doi.org/10.36721/PJPS.2020.33.1.REG.121-127.1

Liquid chromatographic method for simultaneous determination of alprazolam with NSAIDs in bulk drug, pharmaceutical formulation and human serum

Saeeda Nadir Ali*, Shumaila Akram*, Amtul Qayoom, Nida Naz and Anjum Ayub
Department of Chemistry, NED University of Engineering and Technology, Karachi, Pakistan

Abstract: High performance liquid chromatography with UV/vis detection was optimized and validated for simultaneous quantification of alprazolam with celecoxib and diclofenac sodium in pharmaceutical formulation and human serum. Chromatographic separation was achieved at detection wavelength of 230 nm on Shimadzu Shim-pack CLC-ODS (M) 25M column employing 80:20 (v/v) methanol: water (pH 3.5) as mobile phase with elution rate 1.0mL min-1. Analytes were quantified in the ranges 0.2-15, 0.3-20 and 0.6-40 µg mL-1 with detection limits 19.76, 17.29 and 11.83ng mL-1 respectively. Recoveries were in the range 98.15-101.15, 99.24-99.90 and 98.87-101.19% in pharmaceutical formulation and 98.05-101.01, 98.72-99.49 and 98.25-99.47% in human serum respectively and precision ranged from 0.19-1.84%. The analytes were successfully detected without any observable interference commonly present in pharmaceutical formulation and human serum demonstrating applicability of method.

Keywords: Alprazolam, celecoxib, diclofenac sodium, HPLC-UV.

INTRODUCTION

Alprazolam (fig. 1) represents benzodiazepine class of drugs having targeting ability of GABA receptor with inhibitory effect on secretion of ACTH and cortisol in order to manage symptoms of anxiety, panic disorder, seizures and muscles spasm and also included in minor tranquilizers (Borrey *et al*., 2001; Giordano *et al*., 2003; Hefnawy, 2002; Laloup *et al*., 2005). It is also recommended as treatment of insomnia (Toyo'oka *et al*., 2003). It has replaced many barbiturates and meprobamatein treatment of anxiety disorder due to its effectiveness and low toxicity but may cause reappearance of old or new symptoms on discontinuing its treatment (Chouinard, 2004; Naveed and Qamar, 2014). Alprazolam has been analyzed in serum, urine and gastric content of patients (Soriano *et al*., 2001) along with most frequently used class of drugs NSAIDs i.e. celecoxib, diclofenac sodium by using sensitive technique of high performance of liquid chromatography (Nasir *et al*., 2011; Störmer *et al*., 2003), GC with mass spectrometry (Fraser *et al*., 1991; Hold *et al*., 1996), DART-TOF Mass Spectrometry (Samms *et al*., 2011), stripping voltammetry (Nunes *et al*., 2015), high and ultra-performance liquid chromatography (Yu *et al*., 2006).

NSAIDs are generally prescribed as anti-inflammatory, antipyretic and analgesic agent (Heydari *et al*., 2013). Celecoxib is highly selective inhibitor of COX-2 enzyme and is frequently used to treat osteoarthritis and rheumatoid arthritis with good bioavailability. It has capability to minimize the side effects ordinarily associated with other NSAIDs (Rao *et al*., 2005). Diclofenac sodium is administered as anti-inflammatory, antipyretic and analgesic drug which completes its action by inhibiting COX enzyme to prevent secretion of prostaglandins (Nasir *et al*., 2011). Literature survey reveals numerous methods for the analysis of celecoxib and diclofenac sodium including HPLC coupled with UV detector (Störmer *et al*., 2003)(Gugulothu and Patravale, 2012)(Pavan Kumar *et al*., 2006), mass spectrometry (Reddy *et al*., 2014), liquid chromatography with tandem mass spectrometric assay (Sparidans *et al*., 2008), spectrophotometry (Saha *et al*., 2002)(Souza and Tubino, 2005) and voltammetry (Mokhtari *et al*., 2012).

