

RP-HPLC Simultaneous Analysis of Glimepride and NSAIDs in Active Pharmaceutical Ingredient, Formulations and Human Serum

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ABSTRACT

The present work describes an innovative reversed phase High Performance Liquid Chromatographic (RP-HPLC) method for the determination of Non-steroidal anti-inflammatory drugs NSAIDs with glimepride antidiabetic drug simultaneously in dosage formulations, active pharmaceutical ingredients and human serum. The chromatographic system consisted of Sil-20A auto sampler, LC-20A pump and SPD-20A UV/visible detector. The quantification was carried out by using a C18 (5 μ m, 250 \times 4.6 mm) column with mobile phase methanol: water (80:20 v/v, pH 3.0) at 228 nm with a flow rate of 1.0 mL \cdot min⁻¹. The retention time of glimepride was 12 min while of diclofenac sodium, ibuprofen and mefenamic acid were found to be 6.0, 8.0 and 10.0 minutes respectively. The method developed was found to be precise, accurate and selective and was validated for accuracy, precision, linearity, specificity, limit of detection and limit of quantitation. There is no such method reported earlier for the determination of glimepride and NSAIDs simultaneously. It was required for assessing the co-administration of both drugs in treatment and can be employed for drug-drug interactions studies.

Keywords: Glimepride, NSAIDs, method validation, RP-HPLC.

INTRODUCTION*

Glimepride (GLP) is a novel oral anti-diabetic drug belonging to the class of sulfonylurea. Its mean advantages include complete bioavailability, effectiveness at low doses, linear pharmacokinetics, and depicting a prolonged effect[1]. GLP is specified for the management of diabetes mellitus (type 2); it increases the production of insulin by the pancreas[2]. The suggested initial dose of GLP tablets USP is 1 mg or 2 mg q.d. Similar to other members of sulfonylureas, GLP acts as an insulin secretagogue. It reduces blood sugar by inducing the release of insulin by pancreatic beta cells and by stimulating increased activity

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of intracellular insulin receptors. Its use is contraindicated in patients prone to allergic reactions with GLP or other sulfonylureas, and during pregnancy. Considerable absorption occurs within an hour and distribution is all over the body. The drug is 99.5% bound to plasma protein. The metabolism of GLP is by oxidative biotransformation. Around 65% of the drug is excreted in the urine and rest is excreted in the feces[3]. NSAIDs are a diverse group of compounds that are mostly used to relief fever, inflammation and pain[4]. They exert pharmacological action by limiting the synthesis of prostaglandins (PGs)[5]. In current study most frequently prescribed NSAIDs ibuprofen (IBF), diclofenac sodium (DCL), and mefenamic acid (MFN) were used. (Table 1)

HPLC methods for estimation of GLP in dosage formulations, active pharmaceutical ingredients and human plasma had already been reported [6-7]. A number of methods have also been reported for the determination of NSAIDs [8-9]. To the best of authors' knowledge simultaneous estimation of GLP and NSAIDs using HPLC in dosage formulations, active pharmaceutical ingredients and human serum has not been reported earlier. The present study was conducted with the aim to focus on the development and validation of an accurate, rapid as well as cost effective method for the quantification of GLP and NSAIDs by RP-HPLC method in dosage formulations, active pharmaceutical ingredients and human serum. The developed method was validated as per the ICH (Q2A 1995) guiding principles[10].

EXPERIMENTAL:

Materials:

Tablet formulations of all active pharmaceutical ingredients (APIs) were purchased from local pharmacy. The expiry date of all drugs was evaluated at the time of study. Serum was collected from a healthy person at Fatmid Foundation Karachi. All other reagents used were of HPLC grade.

Equipment:

HPLC system (LC 20A, Shimadzu Corp., Japan), Communication Bus Module (CBM 102, Shimadzu Corp., Japan), pump (LC 20A, Shimadzu Corp., Japan), UV-spectrophotometric detector (SPD-20A, Shimadzu Corp., Japan), Class GC software (GC 10, 5.03), Spalco® C18 (5 µm, 250 × 4.6 mm) and C18 (Purospher® Star, 5 µm, 250 × 4.6 mm). UV-visible spectrophotometer (Shimadzu 1800) was also used for the evaluation of isosbestic and individual point of analytes.

