Development and Validation of a Simple and Efficient RPLC Method for Analysis of Captopril, Metformin, Pioglitazone and Glibenclamide in API, Formulations and Human Serum

Najma Sultana¹, Safila Naveed^{1,2*} and Saeed Arayne M³

¹Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Karachi, Karachi, Pakistan

²Faculty of Pharmacy, Jinnah University for Women, Karachi, Pakistan

³Department of Chemistry, University of Karachi, Karachi, Pakistan

Abstract

The association between the use of ACE inhibitors and the incidence of hypoglycemia is controversial. A recent study reported that 14% of all hospital admissions for hypoglycemia might be attributable to ACE inhibitors. In this paper, a novel, precise, specific, accurate and rapid reversed-phase high performance liquid chromatographic method was developed, optimized and validated for determining captopril and hypoglycemic (metformin, pioglitazone and glibenclamide) in bulk, pharmaceutical formulations and human serum with the best chromatographic peak resolution, reduced run time and low cost of analysis. The method was validated according to the US Food and Drug Administration (FDA) and ICH guidelines for the parameters: specificity, stability, limits of detection (LOD), limits of quantification (LOQ), linearity, accuracy, precision and recovery. This method showed the best resolution by using Hypersil ODS, C18 (150×4.6 mm, 5 micron) column using mobile phase, methanol: water (70: 30 v/v) adjusted to pH 3 via ortho phosphoric acid 85% with flow rate of 1 mLmin⁻¹ at ambient temperature and wavelength of 230 nm. The signal-to-noise ratio (S/N) was employed as a quality measurement. This tool permits to establish the influence of some selected factors (methanol: water ratio, pH, and flow rate) on two responses (peak areas and retention time). The LOD and LOQ values for CAP, MET, PGL and GLB were found to be 2.3, 1.5, 2.3 and 2.3 and 0.7, 0.4, 0.7, and 0.7 µg mL⁻¹ respectively. Calibration curves were linear in the concentration range of 2.5-100 µg mL⁻¹ for hypoglycemic and captopril with regression coefficient (*r*²) value of 0.999 for all drugs. The data for accuracy, precision and recovery were within the FDA limits. Intra ad inter-day precision and accuracy results were 98.0 to 102%. Retention time for captopril was found to be 3.3 minute and for metformin, pioglitazone and glibenclamide 2.4, 2.8, 7.2 minutes respectively. Proposed method was selective, precise and accurate short time analysis therefore can be used for routine, quality control and clinical study.

This is the first full report of a method for the simultaneous determination of these four drugs: captopril, metformin, pioglitazone and glibenclamide in API, formulations and serum. The newly developed method is useful for future routine analysis of these drugs and could be used in therapeutic drug monitoring and adherence to medicine studies, which would be helpful in decision making regarding treatment change in combination therapies.

Keywords: Captopril; Metformin; Glibenclamide; Pioglitazone; RP-HPLC determination

Introduction

Captopril (Figure 1) is 1-[(2S)-3-mercapto-2-methyl-1-oxo-propionyl]-L-proline is an orally active inhibitor of the angiotensin-converting enzyme and widely used for the treatment of hypertensive diseases on its own or in combination with other drugs [1]. ACEs lower the blood pressure in hypertensive patients as well as in salt-depleted normotensive patients. ACE inhibitors are highly selective drugs, do not interact directly with other components of the rennin angiotensin system, and the principle pharmacological and clinical effects of ACE inhibitors seen to arise from suppression of synthesis of angiotensin II [2]. ACE inhibitors produce vasodilation by inhibiting the formation of angiotensin II. This vasoconstrictor is formed by the proteolytic action of renin (released by the kidneys) acting on circulating angiotensinogen to form angiotensin I which is then converted to angiotensin II by angiotensin converting enzyme. ACE inhibitors, by blocking the breakdown of bradykinin, increase its levels, which can contribute to the vasodilator action of ACE inhibitors.

