Development and Validation of HPLC Method for Simultaneous Determination of Gliclazide and Enalapril Maleate in Tablet Dosage Form

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ABSTRACT: This paper describes a simple, sensitive and selective high performance liquid chromatographic (HPLC) method for the separation and determination of gliclazide (GLI) and enalapril (ENA) maleate simultaneously in tablet dosage forms. The chromatographic separation was performed on an ODS column (250 mm × 4.6 mm i.d., 5 µm particle size) at 40 ± 0.5°C, with a mobile phase composed of phosphate buffer (pH 4.4), acetonitrile and methanol in the ratio of 45:40:15, (v/v/v). The flow rate was maintained at 1.5 ml/min, injected volume was 20 µl and detection wavelength was 217 nm. The method was validated according to ICH Q2 (R1) guidelines and found to be linear over a range of 40-120 µg/ml (R^2 = 0.991) and 2.5-7.5 µg/ml (R^2 = 0.998) for gliclazide and enalapril maleate, respectively. The proposed method was applied successfully for the assay of these two drugs in their combined in-house developed tablet formulations and could be applicable for routine quality control analysis.

Key words: Gliclazide, enalapril maleate, HPLC, method development, validation

INTRODUCTION

Patients with diabetes mellitus have an increased prevalence of hypertension and associated cardiovascular disease (CVD).[1] An international investigation revealed that hypertension affects about 60% of patients with type II diabetes.[2] A limited survey on the prescribing pattern of diabetic-hypertensive patients also disclosed the same trend in our country.[3] In Bangladesh as well as globally, the physicians are prescribing the ACE inhibitors at a greater frequency in diabetic-hypertensive patients.[2-5]

The multi-component formulations have gained a lot of importance due to obvious benefits like increased patient compliance, convenience and cost savings. In recent days, the pharmaceutical industries are placing a greater emphasis on FDC dosage forms and lots of such products are available in the consumer market, both locally and globally.[6,7] Considering the prevalence of diabetic-hypertension as well as the prescribing pattern of drugs in patients suffering from such complications, it is apparent that antidiabetic and antihypertensive drugs seem to be potential candidates for incorporating in a FDC product. Based on a feasibility study, such a combination product was developed recently in our laboratory containing an oral hypoglycemic agent and an ACE inhibitor. Accordingly, an immediate release tablet containing 80 mg gliclazide (GLI, a second generation sulfonylurea, Figure 1) and 5 mg enalapril maleate (ENA, an oral prodrug that is converted by hydrolysis to enalaprilat, which is an ACE inhibitor, Figure 2) was formulated, prepared and evaluated for various pharmaceutical parameters including dissolution and stability testing.[8] During the course of study, two analytical methods - based on UV spectroscopy and HPLC, were developed for

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the simultaneous estimation of these two drugs in the prepared tablets and in the dissolution medium during dissolution testing. The present paper reports the RP-HPLC method developed for the determination of GLI and ENA in combination dosage form as per ICH Q2 (R1) guideline.[9]

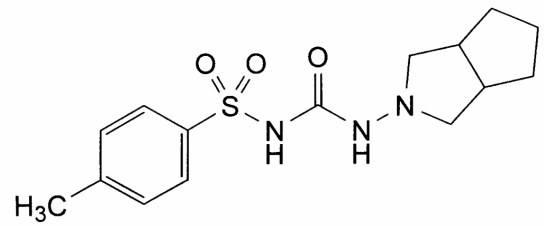

Figure 1. Structure of gliclazide (GLI)

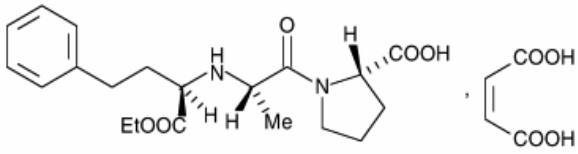

Figure 2. Structure of enalapril maleate (ENA)

MATERIALS AND METHODS

Materials. Gliclazide BP and enalapril maleate USP were of pharmacopeial grade and obtained from Laboratorio Chimico Internazionale SPA, Italy and Chematis Pharma Ltd., China, respectively. Potassium dihydrogen phosphate, acetonitrile and methanol were obtained from Scharlau, Spain. Phosphoric acid was purchased from Merck, Germany. All reagents and solvents were of HPLC or analytical grade.

Instrumentation and chromatographic conditions. A Shimadzu HPLC (Model: LC-2010 HT) integrated with variable wavelength programmable photo diode array (PDA) detector was employed for the analysis. The chromatographic analysis were performed on an ODS column (250 mm length × 4.6 mm ID with 5 µm particle size) and the mobile phase was phosphate buffer of pH 4.4, acetonitrile and methanol (45 : 40 : 15, v/v/v), pumped at a flow rate of 1.5 ml/min. The column temperature was maintained at 40 ± 0.5°C, and the detection wavelength was 217 nm. The injection volume was 10 µL, and the run time was 10 min for each injection.