TDM (therapeutic drug monitoring) of antipsychotics is a reliable tool for dosage optimization of drugs with narrow therapeutic range and establishes therapeutic benefit of de-voiding adverse events including intoxication and non-compliance. TDM empowers the need of an analytical method of high precision, accuracy and reproducibility. This study facilitates a rapid and précised quantitation of alprazolam with co-prescribed NSAIDs in active form, pharmaceutical formulation and human serum as clinical manifestation. Analytical method for alprazolam with NSAIDs (celecoxib and diclofenac sodium) (fig. 1) is developed using HPLC coupled with UV detector and validated by adhering ICH guidelines including internal quality control (precision, linearity, accuracy, recovery). Developed method is accomplished with reliability, time and cost effectiveness.

**Corresponding author:* e-mail: saeeda@neduet.edu.pk, shumailah116@gmail.com

MATERIALS AND METHODS

Reagents and Chemicals

Alprazolam (>98% purity) was kind gift from Pfizer Pakistan Ltd., celecoxib with 99.46% purity was obtained from Getz Pharma Pakistan (Pvt.) Ltd. and diclofenac sodium with purity over 98.23% was acquired from Sami pharmaceuticals (Pvt.) Ltd. Pharmaceutical formulations Xanax® (0.5mg), Celbex® (100mg) and Dicloran® (50mg) were purchased from local pharmacy at Karachi.

STATISTICAL ANALYSIS

For statistical analysis, SPSS Software version 17.0 was used.

Instrumentation

A Shimadzu Corporation, Japan liquid chromatographic equipment consisted of LC-20AT binary gradient solvent delivery pump AOC-20 along with auto-sampler SIL-20AHT/20ACHT UFLC provided with 1µL auto injector AOC-20i and a UV-visible detector SPD-20A/20AV. The system was coupled with Shimadzu CBM-20A Communication Bus Module to attain data. The data was processed with LC solution GPC software version 1.25. A Shimadzu 1800 UV/Vis spectrophotometer was used for wavelength determination.

Chromatographic parameters

Liquid chromatograph was furnished with Shimadzu Shim-pack CLC-ODS (M) 25M column (4.6 mm i.d. × 0.25mm). The components were eluted by a gradient of A) 80% methanol and B) 20% water at 1.0 ml min^{-1} flow rate adjusting detector wavelength 230 nm. Prior to HPLC analysis, Millipore vacuum filter system (0.45 µm pore size filter) and ultrasonic bath (LC 30H) was used for filtration and sonication of mobile phase.

Standard solution preparation

Certified reference standards were deployed to prepare the stock standard solutions of each analyte. Accurately weighed 100 mg alprazolam, celecoxib and diclofenac sodium were separately dissolved in appropriate volume of diluent and volumes were volumes were made up to achieve the concentration of 1mg mL^{-1}. To establish calibration curves, working standard solutions in the concentrations ranges 0.2-15, 0.3-20 and 0.6-40µg mL^{-1} for alprazolam, celecoxib and diclofenac sodium respectively were prepared by serial dilution of stock standard solution into 25mL volumetric flask using 80:20 v/v methanol-water diluent and introduced to auto sampler after 0.45µm millipore filter paper mediated micro filtration. All the solutions were prepared once and stored at -20°C until analysis.

Assay in pharmaceutical formulations

For assay in commercial formulation, ten tablets of each analyte including Xanax (0.5 mg), Celbex (100mg) and Dicloran (50 mg) were individually weighed and then finely triturated with pestle motor. The content equivalent to 100 mg of each was transferred to 100 mL volumetric flask separately, sonicated for 15 min in small amount of diluent using ultra-sonic bath and volumes were completed to obtain the final concentration 1 mg mL^{-1}. Undissolved excipients were removed by filtration through 0.45 µm pore size filter and finally diluted to the desired concentration for analysis.

