed on each day of method validation by ten replicate analysis of standard in order to ratify the column efficiency and resolution of the chromatographic system (Fig. 5a, 5b and 5c). The number of theoretical plates ≥ 2000 and tailing factor ≤ 2 confirms the suitability of method (Table-1).

Table-1: System suitability of method.

Drugs	t_R[a]	N[b]	T[c]	Res[d]
Citalopram	2.3	2510	1.533	0.00
Piroxicam	4.4	4244	1.326	9.619
Celecoxib	5.4	5192	1.203	3.834
Diclofenac sodium	7.2	5809	1.178	4.884

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

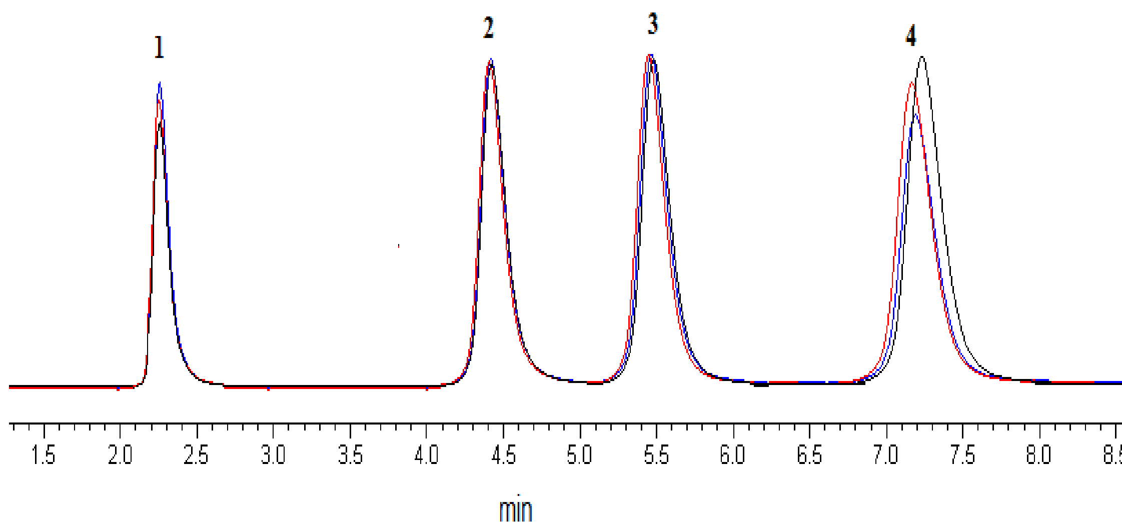

Fig 5a: Representative chromatograms at wavelength 228 nm (black), 230 nm (red) and 232 nm (blue) for citalopram[1], piroxicam[2], celecoxib[3] and diclofenac sodium[4] Three standard solutions in 80% (v/v) methanol in water contained 20, 30, 20 and 38 μg mL^{-1}of the analytes, respectively, maintaining at pH 3.5.

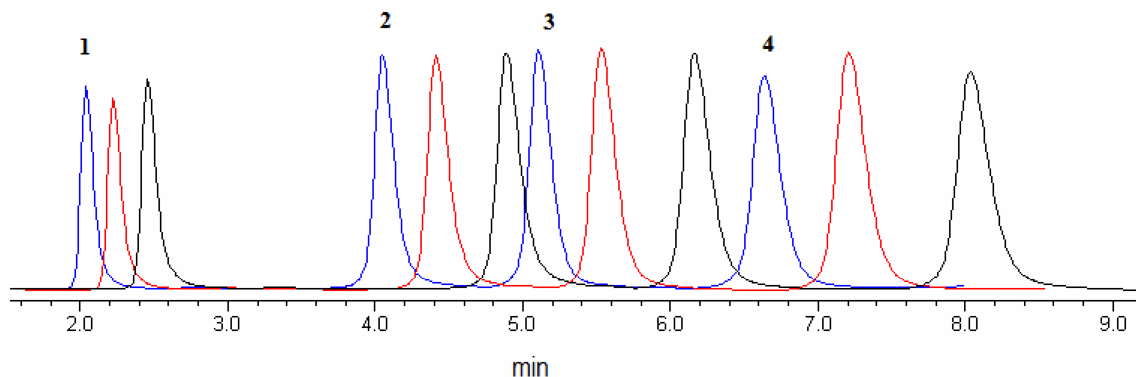

Fig 5b: Representative chromatograms at flow rate 0.9 mL (black), 1.0 mL (red) and 1.1 mL (blue) for citalopram[1], piroxicam[2], celecoxib[3] and diclofenac sodium[4]. Three standard solutions in 80% (v/v) methanol in water contained 20, 30, 20 and 38 µg mL^{-1} of the analytes, respectively, maintaining at pH 3.5 with λ_{max} 230 nm.