Metformin hydrochloride (Figure 1) chemically, N, N dimethyl-imido dicarbonimidic diamide hydrochloride is an antidiabetic agent from biguanide class used in the management of type 2 diabetes. It decrease hepatic glucose production and intestinal absorption of

glucose and improves insulin sensitivity by increasing peripheral glucose uptake and utilization, its predominant effect is to decrease fasting plasma glucose.

Pioglitazone hydrochloride (Figure 1) (±)-5-[p-[2-(5-ethyl-2-pyridyl)-ethoxy]benzyl]-2,4-thiazolidinedione hydrochloride is an oral antidiabetic agent used in the treatment of type 2 diabetes mellitus. PIO decreases insulin resistance in the periphery, liver and resulting in increased insulin-dependent glucose disposal and decreased hepatic glucose output.

Glibenclamide (Figure 1), 5-chloro-N-[2-4[[[(cyclohexylamino)

***Corresponding author:** Safila Naveed, Faculty of Pharmacy, Jinnah University for Women, Karachi, Pakistan, E-mail: safila117@yahoo.com, safila117@gmail.com

Received June 11, 2013; Accepted July 15, 2013; Published July 19, 2013

Citation: Sultana N, Naveed S, Saeed Arayne M (2013) Development and Validation of a Simple and Efficient RPLC Method for Analysis of Captopril, Metformin, Pioglitazone and Glibenclamide in API, Formulations and Human Serum. Pharm Anal Acta 4: 257. doi:10.4172/2153-2435.1000257

Copyright: © 2013 Sultana N, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

carbonyl]-amino]sulphonyl]-henyl]ethyl]-2-methoxy benzamide is oral sulfonylurea antidiabetic agent widely used to lower blood glucose levels in patients with type 2 diabetes mellitus. It acts by inhibiting ATP-sensitive potassium channels in pancreatic beta cells and this inhibition causes cell membrane depolarization, which cause voltage dependent calcium channels to open and increase in intracellular calcium in the beta cell, which stimulates insulin release.

For many patients with type 2 diabetes, mono therapy with an oral antidiabetic agent is not sufficient and the fixed dose combination of metformin, pioglitazone, and glibenclamide showed significant efficacy in improving the glycemic control in type 2 diabetics. Angiotensin converting enzyme inhibitors have been shown to be effective antihypertensive drugs for both patients with diabetes and those without diabetes. Diverse classes of antihypertensive prescription may be used for blood pressure manage in diabetes among these calcium channel blockers, thiazide diuretics and angiotensin converting enzyme inhibitors are common [3]. Various HPLC methods have been reported for estimation of captopril [4-6]. Several methods have also been developed for investigation of anti diabetics with other drugs [7-11]. Our research group has reported a number of methods for the simultaneous determination of these drugs as glipizide and glimepiride [12] and now this method has been used for interaction studies. Metformin has been determined along with captopril, lisinopril and enalapril [13], gliquidone pioglitazone hydrochloride and verapamil has been simultaneously determined [14] and gliquidone, pioglitazone hydrochloride and atorvastatin in formulations and human serum [15]. We have also worked on the interaction of a number of co-administered drugs as interaction of captopril and H₂-receptor antagonists [16,17].

Combinations of metformin with glipizide, gliclazide or glibenclamide are available commercially as single dosage form. A combination tablet formulation is beneficial in terms of its convenience and patient's compliance. The most important purpose of this study was to assess the "in vitro" drug interactions of captopril with commonly prescribed antidiabetic drugs (metformin, glibenclamide and pioglitazone) by utilizing HPLC for the monitoring of diabetic patients who take combination medications and for studying the pharmacokinetics of the combined dosage forms.

Experimental

Materials and reagents

All chemicals and reagents were of analytical grade. Captopril

(purity 99.82%) was a kind gift from Bristol Meyers (Pvt) Limited, Pakistan. Metformin (purity 99.94%), glibenclamide (purity 99.97%) and pioglitazone (purity 99.79%) were gifts from Sanofi Aventis (Pakistan) Ltd, Safe Pharmaceutical (Pvt) Ltd and Ali Gohar Pharmaceuticals (Pvt) Limited, Pakistan. HPLC grade acetonitrile, methanol and phosphoric acid were obtained from Tedia (USA) and Merck Darmstadt, Germany.