Preparation of reference solution:

Ten mg of each API (GLP, DCL, MFN and IBF) was used to prepare 100 mL stock solutions (100 µg/mL) separately. The diluent was used (80:20, methanol: water). Working solutions were further diluted in range of 0.5-50 µg/mL for GLP and 0.25-25 µg/mL for DCL,IBF and MFN.

Preparation of sample solutions from tablets:

For extraction of each active moieties of GLP®, MFN®, DCL® and IBF®, crushed samples of twenty tablets were used separately and then equivalent powder quantity (10 mg) was dissolved in methanol to extract out drugs. Intermittent sonication was performed during 1 hour resting period for each sample. Final solutions were filtered and then serially diluted to prepare working standards in the range of 0.5-50 µg/mL for GLP and 0.25-25 µg/mL for NSAIDs. Diluted solution were further passed through 0.45 micron filter paper and then injected.

Preparation of serum solutions of drugs:

Separation of plasma was performed by shaking and centrifugation (10,000 rpm for 10 min); 1:9 v/v ratio of ACN was used for deproteination with repeated centrifugation at same speed. Supernatant was collected, filtered, stored (refrigerated) at -20 °C 10 and then evaluated for analyte concentration in the range of 0.5-50 µg/mL for GLP and 0.25-25 µg/ mL for NSAIDs.

Chromatographic Conditions:

The mobile phase used was methanol: water (80:20 v/v) (pH 3). It was filtered before use through 0.45µ membrane filter. The flow rate of pump was set at 1ml/min. The assay was carried out on a C18 column at a temperature maintained at 25°C. The sample of 20µl was injected and analyzed under isocratic conditions. Chromatograms were recorded at λ = 228 nm using SPD- 10A VP Shimadzu UV-VIS detector.

Table 1. Chemical structure of GLP and NSAIDS

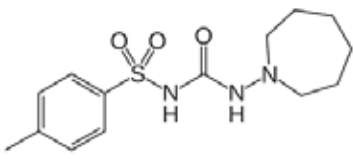
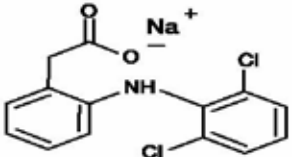
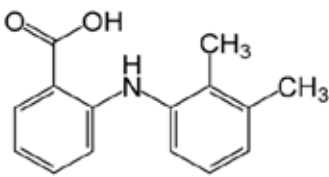
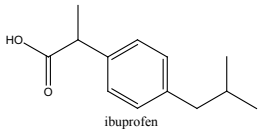
Glimepride		[[p-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-oxamide)ethyl] phenyl] sulfonyl]-3-(trans-4-methylcyclohexyl) urea,
Diclofenac sodium		2-[(2,6-dichlorophenyl)amino] benzene acetic acid monosodium salt
Mefenamic acid		2-[(2,3-dimethyl phenyl amino] benzoic acid and N-(2,3-xylonyl) anthranilic acid
Ibuprofen		(2RS)-2-[4-(2-methylpropyl) phenyl]propanoic acid, C ₁₃ H ₁₈ O ₂

Table 2. Regression equations with LOD, LOQ.

Drugs	r ²	Regression Equation	Conc. (µg mL ⁻¹)	LOD (µg mL ⁻¹)	LOQ (µg mL ⁻¹)
Glimepride	0.999	y=15438x + 3999	2.5-80	0.02	0.04
Diclofenac sodium	0.989	A=34472 x + 643.21	2.5-80	0.02	0.04
Ibuprofen	0.979	A=7784.8x + 10539	2.5-80	0.01	0.03
Mefanamic acid	0.989	A=33176 x + 20209	2.5-80	0.04	0.06

LOD = limit of detection, LOQ = limit of quantification, r² = correlation coefficient.

Table 3. Accuracy of GLP and NSAIDs.

Drugs	Conc ppm	Con %	% Recovery	RSD
Glimepride	30	90	99.06	0.1
	40	100	100.90	0.9
	50	110	99.98	0.8
Diclofenac sodium	30	90	99.09	0.8
	40	100	100.09	0.8
	50	110	101.00	0.7

Drugs	Conc ppm	Con %	% Recovery	RSD
Ibuprofen	30	90	100.93	0.6
	40	100	102.00	0.5
	50	110	100.00	0.9
Mefanamic acid	30	90	98.85	0.1
	40	100	100.96	0.8
	50	110	98.96	0.4