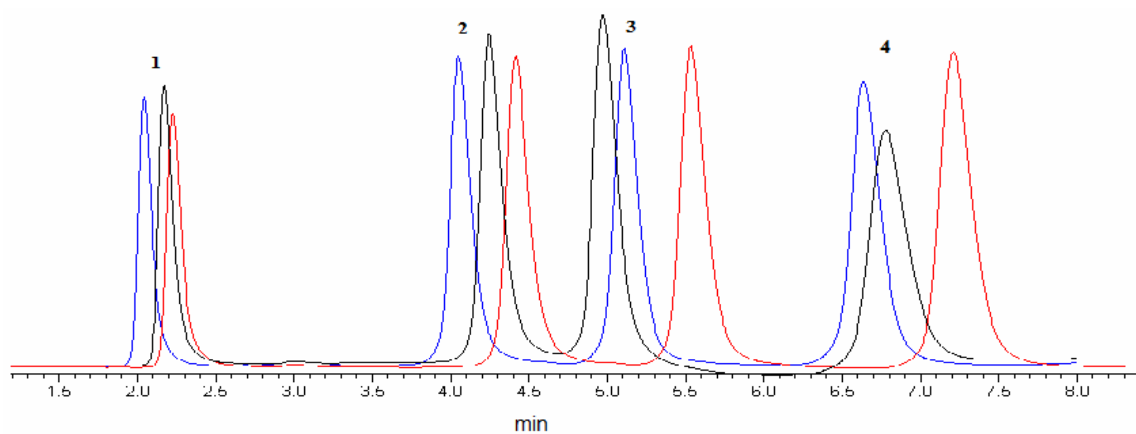

Fig 5c: Representative chromatograms of mobile phase composition at 82:18% (v/v) (blue), 80:20% (v/v) (black) and 78:22 (red) % (v/v) methanol in water for citalopram[1], piroxicam[2], celecoxib[3] and diclofenac sodium[4]. Three standard solutions contained 20, 30, 20 and 38 µg mL^{-1} of the analytes, respectively, maintaining at pH 3.5 with λ_{max} 230 nm.

Table-2: Regression characteristics.

Parameters	Citalopram	Piroxicam	Celecoxib	Diclofenac sodium
Slope	1843.8	2200.1	3795.8	2257.3
Linearity (µg mL^{-1})	0.6-20	0.9-28	0.6-20	1.0-32
Intercept	1665.2	5579	2428.9	2927
Correlation coefficient	0.9999	0.9988	0.9983	0.9984
Standard error	0.1312	0.4769	0.3558	0.6585
Standard error estimate	0.2036	0.8954	0.6934	1.2808
LOD (ng mL^{-1})	16.45	23.33	27.66	14.44
LOQ (µg mL^{-1})	0.04986	0.07071	0.08383	0.04386

Linearity

A linear regression model using least squares regression was applied to evaluate linearity. Excellent linearity was observed over the examined range within an overall correlation coefficient 0.998. The typical regression equations for calibration curves (n=6) of citalopram and NSAIDs along with standard error and standard error estimate are given in Table-2.

Precision

Data for precision is summarized in Table-3. For repeatability, six replicates in the linearity ranging 0.6-20, 0.9-28, 0.6-20 and 1.0-32 µg mL^{-1} for citalopram, piroxicam, celecoxib and diclofenac sodium respectively, were injected to chromatograph under the same operational conditions. The RSD values were found to be in the range 0.31-1.6, 0.23-0.92, 0.32-1.13, and 0.14-1.67% for citalopram, piroxicam, celecoxib and diclofenac sodium respectively for interday precision. Intermediate precision study was extended to two

consecutive days of method validation at mentioned concentration levels. Intra-day precision was determined to be 0.43-1.2, 0.67-1.03, 0.33-1.50, and 0.61-1.31% RSD for citalopram, piroxicam, celecoxib and diclofenac sodium respectively. All the obtained values fit to the acceptance criteria assuring the repeatability and reproducibility of method.

