

Simultaneous Determination of Gliquidone, Fexofenadine, Buclizine, and Levocetirizine in Dosage Formulation and Human Serum by RP-HPLC

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Abstract

In the present paper, a simultaneous method has been developed and validated for estimation of gliquidone in the presence of H₁-receptor antagonists (fexofenadine hydrochloride, buclizine hydrochloride, and levocetirizine dihydrochloride) using reversed-phase high-performance liquid chromatographic technique. A good chromatographic separation between these drugs was achieved using a mobile phase containing methanol–water (80:20 v/v) at pH 3.5 with a flow rate of 1.0 mL/min; and detection was performed at 230 nm with a UV detector. Validation of the method was performed in terms of linearity, accuracy, precision, and limit of detection and quantification. The linearity of the calibration curves for gliquidone, fexofenadine hydrochloride, buclizine hydrochloride, and levocetirizine dihydrochloride were found to be 0.338–50 µg/mL ($r = 0.9964$), 5–50 µg/mL ($r = 0.9956$), 0.325–50 µg/mL ($r = 0.9967$), and 0.553–50 µg/mL ($r = 0.9950$), respectively. There was no significant difference between the amount of drug spiked in serum and the amount recovered, and serum did not interfere in simultaneous estimation. Thus, the proposed method is suitable for the simultaneous analysis of active ingredients in tablet dosage forms and human serum.

Introduction

Diabetes mellitus is a chronic, progressive disease characterized by deteriorating glucose control and increased risk of micro- and macrovascular complications (1,2). In addition, psychological troubles are considered to be risk factors for the future development of diabetes-related complications (3). For patients who are diagnosed with diabetes, a large number of medications become available for appropriate therapy (4). The second most common oral pharmacologic strategy to manage type 2 diabetes include the use of agents that promote insulin release (e.g., sul-

fonylureas) (5). Gliquidone (Figure 1) belongs to the class of sulfonylurea derivatives and is mainly used for the treatment of non-insulin-dependent diabetes mellitus (NIDDM). It lowers the blood sugar level by stimulating the production and release of insulin from the pancreas (6). Gliquidone causes a marked and dose-dependent stimulation of acid production in gastric glands and potentiates the stimulatory effect of both histamine and carbachol, which increases the rate of pepsinogen release in gastric glands (7).

Histamine H₁-receptor antagonists are the mainstays of treatment for several allergic disorders, particularly rhinitis, conjunctivitis, dermatitis, urticaria, and asthma (8). First-generation histamine H₁-receptor antagonists readily penetrate the blood brain barrier and produce histamine blockade at histamine H₁-receptors in the central nervous system. In contrast, second generation histamine H₁-receptor antagonists are associated with a reduced incidence of sedation due to their poor ability to penetrate the blood brain barrier (9). Using immunohistochemical techniques, Stauber et al. (10) found that albumin could easily enter the cerebral cortex and also that second-generation histamine H₁-receptor antagonists may easily penetrate the blood brain barrier and cause potent sedation in diabetics. It has been recognized that patients with diabetes have a higher prevalence of depression than the general population (4).

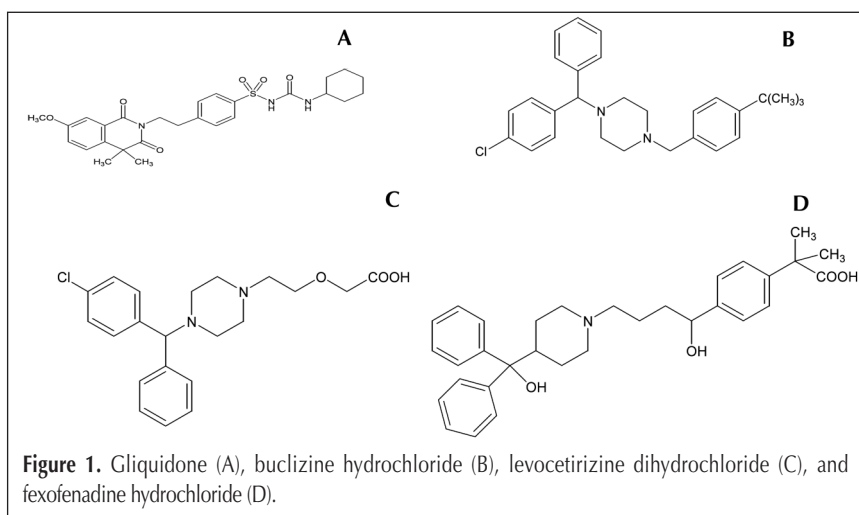


Figure 1. Gliquidone (A), buclizine hydrochloride (B), levocetirizine dihydrochloride (C), and fexofenadine hydrochloride (D).