Table 4: Inter day and intraday precision of GLP and NSAIDs

	Conc. injected $\mu\text{g mL}^{-1}$	Inter-day		Intra-day	
		RSD	%Recovery	RSD	%Recovery
Glimepride	2.5	1.08	100.02	1.41	100.0
	10	1.19	100.06	1.21	100.1
	20	1.41	100.17	1.21	100.2
	40	1.43	99.99	1.31	100.0
	80	1.45	100.10	1.41	1001.7
Diclofenac sodium	2.5	1.61	99.88	1.44	99.9
	10	1.91	99.94	1.42	99.9
	20	1.41	99.99	1.40	100.0
	40	1.43	99.99	1.48	100.0
	80	1.44	99.99	1.47	100.0
Ibuprofen	2.5	1.45	101.12	1.42	101.1
	10	1.31	100.03	1.41	100.0
	20	1.31	100.01	1.33	100.0
	40	1.29	99.91	1.55	99.9
	80	1.21	100.01	1.45	100.0
Mefanamic acid	2.5	1.51	99.72	1.40	99.7
	10	1.41	99.86	1.47	99.9
	20	1.51	100.03	1.46	100.0
	40	1.61	99.93	1.43	99.9
	80	1.01	100.02	1.47	100.0

Method Development:

With the intention of selecting an appropriate mobile phase for the determination of GLP and NSAIDs isocratic elution was applied. The analytical procedure was optimized by altering the flow rate, composition of mobile phase and pH of the mobile phase. Methanol and water

were the mobile phases examined in the study. Optimal retention time for GLP was 12 min and for IBF, DCL and MFN was found to be 8.0, 6.0, 5 and 10.0 min respectively. The optimum resolution was achieved by using mobile phases methanol: water (80:20 v/v) having pH adjusted to 3.0 with phosphoric acid.

RESULTS AND DISCUSSION:

The projected HPLC method involved fewer materials and reagents, simple, less time consuming and may be employed in quality control evaluation in pharmaceutical industries. The chromatograms of GLP and NSAIDs were shown in Figure 1, 2.

Validation procedure:

Present study was conducted to obtain an innovative, simple and affordable method for the estimation of GLP and NSAIDs. The HPLC method development and validation was performed according to the official specifications of Centre of Drug Evaluation and Research (CDER-1994), International Conference on Harmonization and United State Pharmacopeias (ICH, 1997, USP, 2008) [11]. The method validation parameters included system suitability, linearity, accuracy, specificity, limit of detection, limit of quantification, precision and robustness.

System Suitability and Selectivity:

The selectivity of the chromatographic method was determined as it is the significant basis for analytical procedures. The method depicted good resolutions and no interference of excipients employed in products. Hence, the method is particular for GLP and NSAIDs (Figure 1, 2).

Linearity:

A linear relationship must exist over the range of the analytical procedure. The range of an analytical method lies between the highest and lowest analytical concentrations of a sample.

To evaluate the linearity, limit of detection and limit of quantification (LOQ) of the method, serial dilutions of reference drugs were prepared from the standard stock solution (1000 μ g/ml) and resolved in a C18 column with UV detector at 228 nm (Table 2).

Accuracy and Recovery:

Percentage recovery of active drug in pharmaceutical dosage form was evaluated for further validation by preparing known amount of drugs at three concentration levels (in triplicate) and injecting to HPLC (Table 3).

Precision:

The precision interday and intraday reproducibility of the method was established. The intra-and inter-batch precision was estimated by examining the samples (Table 4). The values obtained were within the acceptable range and the method was sufficiently accurate and precise.

Robustness:

For evaluating the robustness of the method, the procedure was repeated. Robustness studies performed on method precision, using a sample concentration by creating slight variations in flow rate, change in pH, detection wave length and proportion of methanol.

Quantification limit:

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method was determined by injecting progressively low concentrations of the standard solutions using the developed methods. The LOD of GLP, IBF, DCL and MFN were found to be 0.02, 0.01, 0.02 and 0.04 μ g/ml respectively. The LOQ of GLP, IBF, DCL and MFN were found to be 0.04, 0.03, 0.04 and 0.06 μ g/ml respectively (Table 2).