Assay for drug serum solution

A 10mL blood sample, adequately withdrawn from a healthy donor at Fatmid Foundation Karachi, was conveyed to a disinfected EDTA glass tube and centrifuged for 10min at 4ºC at 1600xg to separate plasma. A total of 1.0mL plasma was introduced into 9.0 mL acetonitrile; vortexed for 1 min followed by centrifugation at 10,000 rpm for 10min. Clear serum solution obtained was fortified with standard alprazolam, celecoxib and diclofenac sodium in required concentrations for their liquid chromatographic assay.

Method validation

Method validation was accomplished following ICH Q2 (R1) (Guideline and others, 2005) guidelines for system suitability, accuracy, precision, linearity, robustness, quantitation and detection limits, robustness and specificity. All the analyses were performed in triplicates. System suitability was examined in terms of the parameters retention time, tailing factor, theoretical plates and resolution with the purpose to reproduce the result at same experimental condition. Calibration curves were constructed between concentration of each analyte vs. peak response to estimate linearity and regression attributes including slope, correlation coefficient, intercept, standard error and standard error estimate. Accuracy of method was established by evaluating the recovery of pure drug in dosage formulation. Inter-day and intra-day precision was assessed by means of %RSD values at six different concentrations of each standard analyte. Limits of detection and quantitation were documented at signal to noise ratio three times and ten times to the baseline of peak response. Moreover, experimental condition including pH, flow rate, wavelength and mobile phase composition were altered intentionally to validate viability of method. Specificity of the method was assessed by comparing the chromatograms of bulk drug, pharmaceutical formulation and spiked serum solutions to determine the interfering species being eluted at the same retention time of analyte.

Ethical approval

As per ICH Q2 (R1) (Guideline and others, 2005)

RESULTS

Method development is an essential step while undertaking development of new products, evaluating

quality integrity and routine quantitation. This step is of great significance regarding pharmaceutical industries as it has direct effect on human lives. Present study provides content for a newly developed and validated analytical method aided with HPLC-UV for simultaneous determination of alprazolam, celecoxib and diclofenac sodium in active drug, pharmaceutical formulation and human serum.

Acquisition of required data was met by evaluating operational conditions. Primarily, different organic solvents e.g. acetonitrile and methanol were tried with water as mobile phase in gradient system. Using acetonitrile:water, all the analytes were eluted with less analysis time though with inappropriate resolution. Methanol and water was found suitable eluent mixture for the analysis. Ensuring the finest result with maximum resolution and least analysis time mobile phase ratio was selected by screening. Increasing percent volume of methanol with water as 50:50, 60:40, 70:30 80:20 and 90:10 lessens the static retention and thus and decrease the retention time of analyte. Therefore, the most efficient mobile phase mixture methanol: water (80:20 v/v) was used throughout the analysis at constant flow of 1.0mL min^{-1}. In contrast with mobile phase, pH of solvent system was taken in account for optimized output by scanning effective range of pH scale between 2.0 to 4.0. Sharp and well-separated peaks at pH 3.5±0.2 letting fit for the reported purpose. For the appropriate detection wavelength, UV spectra of each drug was acquired by using Shimadzu-1800 UV-visible spectrophotometer (fig. 2). The isosbestic point of 230 nm reflected high sensitivity for individual drug affirming the effectiveness of the newly developed method. The representative chromatogram is given in fig. 3.

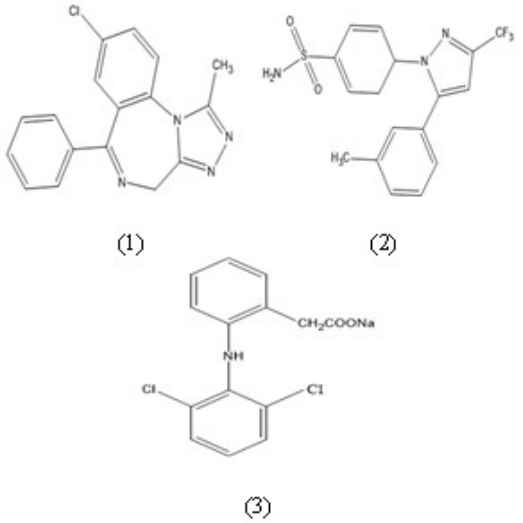

Fig. 1: Alprazolam[1], diclofenac sodium[2], celecoxib[3]

Method validation

Method validation was performed following ICH Q2 (R1) (Guideline and others, 2005) guidelines for system suitability, linearity, accuracy, precision, robustness, quantitation and detection limits, robustness and specificity.