Determination of analytical wavelength. In order to determine a suitable wave length to be used for the combined analysis of the drugs, 80 mg of each of GLI and ENA were taken in a clean and dry 100 ml volumetric flask; suitably diluted with acetonitrile and mix well. 5 ml of this solution was taken in a clean and dry 50 ml volumetric flask and the volume was made up to the mark with acetonitrile. Then this solution, containing 80 µg/ml of each of GLI and ENA, was filtered through 0.45 µ Whatman filter paper, injected and scanned in the range of 200-240 nm by PDA detector.

Preparation of mobile phase. Accurately weighted 6.8 g monobasic potassium phosphate was taken in a 1000 ml volumetric flask, and the volume was made up to mark with distilled water and finally pH was adjusted to 4.4±0.1 with suitably diluted orthophosphoric acid.

Preparation of working standard solution. Standard solution was prepared by transferring accurately weighed 80 mg of GLI and 5 mg of ENA in a 100 ml clean and dry volumetric flask. About 70 ml of mobile phase was added as diluting solution and mixed well. Then the volume was made up to the mark. Accurately measured 5 ml of this solution was further diluted to 50 ml with same diluting solution to prepare a concentration of 80 µg/ml of GLI and 5 µg/ml of ENA. After filtering through a 0.2 µ disc filter, the solution was used as nominal standard solution for analysis.

Method validation

System suitability. The nominal standard solution containing 80 µg/ml of GLI and 5 µg/ml of ENA was injected and repeated six times. The peak area (A), resolution (R_s), number of theoretical plates (N), tailing factor (t_f) and retention time (R_t) were calculated to determine whether the result complies with the recommended limit.

Linearity. The linearity of measurement was analyzed through the standard curves ranging from 40 - 120 µg/ml for GLI and 2.5 - 7.5 µg/ml for ENA and carried out in triplicate. Three calibration curves were prepared at five different concentrations in the

same day. The linearity was evaluated by linear regression analysis, which was calculated by the least-square regression analysis.

Specificity. Specificity of the method was determined by comparison between working standard solution and sample solution. Standard solution and sample solution of 80 µg/ml of GLI and 5 µg/ml of ENA were injected to the HPLC system for triplicates and were analyzed. Peak purity tool was used to investigate the interference of any other peak(s) with the standard chromatograms.

Noninterference of placebo and blank solution. Placebo solution was prepared in the same way of the sample solution in presence of all inactive ingredients of the experimental tablet formulation without the incorporation of two active ingredients (e. g. GLI and ENA) to check the noninterference of placebo. Blank solution was treated in the same way of the standard solution and was injected to check the noninterference of blank (if any).

Accuracy (Recovery test). Accuracy study was carried out for both drug and drug-matrix solutions. In case of drug solution, standard solutions of GLI and ENA, corresponding to 80, 100 and 130% of the nominal analytical concentration of the drugs were compared with reference standard solution of GLI and ENA of known purity (80 µg/ml of GLI and 5 µg/ml of ENA), and the percent recoveries (mean ± %RSD) of both drugs in pure form were calculated (n=3). In case of drug-matrix solution, accuracy study of the proposed method was carried out by the recovery test, which consisted of adding known amounts of working standards, e.g. GLI and ENA, to the placebo solutions in the beginning of the process. This test was realized by assaying three different solutions, three replicates each, containing 50, 100, and 150% of the nominal analytical concentrations of the working standards (80 µg/ml of GLI and 5 µg/ml of ENA), and then percent recoveries (mean ± %RSD) in drug-matrix solutions were calculated (n=3).

Precision. The repeatability (intra-day precision) of the method was evaluated by analyzing separately six nominal standard solutions of GLI and ENA on the same day and the intermediate precision (inter-day precision) was evaluated at same nominal standard concentrations daily for six times in three consecutive days. The concentrations of GLI and ENA were then determined and the standard deviations (SD) and relative standard deviations (RSD) were calculated.

Sensitivity. To calculate limits of detection (LOD) and limits of quantitation (LOQ) of GLI and ENA, sequential dilutions were done and analyzed by the proposed method. The LOD (k = 3.3) and LOQ (k = 10) were calculated by calibration curve method using the following equation:

A = kσ/S where, 'A' is LOD or LOQ, σ is the standard deviation of the response, and 'S' is the slope of the calibration curve.