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Antidiabetic drugs and H₁-receptor antagonists can be coadministered in a number of cases. The main objective of this study was to develop a new method for the simultaneous determination of gliquidone with H₁-receptor antagonists (fexofenadine hydrochloride, buclizine hydrochloride, and levocetirizine dihydrochloride) (Figures 1).

Literature survey revealed that quantification of gliquidone has been achieved by UV spectrophotometry (11), atmospheric pressure chemical ionization liquid chromatographic–mass spectrometry (APCI-LC–MS), LC–MS (12), and high-performance liquid chromatography (HPLC) (13–15). Numerous workers have reported determination of H₁-receptor antagonists by different techniques. There were also HPLC methods reported for the quantitation of cetirizine dihydrochloride or fexofenadine hydrochloride with pseudoephedrine in combined pharmaceutical dosage forms (16–18), cetirizine dihydrochloride or levocetirizine dihydrochloride with cefpirome (19), fexofenadine hydrochloride (20), and buclizine hydrochloride (21,22); but the method of all the previously mentioned coadministered drugs in active and dosage form are not reported simultaneously by HPLC. Such a method is needed as the coadministration of both drugs is possible in multiple-drug therapy. The proposed method was successfully applied to the determination of these drugs in commercial tablets and human serum. The established method was validated with respect to linearity, limit of detection and quantification, precision, accuracy, specificity, and robustness.

Experimental

Materials

The gliquidone reference standard was kindly gifted by Pharmatec Limited Karachi (Karachi, Pakistan). The H₁-receptor antagonists (fexofenadine hydrochloride, buclizine hydrochloride, levocetirizine dihydrochloride) of pharmaceutical purity were obtained from AGP Limited Karachi (Karachi, Pakistan). Glurenor (30 mg), Fexet (30 mg), Longifene (25 mg), and Xyzal (45 mg) tablets were purchased from the local pharmacy. Methanol used was HPLC-grade.

Equipments

A Shimadzu HPLC (Kyoto, Japan) system equipped with LC-10 AT VP pump, Rheodyne manual injector fitted with a 20- μ L loop, Purospher® STAR RP₁₈ end-capped column (25 cm \times 0.46 cm, 5 μ m) and SPD-10 A VP UV–Vis detector was utilized. The chromatographic system was integrated via Shimadzu model CBM-102 to PIV computer. Shimadzu CLASS-GC software (Version 5.03) was used for data acquisition and mathematical calculations.

Solution preparations

Separate stock solutions of gliquidone and H₁-receptor antagonists (fexofenadine hydrochloride, buclizine hydrochloride, and levocetirizine dihydrochloride) were prepared separately in a 100-mL volumetric flask by dissolving 10 mg of each drug, and volume was made up by 100 mL of 80% (v/v) aqueous methanol so that final concentration was 100 μ g/mL. Working solutions of

5, 10, 15, 20, and 50 μ g/mL were prepared by diluting with aqueous methanol (80%, v/v) from the standard solutions.

Assay in formulations

To determine the content of all the drugs in the formulations, 20 tablets of each drug were powdered, and an equivalent to 10 mg of gliquidone and 10 mg of H₁ antagonists (fexofenadine hydrochloride, buclizine hydrochloride, levocetirizine dihydrochloride) were weighed and transferred separately into 100-mL calibrated flasks before 100 mL 80% aqueous methanol was added. The content of the flask was shaken for about 60 min. This solution was filtered through Whatman filter paper to separate out the insoluble excipients, and further dilutions were carried out to obtain the desired concentration. Final solutions were filtered through a 0.45- μ m Millipore filter (Billerica, MA) before injection into the HPLC.

Serum drug analysis

The recovery of gliquidone, fexofenadine hydrochloride, buclizine hydrochloride, and levocetirizine dihydrochloride in human serum was determined by the stated chromatographic conditions. Multiple blood samples (10 mL) of ten healthy volunteers were collected in evacuated glass tubes. The blood was then centrifuged at 3000 rpm for 10 min, and the plasma was separated and deproteinated by acetonitrile. The supernatant obtained was filtered through a 0.45-micron pore size membrane filter. Serum thus obtained was mixed to different aliquots of stock standard solution to produce the desired concentrations.