System suitability test

An essential part of method validation is system suitability test to generate reproducible results with acceptable precision and accuracy range. It was performed daily until analysis in triplicate to confirm the column efficiency and reported in terms of theoretical plates (N), tailing factor (T) and resolution factor (Res) (Table 1). Numbers of theoretical plates greater than two thousand and tailing factor less than two establishes the suitability of proposed method.

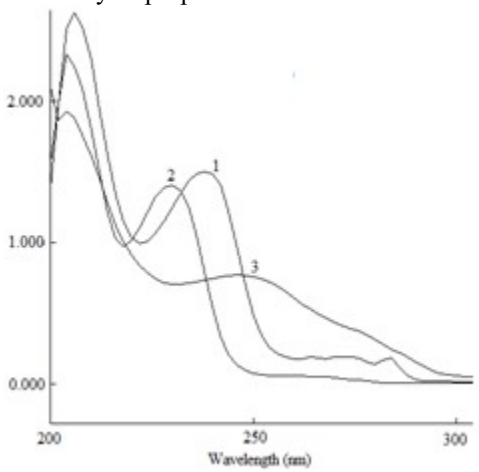

Fig. 2: UV spectra of alprazolam[1], celecoxib[2], diclofenac sodium[3]

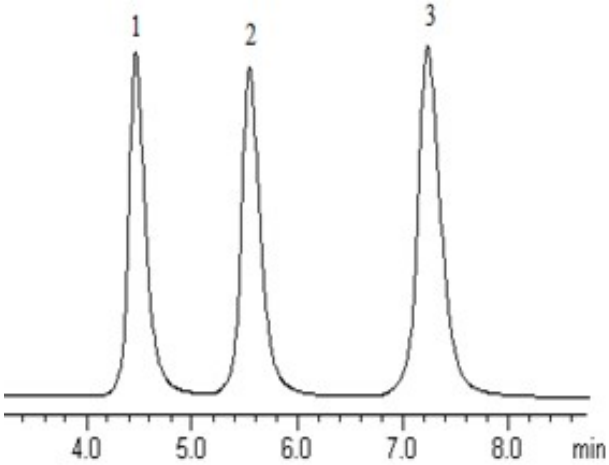

Fig. 3: Chrotaogram of alprazolam[1], celecoxib[2], diclofenac sodium[3] in API

Table 1: system suitability of method

Drugs	N[a)]	T[b)]	Res[c)]
Alprazolam	5243.254	1.365	--
Celecoxib	3801.956	1.283	3.499
Diclofenac sodium	4528.821	1.203	4.680

[a]Theoritical plates, [b]Tailing factor, [c]Resolution

Linearity and range

To evaluate the linearity, linear regression model was employed. Calibration curves were plotted in between detector response vs. concentration range of 0.2-15, 0.3-20 and 0.6-40 µg mL^{-1} for alprazolam, celecoxib and diclofenac sodium respectively. The linear functions were y = 8386.2x + 4847.6, y=6881.8x-640.04 and y= 4461.5x-1029.5 respectively. The data along with standard error and standard error estimate are given in table 2.

Precision

In table 3, precision of the method in active pharmaceutical ingredients are shown. Establishing the same chromatographic conditions, repeatability was evaluated by six replicates within the linearity range 0.2-15, 0.3-20 and 0.6-40 µg mL^{-1} for alprazolam, celecoxib and diclofenac sodium respectively.