Pharmaceutical dosage form

Capoten™ (Captopril 25 mg tablets by Bristol Meyers (Pvt) Ltd), Neodipa™ (metformin 250 mg tablets by Sanofi Aventis (Pvt) Ltd), Diaze™ Glibenclamide 5mg from Safe Pharmaceutical (Pvt) Ltd and Poze™ (45 mg mg tablets from Ali Gohar pharmaceuticals Pakistan Limited), were purchased from the local pharmacies. All these drugs had an expiry of not less than 1 year at the time of study.

Instrumentation

A Shimadzu HPLC system equipped with LC- 10 AT VP pump and SPD-10 A VP UV-VIS detector and other HPLC system was equipped with LC-20AT and SPD-20A UV/VIS detector was utilized. Chromatographic system was integrated via Shimadzu model CBM-102 to P-IV computer loaded with Shimadzu CLASS-VP software (Version 5.03) for data acquisition and mathematical calculations. Rheodyne manual injector fitted with a 20 µL loop, Hypersil, ODS, C18 (150×4.6 mm, 5 micron) and Purospher® STAR RP-18 column and DGU-14 AM on-line degasser. In addition, Mattler Toledo electronic balance, micro liter syringe and micro pore filtration assembly were used in this study.

Preparation of standard and sample solutions

Standard preparation: Stock standard solutions 100 ppm of CAP, MET, PGL and GLB were prepared in 100 mL mobile phase as solvent. Working solutions were prepared separately by making serial dilutions from the standard solutions to obtain concentration between 2.5-100 µg mL⁻¹ for hypoglycemics and captopril. These solutions were stored at 20°C. Once prepared, analyzed daily for inter and intra-day variations of the method. 20 µL of these solutions were injected into LC system and chromatographed.

Preparation of solutions: Standard solutions of captopril and anti diabetic drugs were prepared by dissolving appropriate amounts of each in mobile phase methanol: water (70:30v/v, pH 3) to obtain final drug concentrations of 100 µg mL⁻¹. For the calibration standards, seven calibrators of each drug were prepared by making serial dilutions from stock solutions. For the assay preparation the content of 20 tablets were powdered weighed portion of the powder equivalent to the suitable amount of drug (according to the labeled claimed) was transferred into a 50 mL volumetric flask. The drug was fully dissolved in mobile phase and then diluted with this solvent up to the mark, seven dilutions of each drug were prepared portion of this solution was filtered through a disposable 0.45 µm filter and then injected.

Serum drug analysis: Blood samples were collected from healthy volunteers and after coagulation centrifuged at 3000 rpm for 10 minutes. The supernatant (serum) obtained was stored at -20°C. After thawing, serum was deproteinated by acetonitrile and spiked daily with working solutions to produce desired concentrations of enalapril and anti diabetic drugs. 20 µL volume of each sample was injected and chromatographed under above conditions.

Chromatographic conditions: The chromatographic analysis was performed at ambient temperature with isocratic elution. The mobile

phase consisted of methanol: water (70:30 v/v) with pH adjusted to 3 with phosphoric acid (85%). The pump was set at a flow rate of 1.0 mL min⁻¹, sample volume of 20 µL was injected in triplicate onto the HPLC column and elute was monitored at 230 nm. Optimal retention times for captopril, metformin, Pioglitazone and Glibenclamide was found to be 3.3 2.4, 2.8, 7.2 min respectively.

Results and Discussion

Development of HPLC methods for the determination of drugs has received considerable attention in recent years because of their importance in routine quality control analysis. HPLC methods generally require provision for use and disposal of solvents, labor-intensive sample preparation procedure and personal skilled in chromatographic techniques. The goal of this study was to develop a rapid, more accurate, precise reliable least time consuming HPLC method for the simultaneous determination of captopril and anti diabetic drugs in the form of bulk drug samples, its formulations and human serum using the most commonly employed C-18 column with UV detector.