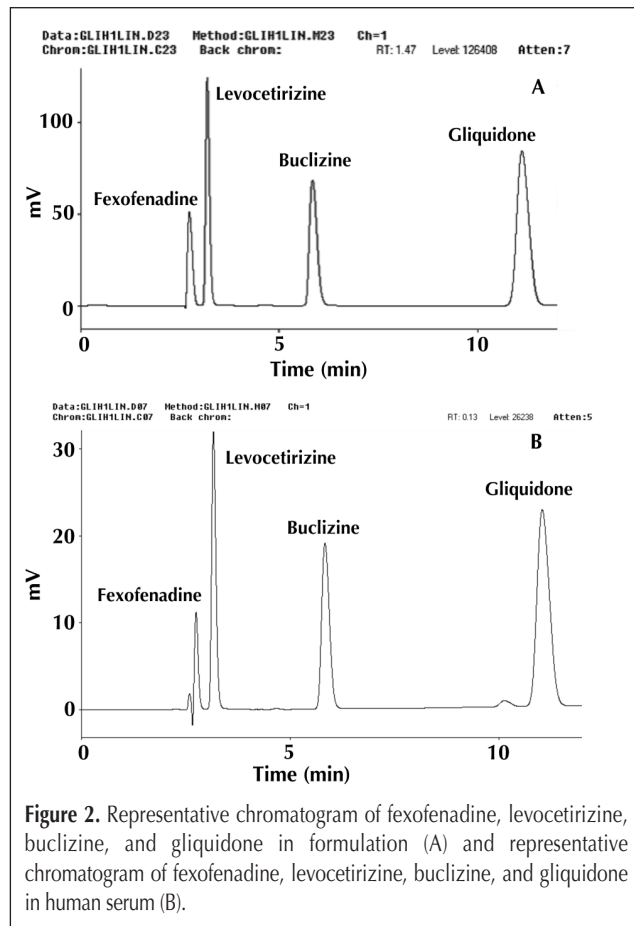


Figure 2. Representative chromatogram of fexofenadine, levocetirizine, buclizine, and gliquidone in formulation (A) and representative chromatogram of fexofenadine, levocetirizine, buclizine, and gliquidone in human serum (B).

These were stored at -20°C , and 10 μL volume of each sample was injected and chromatographed.

Optimization of chromatographic condition

To optimize the operating conditions for isocratic reversed-phase (RP)-HPLC detection, parameters such as mobile phase composition and flow rate were varied. Mobile phase composed of methanol–water (80:20) and a flow rate of 1.0 mL/min were chosen as the optimal settings that gave the retention times 2.71, 3.16, 5.81, and 11.05 min for fexofenadine hydrochloride, levocetirizine dihydrochloride, buclizine hydrochloride, and gliquidone, respectively. The pH effect showed that optimized conditions are reached when the pH value is 3.5 because it produces well-resolved and sharp peaks for all drugs assayed. A representative chromatogram is shown in Figure 2. In addition, the UV spectra of individual drugs were recorded in the wavelength range from 200 to 400 nm and compared. The choice to use

isobestic point set at 230 nm was considered satisfactory, permitting the detection of all drugs with adequate sensitivity.

Result and Discussion

Method validation

The method was validated according to ICH guidelines for validation of analytical procedures (23). The method was validated for the parameters like linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy and precision, specificity, and robustness. The linearity of this method was proved using linear correlation of the peak-area values and appropriate concentrations.

Linearity, limit of detection, and quantification

Under the previously described experimental conditions, linear correlation between the peak area and applied concentration was found in the concentration range 0.3–50 $\mu\text{g/mL}$. The regression statistics are shown in Table I. The LOD and LOQ at concentrations where the signal-to-noise ratios were equal to 3 and 10, respectively, were determined to be 0.10, 0.19, 0.097, 0.16 $\mu\text{g/mL}$ and 0.33, 5.00, 0.32, 0.55 $\mu\text{g/mL}$ for gliquidone, fexofenadine hydrochloride, buclizine hydrochloride, and levocetirizine dihydrochloride, respectively. The correlation coefficient of this dependence was calculated to be 0.9964, 0.9956, 0.9967, 0.9950 for gliquidone, fexofenadine hydrochloride, buclizine hydrochloride, and levocetirizine dihydrochloride, respectively.

Accuracy and precision

Intra-day precision and accuracy of the method were evaluated at three different independent concentrations (i.e., 8, 10, 12 $\mu\text{g/mL}$) ($n = 3$) in synthetic samples using placebo mixtures. The accuracy results (Table II) revealed that the method was accurate for all previously mentioned purposes. %Recoveries and %relative standard deviation (RSD) values were used to express accuracy and precision. Standard addition and recovery experiments were also conducted to determine the accuracy of the present method for the quantification of gliquidone, fexofenadine hydrochloride, buclizine hydrochloride, and levocetirizine dihydrochloride (Table III).