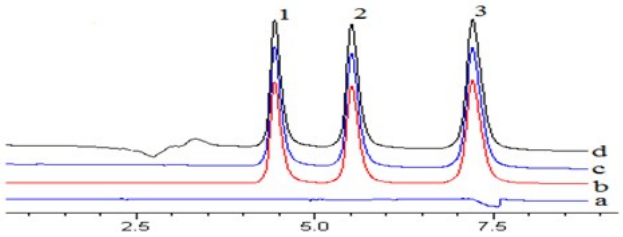

Fig. 4: Chromatograms of (a) placebo (b) spiked excipients solution, (c) pharmaceutical formulation and (d) spiked serum solution in alprazolam[1], celecoxib[2] and diclofenac sodium[3]

The RSD values for within day precision were found to be 0.2-1.84, 0.35-1.07 and 0.19-1.18% respectively. Intermediate precision study was prolonged to two sequential days of method validation at mentioned concentration levels which was established to be 0.19-1.66, 0.33-1.72 and 0.03-1.84% respectively. The data represented in table 3 endorse the repeatability and reproducibility of the proposed method.

Accuracy

Accuracy of the developed method was established by performing recovery experiments. All the studied analytes were injected to the chromatograph and recovery values were reviewed at three concentration levels in the range of 1.6-7.5, 2.5-10 and 5-20µg mL^{-1} for alprazolam, celecoxib and diclofenac sodium respectively in pharmaceutical formulation and human serum. Percent recovery was determined to be in the range 98.15-101.15, 99.24-99.90 and 98.87-101.19% and98.05-101.01, 98.72-99.49 and 98.25-99.47% respectively. Recovery values of each drug in pharmaceutical formulation and in human serum are shown in table 4.

Sensitivity

Sensitivity of the proposed method has been reported in terms of limits of detection (LOD) and quantitation

Table 2: Regression characteristics

Parameters	Alprazolam	Celecoxib	Diclofenac sodium
Slope	8386.2	6881.8	4461.5
Intercept	4847.6	- 640.04	- 1029.5
Linearity (µg mL^{-1})	0.2-15	0.3-20	0.6-40
R^{2a}	0.9984	0.9995	0.9993
SE[b]	0.2080	0.1817	0.4210
SEE[c]	0.4045	0.3701	0.8588
LOD (ng mL^{-1})	19.76	17.29	11.83
LOQ (µg mL^{-1})	0.0598	0.0524	0.0358

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

Table 3: Precision of method

Alprazolam			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]
15	1.84	1.43	20	0.35	1.46	40	0.43	1.27
7.5	0.20	0.31	10	0.39	0.33	20	0.57	0.03
3.25	1.13	1.33	5	0.80	1.22	10	0.19	0.72
1.6	0.33	1.36	2.5	0.95	1.72	5	1.18	1.25
0.8	1.50	0.66	1.25	1.01	0.40	2.5	0.66	1.39
0.4	0.74	1.44	0.6	1.07	0.44	1.25	0.80	1.84
0.2	1.63	0.19	0.3	1.00	1.66	0.6	0.82	0.40
Human serum								
Alprazolam			Celecoxib			Diclofenac sodium		
Conc µg mL^{-1}	%RSD		Conc µg mL^{-1}	%RSD		Conc µg mL^{-1}	%RSD	
7.5	0.932		10	0.33		20	0.69	
3.3	1.383		5	0.99		10	1.63	
1.6	0.818		2.5	0.44		5	1.65	

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

(LOQ). These are the concentration in response to signal-to-noise ratio greater than or equal to three and ten respectively. The LOD and LOQ for alprazolam and studied NSAIDs including celecoxib and diclofenac sodium were found to be 19.76, 17.29 and 11.83ng mL^{-1} and 0.05987, 0.05240 and 0.03586µg mL^{-1} respectively. The detailed LOD and LOQ data for each analyte is represented in table 2.

Robustness

Robustness of the proposed method was evaluated by making small variation in operating conditions. Alteration in chromatographic parameters were intentionally made like mobile phase composition up to ±2mL, pH up to ± 0.1, flow rate in the range 0.7-1.2mL min^{-1} and wavelength up to ±2nm and instrumental response was observed. Expected differences in the analytical results were obtained assuring consistency of the proposed method. The detailed robustness data is shown in table 5.