Accuracy

Recovery experiments were performed to estimate accuracy of the developed method. It was calculated by reviewing the recovery values at three concentration levels of each bulk drug in the range of 1.25-5.0, 1.6-7.0, 1.25-5.0 and 2.0-4.0 µg mL^{-1} for citalopram, piroxicam, celecoxib and diclofenac sodium respectively in pharmaceutical formulation and human serum. Recovery values were found to be 99.64-100.86%, 99.61-100.03%, 99.86-100.54% and 99.93 - 100.36% for citalopram, piroxicam, celecoxib and diclofenac sodium respectively. Table 4 represents good %recovery values of each drug in pharmaceutical formulation and in human serum (Fig. 4a and 4b).

Specificity

The specificity of method was assessed by observing the absence of interfering peaks at the retention times of analytes. Chromatograms were recorded for pure solvent, reference standard, pharmaceutical formulation, blank serum solution and spiked serum sample, and it was observed that there was no endogenous interference at the retention time of studied analyte and chromatograms for all the injected solutions exhibited good specificity (Fig. 6).

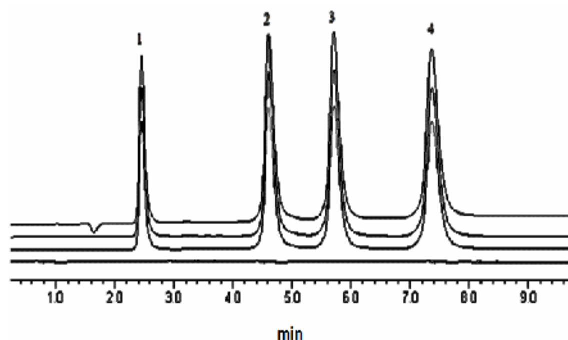

Fig 6: Chromatograms of (a) placebo (b) spiked excipients solution, (c) pharmaceutical formulation and (d) spiked serum solution in citalopram[1], piroxicam[2], celecoxib[3] and diclofenac sodium[4]. Three standard solutions in 80% (v/v) methanol in water contained 10, 14, 10 and 16 µg mL^{-1}of the analytes, respectively, maintaining at pH 3.5 with λ_{max} 230 nm.

Table-3: Precision of method.

Citalopram			Piroxicam			Celecoxib			Diclofenac sodium		
Conc µg mL^{-1}	%RSD^a	%RSD^b	Conc µg mL^{-1}	%RSD^a	%RSD^b	Conc µg mL^{-1}	%RSD^a	%RSD^b	Conc µg mL^{-1}	%RSD^a	%RSD^b
20	0.31	1.09	28	0.23	0.90	20	0.32	0.46	32	0.34	0.67
10	1.60	1.20	14	0.92	0.72	10	0.84	0.33	16	0.14	0.61
5	0.16	0.43	7	0.45	0.67	5	0.86	1.33	8.0	1.47	0.51
2.5	1.49	0.50	3.5	0.61	1.03	2.5	0.83	0.40	4.0	1.67	1.31
1.25	0.57	1.40	1.6	0.36	0.71	1.25	0.96	0.52	2.0	0.68	0.78
0.6	0.88	1.11	0.9	0.80	1.02	0.6	1.13	1.50	1.0	0.85	0.94
Human Serum											
Citalopram		Piroxicam		Celecoxib		Diclofenac sodium					
Conc. µg mL^{-1}	%RSD	Conc. µg mL^{-1}	%RSD	Conc. µg mL^{-1}	%RSD	Conc. µg mL^{-1}	%RSD				
10	1.43	14	1.73	10	0.78	16	0.67				
5	0.09	7	1.50	5	0.36	8.0	1.61				
2.5	1.69	3.5	0.97	2.5	1.56	4.0	0.26				

^{a} inter-day precision and ^{b} intra-day precision for n=6

Table-4: Recovery of method.