Specificity

A representative chromatogram (Figure 2) was generated to show that other components, which could be present in the sample matrix, are resolved from the parent analyte. No significant changes in retention times of the drugs in the presence and the absence of excipients clearly indicated the specificity of the method.

Robustness

The robustness was evaluated by minor modifications in the composition of the mobile phase used in the proposed method. The factors selected to examine were pH of mobile phase and temperature ($^{\circ}\text{C}$). A change of ± 0.1 unit of

Drug	Regression equation	R^2	LOD ($\mu\text{g/mL}$)	LOQ ($\mu\text{g/mL}$)
Gliquidone	$y = 17786x - 36988$	0.9964	0.10	0.33
Fexofenadine	$y = 6940.8x - 46043$	0.9956	0.19	5.00
Buclizine	$y = 9493.2x - 21326$	0.9967	0.09	0.32
Levocetirizine	$y = 8454.4x - 1434.7$	0.9950	0.16	0.55

Analyte	Spiked conc. ($\mu\text{g/mL}$)	Precision (% RSD)	Accuracy (%)
Gliquidone	8	0.49	100.0
	10	2.46	100.5
	12	0.93	100.7
Fexofenadine hydrochloride	8	2.11	99.8
	10	3.26	96.9
	12	0.96	100.4
Buclizine hydrochloride	8	0.49	102.2
	10	0.92	99.8
	12	1.03	97.8
Levocetirizine dihydrochloride	8	0.90	99.6
	10	1.00	98.4
	12	1.80	99.5

Conc. $\mu\text{g/mL}$	Gliquidone		Fexofenadine		Buclizine		Levocetirizine	
	Found $\mu\text{g/mL}$	Recovery %	Found $\mu\text{g/mL}$	Recovery %	Found $\mu\text{g/mL}$	Recovery %	Found $\mu\text{g/mL}$	Recovery %
5	4.96	99.3	5.10	102.0	5.01	100.2	5.01	100.1
10	10.05	100.5	9.79	97.9	9.98	99.8	9.94	99.4
15	14.93	99.6	15.13	100.9	15.27	101.8	14.88	99.2
20	20.01	100.1	19.97	99.8	19.84	99.2	19.08	95.4
25	24.85	99.4	25.04	100.2	25.15	100.6	24.35	97.4

Table IV. Accuracy and Precision in Human Serum

Analyte	Spiked conc. ($\mu\text{g/mL}$)	Precision (% RSD)	Accuracy (%)
Gliquidone	8	1.55	102.0
	10	1.36	101.7
	12	1.45	100.8
Fexofenadine hydrochloride	8	0.98	101.7
	10	2.11	97.9
	12	1.56	101.4
Buclizine hydrochloride	8	0.41	101.5
	10	1.92	102.0
	12	0.93	99.8
Levocetirizine dihydrochloride	8	1.50	99.9
	10	1.31	99.4
	12	1.20	101.0

pH (pH of mobile phase) had no considerable impact on chromatographic performance. The effect of column temperature on resolution was studied at 25°C and 32°C instead of room temperature (28°C). It can be seen that at every employed condition, the chromatographic parameters are in accordance with the established value.

The results of the present study demonstrate that simultaneous determination of gliquidone, fexofenadine hydrochloride, buclizine hydrochloride, and levocetirizine dihydrochloride are very beneficial for pharmaceutical companies, clinicians, and physicians and also can be beneficial for the studies of drug interaction.

Application in human serum

It was observed after spiking the analyte in the serum sample that there was no significant difference between the amount of drug spiked in serum and the amount recovered. Previously developed HPLC procedures for the determination of gliquidone, fexofenadine hydrochloride, buclizine hydrochloride, and levocetirizine dihydrochloride in plasma are based on liquid-liquid extraction from plasma samples. The method applied in our study involved the direct injection of the plasma samples after precipitation of protein with acetonitrile. The recovery values (Table IV) in human serum clearly indicate the applicability of the present method for the required purpose (Figure 2B).

Conclusion

After studying all the results obtained by HPLC studies, it was concluded that the present method was fast and easy to perform. The linearity range, LOD and LOQ, precision, accuracy, and specificity were processed to determine the suitability of the method, and the confirmed results were obtained. HPLC has several superiorities compared with UV spectrophotometry, such as smaller detection and quantification limits, small sample volumes, and specificity. The validity of the method makes it an acceptable for clinical studies in patients taking these medications simultaneously. Thus, the developed HPLC method is

rapid, reliable, cost-effective, and can be proposed for routine analysis laboratories and quality control purposes.

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