RESULTS AND DISCUSSION

Selection of analytical wavelength. A chart of GLI and ENA representing the relation between the peak area (y) versus experimental wavelengths (x) studied in the range of 200-240 nm was constructed. The result, shown in Figure 3, represents that at 217 nm both drugs provided equal response and was taken as the experimental wavelength during subsequent analytical study.

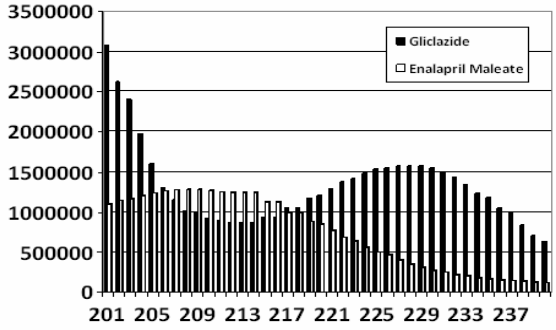

Figure 3. Chart representing the relationship between peak area *vs.* experimental wavelength

Method validation

System suitability. The chromatographic parameters, such as peak area, resolution, number of theoretical plates, tailing factors and retention times were calculated for the standard solution. The results

(Mean ± %RSD) are shown in Table 1, indicating the good performance of the system.

Linearity. The linear regression equation for GLI was found to be Y = 18870X + 13575; whereas for ENA was Y = 2200X + 1635 with correlation coefficients (R^2) were 0.991 and 0.998 for GLI and ENA, respectively. The results of the regression analysis were statistically significant. The validity of the assay was verified by means of ANOVA and no deviation from linearity was found ($P < 0.05$).

Specificity. Standard solution and sample solution of 80 µg/ml of GLI and 5 µg/ml of ENA were injected to the HPLC system and the chromatograms were recorded to check the peak purity. In every case, the peak purity was found 99.99% demonstrating that other added compounds did not co-elute with the main peaks, hence the chromatograms of GLI and ENA were pure in all cases. Figure 4 shows the typical standard and sample chromatograms of GLI and ENA by the proposed method.

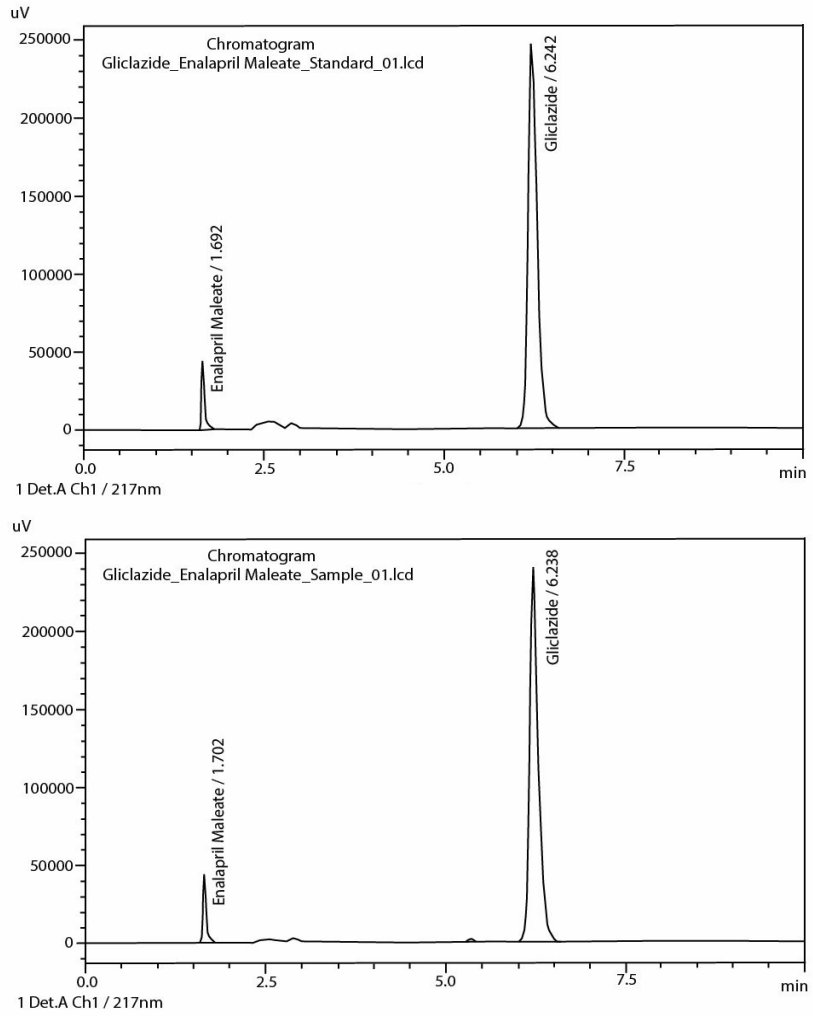

Figure 4. Chromatograms of standard and sample solutions at 217 nm. Mobile phase was phosphate buffer (pH 4.4), acetonitrile and methanol in the ratio of (45:40:15, v/v/v), flow rate was 1.5 ml/min and column temperature was 40 ± 0.5°C.