Pramcit®(20 mg)			Feldene(10 mg)			Celbex(100 mg)			Dicloran(50 mg)		
Conc. µg mL^{-1}	%Rec	%Error	Conc. µg mL^{-1}	%Rec	%Error	Conc. µg mL^{-1}	%Rec	%Error	Conc. µg mL^{-1}	%Rec	%Error
10	99.64	-0.36	14	100.03	0.03	10	100.05	0.05	16	99.93	-0.07
5	100.86	0.85	7	99.68	-0.32	5	99.86	-0.14	8.0	100.28	0.28
2.5	99.73	-0.27	3.5	99.61	-0.39	2.5	100.54	0.54	4.0	100.36	0.35
Human Serum											
Citalopram			Piroxicam			Celecoxib			Diclofenac sod		
Conc. µg mL^{-1}	%Rec	%Error	Conc. µg mL^{-1}	%Rec	%Error	Conc. µg mL^{-1}	%Rec	%Error	Conc. µg mL^{-1}	%Rec	%Error
10	98.54	-1.486	14	99.43	-0.570	10	100.87	0.860	16	98.54	-1.486
5	98.63	-1.390	7	98.45	-1.575	5	99.89	-0.114	8.0	98.08	-1.961
2.5	101.07	1.060	3.5	98.25	-1.782	2.5	99.47	-0.531	4.0	100.79	0.786

no. of analysis n=6

Table-5: Robustness of the proposed method.

Parameters		Citalopram		Piroxicam		Celecoxib		Diclofenac sod	
		T	N	T	N	T	N	T	N
Flow rate (mL min^{-1})	0.9	1.764	2425	1.500	4042	1.352	4895	1.356	5597
	1.0	1.714	2285	1.429	3974	1.294	4858	1.243	5495
	1.1	1.692	2126	1.401	3828	1.274	4788	1.221	5367
Wave length (nm)	228	1.756	2226	1.509	3800	1.366	4640	1.321	5255
	230	1.766	2234	1.500	3831	1.366	4692	1.319	5327
	232	1.757	2233	1.509	3853	1.363	4713	1.313	5362
pH	3.4	1.942	1979	1.520	3485	1.309	4498	1.202	5281
	3.5	1.647	2429	1.443	4092	1.312	5079	1.256	5704
	3.6	1.832	2129	1.404	3534	1.304	4512	1.198	5239
Mobile phase	78:22	1.775	2222	1.711	3746	1.286	4322	1.270	3476
	80:20	1.746	2283	1.324	3790	1.365	5831	1.283	6377
	82:18	1.748	2268	1.463	3919	1.351	5252	1.306	6445

no. of analysis n=10

Sensitivity

Regression data was used to find standard deviation and slope to evaluate the sensitivity of method. The limits of detection (LOD) and quantitation (LOQ) are the concentration resulting in a signal-to-noise ratio greater than or equal to three and ten respectively. The LOD of citalopram and studied NSAIDs i.e. piroxicam, celecoxib and diclofenac sodium were found to be 16.45, 23.33, 27.66 and 14.44 ng ml^{-1} and 0.04986, 0.07071, 0.08383 and 0.04386 µg mL^{-1} respectively. Table 2 represents the detailed LOD and LOQ data for each analyte.

Robustness

Robustness study was estimated by considering effect of changes in the operating parameters of the established analytical method which involved variation in pH of solvent, composition of mobile phase, flow rate range and wavelength. Robustness data confirmed the reliability of the proposed method throughout normal practice. It was observed that the method stood up to deliberate changes made in chromatographic parameters. Table 5 shows robustness data.

Assay in pharmaceutical formulation

The validated LC method was found to be accurate and applicable for the simultaneous determination of citalopram (Pramcit 20 mg) with piroxicam (Feldene 10 mg), celecoxib (Celbex 100 mg) and diclofenac sodium (Dicloran 50 mg) in commercial formulations showing good separation with high resolution for all the analytes at the isosbestic point. Proposed method exhibited good percent recoveries and %RSD values (Table 3 and 4). Furthermore, resulted chromatograms confirmed elution of analytes was not interrupted by any excipient in the drug formulation. Hence, developed method is reliable and sensitive for the simultaneous determination of citalopram with piroxicam, celecoxib and diclofenac sodium in commercial formulation without any interference of unwanted species.

Assay in human serum

Proposed method for simultaneous determination and quantification of citalopram with piroxicam, celecoxib and diclofenac sodium in human serum was assessed for routine analysis. Results showed good separation and percent recoveries within the satisfactory limits (Table 3 and 4). Any interference of endogenous components of serum was not detected. Therefore, it is confirmed that the validated LC method is appropriate for simultaneous determination of citalopram with piroxicam, celecoxib and diclofenac sodium in human serum and applicable for routine and clinical analysis.