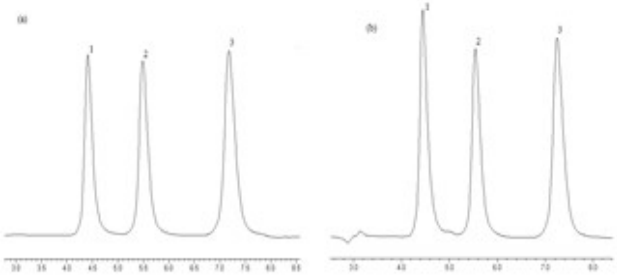

Fig. 5: Chromatograms representing alprazolam[1], celecoxib[2] and diclofenac sodium[3] in pharmaceutical formulation[a] and human serum[b]

Specificity

All the analytes were studied in commercial formulation and spiked serum solution in contrast with bulk drug and blank serum solution to demonstrate specificity of developed method. Results extracted from typical chromatogram of placebo, spiked excipients solution, pharmaceutical formulation and spiked serum sample showed good agreement between data even in presence of excipients and interfering species (fig. 4).

DISCUSSION

Assay in pharmaceutical formulation

Liquid chromatographic method for simultaneous quantitation of alprazolam, celecoxib and diclofenac sodium in bulk drug was developed and validated in committed study. Method was found equally potent and compatible in commercially formulated dosage inclusive of Xanax 0.5 mg, Celbex 100 mg and Dicloran 50 mg proceeding with identical experimental conditions. Chromatograms regarding formulation show well resolved and separated peaks with appropriate time interval having appreciable accuracy in terms of statistical evaluation (table 4 and fig. 5) concluding high sensitivity and reliability. All the studied analytes were resolved without intrusion of formulation excipients.

Table 4: Recovery of method

Pharmaceutical formulation								
Xanax			Celbex			Dicloran		
Conc	%Recovery	%Error	Conc	%Recovery	%Error	Conc	%Recovery	%Error
7.5	99.89	-0.11	10	99.72	-0.28	20	100.86	0.86
3.3	98.15	-1.89	5	99.24	-0.77	10	101.19	1.18
1.6	101.15	1.14	2.5	99.90	-0.10	5	98.87	-1.14
Human serum								
Xanax			Celbex			Dicloran		
7.5	98.05	-1.991	10	99.49	-0.51	20	99.47	-0.53
3.3	101.01	0.998	5	98.72	-1.30	10	98.25	-1.78
1.6	99.98	-0.015	2.5	99.44	-0.57	5	98.49	-1.54

Table 5: Robustness of method

Parameters		Alprazolam		Celecoxib		Diclofenac sodium	
		T	N	T	N	T	N
Flow rate (mL min^{-1})	0.9	1.230	3845.190	1.361	4898.001	1.318	5542.691
	1.0	1.365	3801.956	0.999	2355.293	0.934	2560.776
	1.1	1.154	3761.011	1.355	4661.588	1.287	5256.363
Wave length (nm)	228	1.252	3721.772	1.250	4560.490	1.161	5291.947
	230	1.365	3801.956	1.283	4528.721	1.203	5243.254
	232	1.295	3710.744	1.261	4588.321	1.181	5273.158
pH	3.4	1.366	3746.699	1.287	4529.818	1.196	5283.407
	3.5	1.345	3737.238	1.266	4483.258	1.197	5204.998
	3.6	1.379	3722.602	1.290	4505.704	1.202	5243.625
Mobile phase	78:22	1.188	3748.992	1.169	4718.8	1.121	5344.482
	80:20	1.340	3818.216	1.379	4535.187	1.188	5101.448
	82:18	1.384	3376.553	1.224	4029.750	1.144	4793.599

Assay in human serum

Newly designed method for the assay of alprazolam, celecoxib and diclofenac sodium was also investigated for its serological application. Highly resolved and well-separated peaks were obtained without significant interference of endogenous components of serum. All the studied statistical parameters showed good compliance with the acceptable limits including percent recovery and RSD (table 4 and fig. 5). Hence it can be deduced that proposed LC method is suitable for simultaneous analysis of alprazolam, celecoxib and diclofenac sodium in human serum and method of choice for routine clinical scanning.