Noninterference of placebo and blank solution. Separately prepared placebo and blank solution were injected and their chromatograms were recorded to check the interference of inactive ingredients and diluting solution within the retention times around 1.7 min (ENA) and 6.25 (GLI) min. But the chromatograms revealed no peaks around the aforementioned times indicating that peaks of GLI

and ENA by the proposed method were clearly separated from the response of any unwanted peak(s).

Accuracy. The overall results of percent recoveries (Mean ± %RSD) at three levels of solutions of GLI and ENA in pure and drug-matrix solutions are given in Table 2, indicating that the proposed method is highly accurate and suitable for the intended use**.** The calculated recovery values of GLI and ENA ranged from 99.942% (±0.064%) to 100.057% (±0.059%) and 99.846% (±0.267%) to 101.6% (±1.052%) in pure forms, respectively, and from 100.203% (±0.062%) to 100.308% (±0.104%) and 101.156% (±0.201%) to 101.6% (±0.682%) in drug-matrix solutions, respectively.

Precision. The results obtained from intra-day and inter-day precision are presented in Table 3. Table 3 shows %RSD values with 2% which indicate that there was no significant difference between assays results of both GLI and ENA either within-day or between days, therefore, the proposed method was reliable and precise.

Table 1. System suitability parameters

Parameters	Values (Mean ± % RSD)*	
	GLI	ENA
Peak area (A)	1657393 ± 0.045	111515 ± 0.126
Resolution (R_s)	15.517 ± 0.228	-
No. of theoretical plate (N)	3844 ± 0.199	1427 ± 0.423
Tailing factor (t_f)	1.17 ± 0.936	1.08 ± 0.754
Retention time (R_t)	6.25 ± 0.351	1.71 ± 0.953

*Mean and % Relative Standard Deviation of six replicates

Table 2. Accuracy studies of the proposed method in pure and drug-matrix solutions

Drugs	Conditions	Added amount (µg/ml)	Amount recovered (Mean ± % RSD)*	% Recovery (Mean ± % RSD)*
GLI	Standard solution	64	64.037 ± 0.059	100.057 ± 0.059
		80	79.953 ± 0.064	99.942 ± 0.064
		104	104.270 ± 0.044	100.260 ± 0.044
	Drug-matrix solution	40	40.123 ± 0.104	100.308 ± 0.104
		80	80.180 ± 0.057	100.225 ± 0.057
		120	120.243 ± 0.062	100.203 ± 0.062
ENA	Standard solution	4	4.000 ± 1.732	100.000 ± 1.732
		5	5.030 ± 1.052	100.600 ± 1.052
		6.5	6.490 ± 0.267	99.846 ± 0.267
	Drug-matrix solution	2.5	2.54 ± 0.682	101.600 ± 0.682
		5	5.060 ± 0.523	101.200 ± 0.523
		7.5	7.587 ± 0.201	101.156 ± 0.201

*****Mean ± % RSD of three replicates

Table 3. Summary of intra-day and inter-day precision data

Drugs	Spike level (%)	Intra-day** (Mean ± % RDS)	Inter-day** (Mean ± % RDS)			Inter-day*** (Overall Mean ± RDS)
			Day-1	Day-2	Day-3	
GLI	100*	101.268±0.486	101.268±0.486	100.037±1.128	100.093±1.26	100.466±1.115
ENA	100*	100.677±0.516	100.677±0.516	99.96±0.965	99.138±0.713	99.925±0.959

*Spike level 100% indicates 80 µg/ml for GLI and 5 µg/ml for ENA.
** Mean and % RSD value of six determinations.
***(Overall Mean ± % RDS) means the arithmetic mean of inter-day results.

Sensitivity. According to the proposed method, the limit of detection (LOD) and limit of quantitation (LOQ) were calculated based on signal-to-noise ratio method and the values were found to be 11 ng/ml and 35 ng/ml for GLI, respectively, and 41 ng/ml and 127 ng/ml for ENA, respectively.

CONCLUSION

The proposed RP-HPLC method was used for the simultaneous estimation of GLI and ENA in tablet dosage form was found to be simple, sensitive, accurate, and precise. The method was validated according to the ICH guidelines. A good separation was performed for ENA at 1.7 min and GLI at 6.2 min with correlation coefficients (R^2) were 0.998 and 0.991, respectively. Hence, the present RP-HPLC method may be used for investigational analysis and routine quality control analysis of GLI and ENA in pure and fixed dose combination tablet dosage forms.

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

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