Conclusion

The assay defined herein for simultaneous liquid chromatographic method with UV detection determination of citalopram with NSAIDs in bulk drug, pharmaceutical formulation and human serum. The method was found to be reliable, sensitive, specific and robust and showed satisfactory linearity and accuracy for all the studied analytes. Based on validation and applicability, it is concluded that the method is well suited and meets the need for routine analysis of studied analytes as well as for laboratory applications.

Acknowledgement

The authors highly acknowledge the support of Prof. Dr. Saqib Anjum, Chairman, Department of Chemistry and Dr. Zahoor-ul-Hassan Awan, Assistant Professor, Department of Chemical

Engineering, NED University of Engineering and Technology, Karachi. Authors acknowledge Higher Education Commission, Pakistan for SRGP research grant (21-250/SRGP/R&D/HEC/2014).

References

1. F. Tanwir, G. Marrone, A. Tariq and C. S. Lundborg, Diagnosis and Prescribing Pattern of Antibiotics and Painkillers Among Dentists, *Oral Health Prev. Dent.*, **13**, 75 (2015).
2. A. L. Riza, F. Pearson, C. Ugarte-Gil, B. Alisjahbana, S. van de Vijver, N. M. Panduru, P. C. Hill, R. Ruslami, D. Moore, R. Aarnoutse, J. A. Critchley and R. van Crevel, Clinical Management of Concurrent Diabetes and Tuberculosis and the Implications for Patient Services, *Lancet Diab. & Endo.*, **2**, 740, (2014).
3. P. F. Pereira, M. C. Marra, R. R. Cunha, W. P. da Silva, R. A. A. Munoz and E. M. Richter, Two Simple and Fast Electrochemical Methods for Simultaneous Determination of Promethazine and Codeine, *J. Electro. Ana. Chem*, **713**, 32 (2014).
4. A. B. Ahmed, N. S. Abdelwahab, M. M. Abdelrahman and F. M. Salama, Simultaneous Determination of Dimenhydrinate, Cinnarizine and Cinnarizine Impurity by TLC and HPLC Chromatographic Methods, *Bull.Fac.Pharm, Cairo University*, (2017).
5. Z. R. Tan, D. S. Ouyang, G. Zhou, L. S. Wang, Z. Li, D. Wang and H. H. Zhou, Sensitive Bioassay for the Simultaneous Determination of Pseudoephedrine and Cetirizine in Human Plasma by Liquid-Chromatography–Ion Trap Spectrometry, *J. Pharm. and Bio. Ana.,* **42**, 207 (2006).
6. H. Lou, H. Yuan, Z. Ruan and B. Jiang, Simultaneous Determination of Paracetamol, Pseudoephedrine, Dextrophan and Chlorpheniramine in Human Plasma by Liquid Chromatography–Tandem Mass Spectrometry', *J. Chromatogr.* B, **878**, 682 (2010).
7. S. Akram, S. N. Ali, A. Qayoom, S. Iqbal, N. Naz and I. Memon, High Performance Liquid Chromatographic Method for Simultaneous Determination of Alprazolam with Antihistamines in Bulk Drug, Pharmaceutical Formulation and Human Serum, *Sindh Uni. Res. J.-SURJ (Science Series)*, **49**, 7 (2017).