Method development

In the present investigation the best separation of captopril and antidiabetic drugs was achieved using a Hypersil, ODS, C18 (150×4.6 mm, 5 micron) column which provides efficient and reproducible separation of the components. Using other type of column under similar experimental condition, the separation lasted about 8 minutes. A mobile phase of methanol: water (70:30 v/v) having pH adjusted with phosphoric acid to 3 provided a reproducible, baseline resolved peak. Small changes in pH of the mobile phase had a great influence to the chromatographic behavior of these substances, higher pH of the mobile phase also results in peak tailing and at a lower pH retention time of antidiabetic drugs and captopril was delayed. It is obvious from the chromatogram (Figure 2 and 3) that antidiabetic drugs and captopril eluted out forming symmetrical peaks and were well separated from each other. The method was found to be rapid as the drugs separated in a very short time i.e. captopril 3.3 mins and metformin, pioglitazone and glibenclamide 2.4, 2.8, 7.2 minutes respectively. The advantages of this method are ease of operation, short analysis time (total run time < 8 minutes), utilization of readily available cost-effective solvents, no matrix interferences and satisfactory limit of quantification to enable pharmacokinetic studies of captopril and hypoglycemic drugs. Rapidness, sensitivity, simplicity, economical nature, acceptable resolution, good recovery and precision of this method give it an advantage over the other reported HPLC methods for the determination of captopril and (non insulin dependent diabetes mellitus drugs) NIDDMs drugs.

Method validation

The newly developed method has been validated and holds well for the determination of drug in raw materials, dosage formulations and serum. For validation of analytical methods, the guidelines of the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use [16] have recommended the accomplishment of selectivity, specificity, linearity, accuracy test, precision, sensitivity, limit of detection and quantification of the method.

Selectivity and specificity

The specificity of the chromatographic method was determined to ensure separation of captopril and antidiabetic as shown in Figure

3. Specificity was also determined by screening four different samples of controlled human serum, which were free from interfering endogenous plasma components. Solutions of placebo, captopril and antidiabetic were prepared and then injected to check for interference from common exceptions (Table 1).

Range and linearity

Linearity is generally reported as the variance of the slope of the regression line. Linearity was tested with known concentrations of captopril, metformin, Pioglitazone and Glibenclamide i.e. 2.5, 5, 10, 25,

Figure 2: A representative chromatogram of (a) metformin (b) pioglitazone (c) captopril and (d) glibenclamide in API.

Figure 3: A representative chromatogram of (a) metformin (b) pioglitazone (c) captopril and (d) glibenclamide in formulation.

Figure 4: A representative chromatogram of (a) metformin (b) pioglitazone (c) captopril and (d) glibenclamide in serum.

50 and 100 $\mu\text{g mL}^{-1}$, respectively. Five runs were performed for every concentration. Injected concentrations versus area were plotted and the correlation coefficients were calculated which are shown in Table 2.

Accuracy and recovery

The accuracy of an analytical procedure measures the closeness of measured values to the true values. It was evaluated as percentage relative error between the measured mean concentrations and taken concentrations [17,18]. Minimal of three concentration levels covering the specified ranges were selected and three runs were performed for every concentration and then peak area was calculated as given in Table 3.

Precision

The intra- and inter-day precision was evaluated by assaying the samples (Table 4). In this assay, the intra-day precision and the inter-day precision recovery was 98-102% in bulk materials and in human serum (Figure 4). Intra and interday precision was performed and % RSD was found to be less than 2 which indicate that the method was sufficiently accurate and precise.