CONCLUSION

Highly sensitive assay technique of HPLC paired with UV detector was hired to established the practical implementation between proposed study and laboratory outcome. Simultaneous determination for alprazolam with celecoxib and diclofenac sodium was duly developed and validated. This method showed acceptable result for all the pre-defined parameters of intstrumentental response. All the analytes were analysed in active pharmaceutical ingredient (API), pharmaceutical dosage and human serum precisely and accurately showing good sensitivity along with reduced analysis time. Concisely it is inferenced that the developed method is preceived to be sensitive and specefic with high reliability index and laboratory application.

ACKNOWLEDGEMENT

The unconditional 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 is highly acknowledged. Authors acknowledge Higher Education Commission, Pakistan for SRGP research grant for project (Project # 21-250/SRGP/RnD/HEC/2014).

REFERENCES

Borrey D, Meyer E, Lambert W, Van Peteghem C and De Leenheer AP (2001). Simultaneous determination of fifteen low-dosed benzodiazepines in human urine by solid-phase extraction and gas chromatography--mass spectrometry. *J Chromatogr-Biomed,* **765**(2): 187-197.

Chouinard G (2004). Issues in the clinical use of benzodiazepines: Potency, withdrawal, and rebound. *J Clin Psychiat*, **65**(5): 7-12.

Fraser AD, Bryan W and Isner AF (1991). Urinary screening for alprazolam and its major metabolites by the Abbott ADx and TDx analyzers with confirmation by GC/MS. *J Anal Toxicol*, **15**(1): 25-29.

Giordano R, Grottoli S, Brossa P, Pellegrino M, Destefanis S, Lanfranco F, Gianotti L, Ghigo E and Arvat E (2003). Alprazolam (a benzodiazepine activating GABA receptor) reduces the neuroendocrine responses to insulin-induced hypoglycaemia in humans. *Clin Endocrinol,* **59**(3): 314-320.

Gugulothu DB and Patravale VB (2012). A new stability-indicating HPLC method for simultaneous determination of curcumin and celecoxib at single wavelength: an application to nanoparticulate formulation. *Pharm Anal Acta*, **3**(4): 1-6

Guideline, I.C.H.H.T., others (2005). Validation of analytical procedures: Text and methodology. Q2 (R1) 1.

Hefnawy MM (2002). Analysis of certain tranquilizers in biological fluids. *J. Pharmaceut. Biomed.,* **27**(5): 661-678.

Heydari R, Shamsipur M and Naleini N (2013). Simultaneous determination of EDTA, sorbic acid and diclofenac sodium in pharmaceutical preparations using high-performance liquid chromatography. *AAPS Pharm. Sci. Tech.*, **14**(2): 764-769.

Höld KM, Crouch DJ, Rollins DE Wilkins DG, Canfield, DV and Maes RA (1996). Determination of Alprazolam and $α$-Hydroxyalprazolam in human plasma by gas chromatography/negative-ion chemical ionization mass spectrometry. *J. Mass Spectrom,* **31**(9): 1033-1038.

Laloup M Fernandez M del MR, De Boeck G Wood M, Maes V and Samyn N (2005). Validation of a liquid chromatography-tandem mass spectrometry method for the simultaneous determination of 26 benzodiazepines and metabolites, zolpidem and zopiclone, in blood, urine, and hair. *J. Anal. Toxicol.,* **29**(7): 616-626.

Mokhtari A, Karimi-Maleh H, Ensafi AA & Beitollahi H., (2012). Application of modified multiwall carbon nanotubes paste electrode for simultaneous voltammetric determination of morphine and diclofenac in biological and pharmaceutical samples. *Sensor Actuat B-Chem*, **169**: 96-105.