8. S. N. Ali, N. Sultana, M. S. Arayne and A. Qayoom, Liquid Chromatographic Determination of Alprazolam with ACE Inhibitors in Bulk, Respective Pharmaceutical Products and Human Serum, *Sci. Int. (LHR)*, **28**, 3815 (2016).
9. A. Westphal, M. H and L. Kriston, Selective Serotonin Reuptake Inhibitors and Tricyclic Antidepressants in the Acute Treatment of Chronic Depression and Dysthymia : A Systematic Review and Meta-Analysis, *J. Affect. Disord.*, **144**, 7 (2013).
10. Y. S. Mineur, A. Obayemi, M. B. Wigestrand, G. M. Fote, C. A. Calarco, A. M. Li and M. R. Picciotto, Cholinergic Signaling in the Hippocampus Regulates Social Stress Resilience and Anxiety and Depression-like Behavior.', *Proc. Natl. Acad. Sci. U. S. A.*, **110**, 3573 (2013).
11. S. Andersen, T. G. Halvorsen, S. Pedersen-Bjergaard, K. E. Rasmussen, L. Tanum and H. Refsum, Stereospecific Determination of Citalopram and Desmethylcitalopram by Capillary Electrophoresis and Liquid-Phase Microextraction, *J. Pharm. Biomed. Anal.*, **33**, 263 (2003).
12. J. Rodríguez, G. Castañeda and L. Muñoz, Rapid Determination of Letrozole, Citalopram and their Metabolites by High Performance Liquid Chromatography-Fluorescence Detection in Urine: Method Validation and Application to Real Samples, *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.*, **913**, 12 (2013).
13. T. A. Ali, G. G. Mohamed and M. A. Migahed, A New Screen-Printed Ion Selective Electrode for Determination of Citalopram Hydrobromide in Pharmaceutical Formulation, *Chinese J. Anal. Chem.*, **42**, 565 (2014).
14. H. Ghaedi, A. Afkhami, T. Madrakian and F. Soltani-Felehgari, Construction of Novel Sensitive Electrochemical Sensor for Electro-Oxidation and Determination of Citalopram Based on Zinc Oxide Nanoparticles and Multi-Walled Carbon Nanotubes, *Mater. Sci. Eng.* C, **59**, 847 (2016).
15. M. N. Khan, M. R. Jan, J. Shah and S. H. Lee, A Validated Silver-Nanoparticle-Enhanced Chemiluminescence Method for the Determination of Citalopram in Pharmaceutical Preparations and Human Plasma, *Luminescence*, **29**, 266 (2014).
16. A. Raza and T. M. Ansari, Spectrophotometric Determination of Citalopram Hydrobromide in Tablet Dosage form using Chloranil, *Pak. J. Pharm. Sci*, **27**, 255 (2014).
17. M. N. Khan, J. Shah, M. R. Jan and S. H. Lee, A Validated Spectrofluorimetric Method for the Determination of Citalopram in Bulk and Pharmaceutical Preparations based on the Measurement of the Silver Nanoparticles-Enhanced Fluorescence of Citalopram/Terbium Complexes, *J. Fluoresc.*, **23**, 161 (2013).
18. J. Rodríguez Flores, A. M. C. Salcedo, M. J. V. Llerena and L. M. Fernández, Micellar Electrokinetic Chromatographic Method for the Determination of Letrozole, Citalopram and their Metabolites in Human Urine, *J. Chromatogr.* A,