Robustness

To evaluate robustness of the developed RP-HPLC method, small deliberate variations in the optimized method parameters were done. The effect of change in flow rate, pH and mobile phase ratio on the

Analytes	Retention time (T_R) (min)	Capacity factors (K')	Theoretical plates (N)	Tailing factor (T)	Resolution (R)	Separation factor
CAP	3.3	2.13	3200	1.23	3.4	2.48
MET	2.4	2.25	3250	1.25	3.5	2.36
PGL	2.8	2.36	3250	1.36	3.6	2.59
GLB	7.2	2.36	3246	1.69	3.3	2.56

captopril (CAP), metformin (MET), Pioglitazone (PGL) and Glibenclamide (GLB)

Table 1: System suitability parameters.

Drugs	Conc. $\mu\text{g mL}^{-1}$	Regression Equation	r^2	LLOD $\mu\text{g mL}^{-1}$	LLOQ
CAP	2.5-100	$A = 2501.7x + 3073.7$	0.9995	0.7	2.3
MET	2.5-100	$A = 3841.3x + 4744.2$	0.9998	0.4	1.5
PIO	2.5-100	$A = 2419.8x + 2988.8$	0.9995	0.7	2.3
GLB	2.5-100	$A = 2419.8x + 2988.8$	0.9995	0.7	2.3

captopril (CAP), metformin (MET), Pioglitazone (PGL) and Glibenclamide (GLB)

Table 2: Regression characteristics.

Analyte	Assay (spiking method)			Assay in serum	
	Conc. $\mu\text{g mL}^{-1}$	%RSD	% Rec	%RSD	%Rec
CAP	8	0.01	99.98	0.002	102
	10	0.33	100.04	0.02	101
	12	0.36	99.97	0.03	101
MET	8	0.01	100	0.002	101
	10	0	100.02	0.002	101.3
	12	0.3	99.98	0.02	100.3
PGL	8	0.22	99.3	0.03	100.2
	10	0.4	99.98	0.036	100.6
	12	99.73	79.79	0.3	101.3
GLB	8	0.01	99.73	0.06	101.3
	10	0.3	100.24	0.05	101.6
	12	0.5	100.06	0.06	102.0

Table 3: Accuracy of captopril and antidiabetic drugs.

Drugs	Conc. injected $\mu\text{g mL}^{-1}$	Inter-day		Intra-day	
		%RSD	%Rec	%RSD	%Rec
CAP	2.5	0.0073	101.11	0.073	101.11
	5	0.0109	102.36	0.009	102.36
	10	0.3261	100	0.361	100.02
	25	0.0009	100	0.09	100.03
	50	0.0005	99.826	0.005	99.26
	100	0.0002	99.998	0.002	99.98
MET	1.25	0.0047	99.997	0.047	99.9
	2.5	0.0071	99.988	0.071	99.88
	5	0.0024	100.12	0.024	100.1
	10	0.0006	99.983	0.006	99.93
	25	0.0006	99.968	0.006	99.98
	50	0.0003	99.991	0	99.91
PGL	1.25	0.0075	98.72	0.007	98.72
	2.5	0.0075	99.98	0.075	99.98
	5	0.0019	99.73	0.019	99.73
	10	0.0012	99.97	0.012	99.87
	25	0.0005	99.97	0.005	99.87
	50	0.0003	100.02	0.003	100.1
GLB	1.25	0.008	100.02	0.08	100.2
	2.5	0.008	99.783	0.008	99.7
	5	0.002	99.983	0.002	99.9
	10	0.001	99.972	0.001	99.9
	25	0.05	99.996	0.05	99.9
	50	0.03	99.997	0.03	99.9

Table 4: Inter day and intraday precision of captopril and NIDDM drugs.