Nasir F, Iqbal Z, Khan A, Ahmad L, Shah Y, Khan AZ, Khan JA and Khan S (2011). Simultaneous determination of timolol maleate, rosuvastatin calcium and diclofenac sodium in pharmaceuticals and physiological fluids using HPLC-UV. *J. Chromatogr. B*, **879**(30): 3434-3443.

Naveed S and Qamar F (2014). Comparative study of different brands of Alprazolam. *Glob. J. Med. Res*, **14**(3): 25-28.

Nunes CN, Pauluk LE, Dos Anjos VE, Lopes MC and Quináia SP (2015). New approach to the determination of contaminants of emerging concern in natural water: study of alprazolam employing adsorptive cathodic stripping voltammetry. *Anal. Bioanal. Chem.,* **407**: 6171-6179.

Pavan Kumar VV, Vinu MCA, Ramani AV, Mullangi R and Srinivas NR (2006). Simultaneous quantitation of etoricoxib, salicylic acid, valdecoxib, ketoprofen, nimesulide and celecoxib in plasma by high-performance liquid chromatography with UV detection.

Biomed. Chromatogr, **20**(1): 125-132.

Rao RN, Meena S and Rao AR (2005). An overview of the recent developments in analytical methodologies for determination of COX-2 inhibitors in bulk drugs, pharmaceuticals and biological matrices. *J. Pharmaceut. Biomed.*, **39**(3-4): 349-363.

Reddy AVB, Venugopal N and Madhavi G (2014). A selective and sensitive LC-MS/MS method for the simultaneous determination of twopotential genotoxic impurities in celecoxib. *J Anal. Sci. Technol*, **5**(18):1-8

Saha RN, Sajeev C, Jadhav PR, Patil SP & Srinivasan N (2002). Determination of celecoxib in pharmaceutical formulations using UV spectrophotometry and liquid chromatography. *J. Pharmaceut. Biomed.*, **28**(3-4): 741-751.

Samms WC, Jiang YJ, Dixon MD, Houck SS and Mozayani A (2011). Analysis of Alprazolam by DART-TOF mass spectrometry in counterfeit and routine drug identification cases. *J. Forensic. Sc.,* **56**(4): 993-998.

Soriano T, Jurado C, Menendez M and Repetto M (2001). Improved solid-phase extraction method for systematic toxicological analysis in biological fluids. *J. Anal. Toxicol.*, **25**(2): 137-143.

Souza RL de and Tubino M (2005). Spectrophotometric determination of diclofenac in pharmaceutical preparations. *J. Brazil. Chem. Soc.*, **16**(5): 1068-1073.

Sparidans RW, Lagas JS, Schinkel AH, Schellens JHM and Beijnen JH (2008). Liquid chromatography-tandem mass spectrometric assay for diclofenac and three primary metabolites in mouse plasma. *J Chromatogr B,* **872**(1-2): 77-82.

Störmer E, Bauer S, Kirchheiner J, Brockmoller J and Roots I (2003). Simultaneous determination of celecoxib, hydroxycelecoxib, and carboxycelecoxib in human plasma using gradient reversed-phase liquid chromatography with ultraviolet absorbance detection. *J. Chromatogr. B,* **783**(1): 207-212.

Toyo'oka T, Kumaki Y, Kanbori M, Kato M and Nakahara Y (2003). Determination of hypnotic benzodiazepines (alprazolam, estazolam and midazolam) and their metabolites in rat hair and plasma by reversed-phase liquid-chromatography with electrospray ionization mass spectrometry. *J. Chromatogr. Biomed.*, **30**(6): 1773-1787.

Yu K, Little D, Plumb R and Smith B (2006). High-throughput quantification for a drug mixture in rat plasma--a comparison of Ultra Performance™ liquid chromatography/tandem mass spectrometry with high-performance liquid chromatography/tandem mass spectrometry. *Rapid Commun. Mass. Sp.*, **20**(4): 544-552.