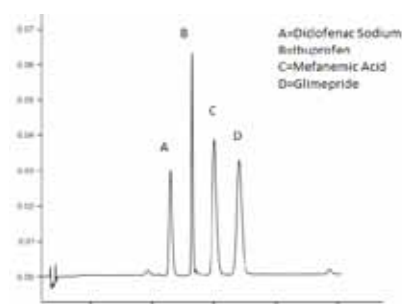


Fig. 1. Chromatogram of diclofenac sodium (6 min) ibuprofen and (8min), mefenamic acid(10 min) and Glimepride (12 min)in API

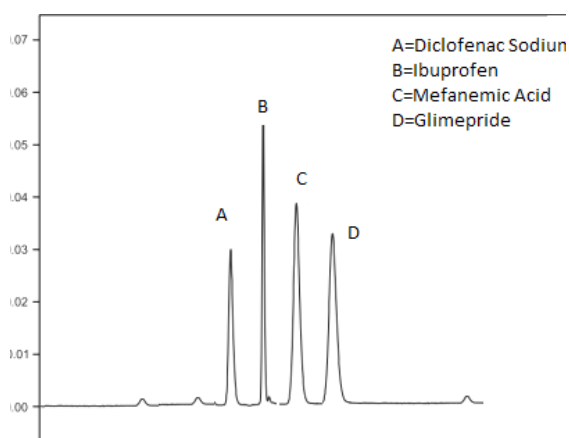


Fig. 2. Chromatogram of diclofenac sodium (6 min) ibuprofen and (8min), mefenamic acid(10 min) and Glimepiride (12 min) in human serum

CONCLUSIONS

The HPLC method developed was an innovative, rapid, accurate and can be employed for the determination of NSAIDs with GLP simultaneously for the analysis in regular quality control evaluation. Additionally, this method is likely to be applied in clinical research of multi-drug combination, pharmacokinetics and interactions.

ACKNOWLEDGMENT

None

REFERENCES:

1. Jain D, Jain S, Jain D, Amin M. Simultaneous estimation of metformin hydrochloride, pioglitazone hydrochloride, and glimepiride by RP-HPLC in tablet formulation. *Journal of chromatographic science*. 2008; 46(6):501-4.
2. Cheng AY, Fantus IG. Oral anti-hyperglycemic therapy for type 2 diabetes mellitus. *Canadian Medical Association Journal*. 2005; 172(2):213-26.
3. Nissen SE, Nicholls SJ, Wolski K, Nesto

R, Kupfer S, Perez A, et al. Comparison of pioglitazone vs glimepiride on progression of coronary atherosclerosis in patients with type 2 diabetes: the PERISCOPE randomized controlled trial. *Jama*. 2008;299(13):1561-73.

4. Qandil AM. Prodrugs of nonsteroidal anti-inflammatory drugs (NSAIDs), more than meets the eye: a critical review. *International journal of molecular sciences*. 2012;13(12):17244-74.
5. Sultana N, Arayne MS, Naveed S. Simultaneous Quantitation of Captopril and NSAID's in API, Dosage Formulations and Human Serum by RP-HPLC. *Journal of the Chinese Chemical Society*. 2010;57(1):62-7.
6. Chakradhar L, Kallem R, Karthik A, Sundari BT, Ramesh S, Mullangi R, et al. A rapid and highly sensitive method for the determination of glimepiride in human plasma by liquid chromatography-electrospray ionization tandem mass spectrometry: application to a pre-clinical pharmacokinetic study. *Biomedical Chromatography*. 2008;22(1):58-63.
7. Salem II, Idrees J, Al Tamimi JJ. Determination of glimepiride in human plasma by liquid chromatography-electrospray ionization tandem mass spectrometry. *Journal of chromatography B*. 2004;799(1):103-9.
8. Sultan M, Stecher G, Stoggl WM, Bakry R, Zaborski P, Huck C, et al. Sample Pretreatment and Determination of Non Steroidal Anti-Inflammatory Drugs (NSAIDs) in Pharmaceutical Formulations and Biological Samples (Blood, Plasma, Erythrocytes) by HPLC-UV-MS and μ -HPLC. *Current medicinal chemistry*. 2005;12(5):573-88.
9. Ibrahim H, Boyer A, Bouajila J, Couderc F, Nepveu F. Determination of non-steroidal

- anti-inflammatory drugs in pharmaceuticals and human serum by dual-mode gradient HPLC and fluorescence detection. *Journal of chromatography B*. 2007;857(1):59-66.
10. Guideline IHT. Validation of analytical procedures: text and methodology. Q2 (R1). 2005;1.
11. Görög S. Drug safety, drug quality, drug analysis. *Journal of pharmaceutical and biomedical analysis*. 2008;48(2):247-53.