	Level	K'	T	(R_s)
A: pH of mobile phase				
2.6	-0.2	2.8	1.22	3.4
2.8	0	2.13	1.23	3.4
3	0.2	2.2	1.4	3.2
Mean $\pm$ S.D (n=6)		2.13 ± 0.3	1.23 ± 0.020	3.4 ± 0.1
B: Flow rate (mLmin ⁻¹)				
0.8	-0.2	2.1	1.45	3.32
1	0	2.3	1.44	3.36
1.2	0.2	2.4	1.42	3.37
Mean $\pm$ S.D (n=6)		4.3 ± 0.212	1.44 ± 0.015	3.36 ± 0.026
C: Percentage of water in mobile phase (V/V)				
25	-5	2.6	1.42	3.38
30	0	2.3	1.43	3.36
35	5	2.5	1.46	3.33
Mean $\pm$ S.D (n=6)		2.36 ± 0.070	1.43 ± 0.025	3.36 ± 0.025
C: Wavelength (nm)				
225	-5	2.5	1.42	3.38
230	0	2.3	1.43	3.36
235	5	2.4	1.45	3.32
Mean $\pm$ S.D (n=6)		2.3 ± 0.070	1.43 ± 0.015	2.36 ± 0.030

Table 5: Robustness of the method (n=6).

retention time and tailing factor were studied. The method was found to be unaffected by small changes like ± 0.2 change in pH, ± 0.2 change in flow rate and ± 5 change in mobile phase (Table 5).

Ruggedness

Ruggedness was established by determining CAP, MET, PGL and GLB using same and different chromatographic systems and using two different columns Hypersil, ODS, C18 (150 $\times$ 4.6mm, 5micron) and Purospher® STAR RP-18 column and Hypersil, ODS, gave the best

HPLC System----->		LC 10			LC 20	
Drugs	Columns	Conc. ($\mu\text{g mL}^{-1}$)	Conc. Found ($\mu\text{g mL}^{-1}$)	Recovery (%)	Conc. Found ($\mu\text{g mL}^{-1}$)	Recovery (%)
CAP	Purospher STAR	30	30.03	100.1	30.03	100.1
		40	40.9	102.25	40.03	100.07
		50	50	100.0	50	100.0
	Hypersil, ODS	30	30.09	100.3	30.04	100.13
		40	40	100.0	40	100.0
		50	50.03	100.06	50.03	100.06
MET	Purospher STAR	30	30.09	100.3	30.04	100.13
		40	40	100.0	40	100.0
		50	50.02	100.04	50.02	100.04
	Hypersil, ODS	30	30.04	100.133	30.04	100.13
		40	40.33	100.825	40.34	100.85
		50	50.23	100.46	50.23	100.46
PGL	Purospher STAR	30	30.22	100.73	30.22	100.73
		40	40.02	100.05	40.02	100.05
		50	50.09	100.18	50.09	100.18
	Hypersil, ODS	30	30.3	101.0	30.3	101.0
		40	40.09	100.22	40.09	100.22
		50	50.09	100.18	50.19	100.38
GLB	Purospher STAR	30	30.04	100.13	30.04	100.13
		40	40.09	100.22	40.09	100.22
		50	50.09	100.18	50.09	100.18
	Hypersil, ODS	30	30.09	100.3	30.06	100.20
		40	40.03	100.07	40.03	100.07
		50	50.06	100.12	50.06	100.12

Table 6: Ruggedness of the method.

results. The assay results indicated that the method was capable with high precision (Table 6).

Lower limit of detection and quantification

The lower limit of quantitation (LLOQ) of the method as signal/noise of captopril, metformin, Pioglitazone and Glibenclamide were found to be 2.3, 1.5, 2.3 and 2.3 $\mu\text{g mL}^{-1}$ respectively. Similarly a signal/noise of 3, a LOD of captopril, metformin, Pioglitazone and Glibenclamide were determined to be 0.7, 0.4, 0.7, and 0.7 $\mu\text{g mL}^{-1}$ respectively.

Conclusion

The novel RP-HPLC method described in this paper provides a simple, universal, short time analysis and reproducible approach for the simultaneous identification and quantification that can be used to determine captopril and any of the three NIDDMs. In summary, the proposed method can be used for drug analysis in routine quality control. In addition, this method has the potential application to clinical research of drug combination, multi-drug pharmacokinetics and drug-drug interactions.