1185, 281 (2008).
19. C. Saravanan, M. Thamizhmozhi, C. A. S. Kumar, C. Sudhakar, B. Rajesh and G. S. Kumar, A Novel and Rapid HPTLC Method for the Analysis of Citalopram Hydrobromide in Tablet Dosage Form – Development and Validation, *J. Adv. Sci. Res.*, **3**, 62 (2012).
20. P. J. Mendes VM, Analysis of Citalopram in Plasma and Hair by a Validated LC MS/MS Method, *J. Chromatogr. Sep. Tech.*, **6**, (2015).
21. R. Geryk, J. Vozka, K. Kalíková and E. Tesařová, HPLC Method for Chiral Separation and Quantification of Antidepressant Citalopram and Its Precursor Citadiol, *Chromatographia*, **76**, 483 (2013).
22. T. Hu, T. Peng, X. J. Li, D. D. Chen, H. H. Dai, X. J. Deng, Z. F. Yue, G. M. Wang, J. Z. Shen, X. Xia, S.-Y. Ding, Y. N. Zhou, A. L. Zhu, and H. Y. Jiang, Simultaneous Determination of Thirty Non-Steroidal Anti-Inflammatory Drug Residues in Swine Muscle by Ultra-High-Performance Liquid Chromatography with Tandem Mass Spectrometry, *J. Chromatogr.* A, **1219**, 104 (2012).
23. P. Emery, H. Zeidler, T. K. Kvien, M. Guslandi, R. Naudin, H. Stead, K. M. Verburg, P. C. Isakson, R. C. Hubbard and G. S. Geis, Celecoxib Versus Diclofenac in Long-Term Management of Rheumatoid Arthritis: Randomised Double-Blind Comparison, *Lancet*, **354**, 2106 (1999).
24. C. A. Szekely, T. Town and P. P. Zandi, In *Inflammation in the Pathogenesis of Chronic Diseases: The COX-2 Controversy*, NSAIDs for the Chemoprevention of Alzheimer's Disease, Springer Netherlands, Dordrecht, p. 229 (2007).
25. A. Zarghi, A. Shafaati, S. M. Foroutan and A. Khoddam, Simple and Rapid High-Performance Liquid Chromatographic Method for Determination of Celecoxib in Plasma using UV Detection: Application in Pharmacokinetic Studies, *J. Chromatogr.* B *Anal. Technol. Biomed. Life Sci.*, **835**, 100 (2006).
26. R. W. Sparidans, J. S. Lagas, A. H. Schinkel, J. H. M. Schellens and J. H. Beijnen, Liquid Chromatography-Tandem Mass Spectrometric Assay for Diclofenac and Three Primary Metabolites in Mouse Plasma, *J. Chromatogr.* B *Anal. Technol. Biomed. Life Sci.*, **872**, 77 (2008).
27. S. P. Puthli and P. R. Vavia, Stability Indicating HPTLC Determination of Piroxicam, *J. Pharm. Biomed. Anal.*, **22**, 673 (2000).
28. D. Arroyo, M. C. Ortiz and L. A. Sarabia, Optimization of the Derivatization Reaction and the Solid-Phase Microextraction Conditions using a D-Optimal Design and Three-Way Calibration in the Determination of Non-Steroidal Anti-Inflammatory Drugs in Bovine Milk by Gas Chromatography – Mass Spectro, *J. Chromatogr.* A, **1218**, 4487 (2011).
29. F. Khan, I. Ahmad, M. Akhtar, H. A. Rauf, H. Altaf and M. M. Hayat, 'RP-HPLC Method Development and Validation for Simultaneous Determination of Esomeprazole and Diclofenac Sodium in Pharmaceutical Dosage Forms', *Pharm. Chem. J.*, **49**, 788 (2016).
30. N. Migowska, P. Stepnowski, M. Paszkiewicz, M. Gołębiowski and J. Kumirska, Trimethylsilyldiazomethane (TMSD) as a New Derivatization Reagent for Trace Analysis of Selected Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) by Gas Chromatography Methods, *Anal. Bioanal. Chem.*, **397**, 3029 (2010).
31. L. Ascar, I. Ahumada, A. López, F. Quintanilla and K. Leiva, Nonsteroidal Anti-Inflammatory Drug Determination in Water Samples by HPLC-DAD under Isocratic Conditions, *J. Braz. Chem. Soc.*, **24**, 1160 (2013).
32. M. Villar Navarro, M. Ramos Payán, R. Fernández-Torres, M. A. Bello-López, M. Callejón Mochón and A. Guiráum Pérez, Capillary Electrophoresis Determination of Nonsteroidal Anti-Inflammatory Drugs in Wastewater using Hollow Fiber Liquid-Phase Microextraction, *Electrophoresis*, **32**, 2107 (2011).
33. T. Nemoto, X. Lee, T. Kumazawa, C. Hasegawa, M. Fujishiro, A. Marumo, Y. Shouji, K. Inagaki and K. Sato, Journal of Pharmaceutical and Biomedical Analysis High-throughput determination of nonsteroidal anti-inflammatory drugs in human plasma by HILIC-MS / MS, *J. Pharm. Biomed. Anal.*, **88**, 71 (2014).
34. A. A. Gouda, M. I. K. El-sayed, A. S. Amin and R. El, Spectrophotometric and Spectrofluorometric Methods for the Determination of Non-Steroidal Anti-Inflammatory Drugs : A Review, *Arab. J. Chem.*, **6**, 145 (2013).
35. H. Y. Jiang, H. Z. Chen, X. J. Hu, Z. H. Yu, W. Yang, M. Deng, Y. H. Zhang and B. Ruan, Use of Selective Serotonin Reuptake Inhibitors and Risk of Upper Gastrointestinal Bleeding: A Systematic Review and Meta-Analysis, *Clin. Gastroenterol. Hepatol.*, **13**, 42 (2015).
36. Y. K. Loke, A. N. Trivedi and S. Singh, Meta-Analysis: Gastrointestinal Bleeding due to Interaction Between Selective Serotonin Uptake Inhibitors and Non-Steroidal Anti-Inflammatory Drugs, *Aliment. Pharmacol. Ther.*, **27**, 31 (2007).
37. I. C. H. Harmonised and T. Guideline, in *Climate Change 2013 - The Physical Science Basis*, Summary for Policymakers, Cambridge University Press, Cambridge, p. 1–30 (2014).