References

- Wilson CO, Gisvold O, Jaime ND, William AR (1991) Wilson and Gisvold's Text Book of Organic Medicinal and Pharmaceutical Chemistry. (9th edn) JB Lippincott Company, USA: 564.
- Myceh MJ (2000) Lippincott's Illustrated Reviews Pharmacology, (2nd edn), Lippincott William & Wilkins.
- Curtis T (2006) Drug interactions of medications commonly used in diabetes. *Diabetes Spectrum* 19: 202-211.
- Sultana N, Arayne MS, Naveed S (2010) Simultaneous Determination of Captopril and Statins in active pharmaceutical ingredients, Pharmaceutical Formulations and in Human Serum by RP-HPLC. *J Chin Chem Soc* 57: 378-383.
- Sultana N, Arayne MS, Naveed S (2010) Simultaneous Quantitation of Captopril and NSAIDs in API, Dosage Formulations and Human Serum by RP-HPLC. *Journal of the Chinese Chemical Society* 57: 62-67.
- Sultana N, Arayne MS, Naveed S (2011) RP-HPLC method for simultaneous determination of captopril and diuretics: application in pharmaceutical dosage forms and human serum. *Chromatography Separation Sciences*. 2 : 4.
- Jain D, Jain S, Jain D, Amin M (2008) Simultaneous estimation of metformin hydrochloride, pioglitazone hydrochloride, and glimepiride by RP-HPLC in tablet formulation. *J Chromatogr Sci* 46: 501-504.
- A K, G S, C MR, Bhat K, A R, et al. (2008) Simultaneous determination of pioglitazone and glimepiride in bulk drug and pharmaceutical dosage form by RP-HPLC method. *Pak J Pharm Sci* 21: 421-425.
- Venkatesh P, Harisudhan T, Choudhury H, Mullangi R, Srinivas NR (2006) Simultaneous estimation of six anti-diabetic drugs--glibenclamide, glipizide, pioglitazone, repaglinide and rosiglitazone: development of a novel HPLC method for use in the analysis of pharmaceutical formulations and its application to human plasma assay *Biomed Chromatogr* 20: 1043-1048.
- Al-Arfaj NA, Al-Abdulkareem EA, Aly FA (2008) A Validated Adsorptive Stripping Voltammetric Determination of Antidiabetic Agent Pioglitazone HCl in Tablets and Biological Fluids. *Int J Biomed Sci* 4: 310-318.
- Lakshmi KS, Rajesh T (2011) Separation and quantification of eight antidiabetic drugs on a high-performance liquid chromatography: its application to human plasma assay. *ISRN Pharm* 2011: 521353.
- Sultana N, Arayne MS, Ali SN, Zuberi MH (2012) Simultaneous determination of glipizide and glimepiride by RP-HPLC in dosage formulations and in human serum. *Medicinal Chemistry Research* 21: 2443-2448.
- Arayne MS, Zuberi MH, Siddiqui FA (2012) Simultaneous RP-HPLC method for the determination of metformin, captopril, lisinopril and enalapril: Its applications in dosage formulations and in human plasma. *Medicinal Chemistry Research* 21: 4542-4548.
- Arayne MS, Sultana N, Mirza AZ (2011) Simultaneous determination of glipizide, pioglitazone hydrochloride, and verapamil in formulation and human serum by RP-HPLC. *J Chromatogr Sci* 49: 114-117.
- Mirza AZ, Arayne MS, Sultana N (2013) RP-LC method for the simultaneous determination of glipizide, pioglitazone hydrochloride, and atorvastatin in formulations and human serum. *J AOAC Int* 96: 56-59.

-
16. Sultana N, Naveed S, Arayne MS (2013) RP-HPLC method for simultaneous determination of captopril and H₂-receptor antagonist: Application to interaction studies *Med Chem* 3: 183-187.
 17. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonised Tripartite Guideline; Validation of Analytical Procedures: Text and Methodology Q2(R 1), Complementary Guideline on Methodology dated 06 November 1996, incorporated in November 2005, London
 18. Shabir GA (2003) Validation of high-performance liquid chromatography methods for pharmaceutical analysis. Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. *J Chromatogr A* 987: 57-66.