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ORIGINAL RESEARCH

Monitoring of in vitro interaction studies of enalapril with hypoglycemic agents by LC-UV

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Abstract: The coadministration of antihypertensive and antidiabetic drugs is common, as both of these ailments are synergistic to each other and often occur together. In the present paper, we describe in vitro drug interactions of enalapril, an antihypertensive drug, with the hypoglycemic agents, metformin, glibenclamide, and glimepiride. These studies were carried out using an isocratic reversed phase high-performance liquid chromatographic method using a C18 column with ultraviolet detection at 230 nm. The system was operated at room temperature using a mobile phase consisting of methanol:water (70:30) adjusted to pH 2.5 with o-phosphoric acid with a flow rate of 1 mL minute^{-1}. The assay was reproducible, linear (concentration range of 2.5–100 µg mL^{-1}) with a correlation coefficient of 0.9999 and an accuracy rate of 98%–102%. The results from the reversed phase high-performance liquid chromatographic method clearly indicated that the availability of enalapril was unaffected by the simultaneous administration of hypoglycemic agents. Hence, the two drugs can be safely administered with one another.

Keywords: enalapril, antihypertensive agent, hypoglycemic agents, drug interactions, RP-HPLC

Introduction

Hypertension in diabetics represents an important health problem as the combination of these ailments is common, and can carry significant morbidity and mortality rates in addition to being difficult to treat. The prevalence of hypertension in diabetic people is probably 1.5–2 times higher than in the general population.[1] Diverse classes of antihypertensive prescriptions may be used for blood pressure management in diabetes; among these, angiotensin II receptor blockers (ARBs), calcium channel blockers, thiazide diuretics, and angiotensin-converting enzyme (ACE) inhibitors are common.[2] Use of the ACE inhibitor, enalapril, was associated with an increased risk of hypoglycemia (odds ratio, 2.4; 95% confidence interval [CI], 1.1–5.3) in sulfonylurea users.[3] Cardiovascular drugs carried a risk of drug–drug interactions (187 drugs, or 49.5%). The most common potential drug–drug interaction observed was between metformin and enalapril (n = 64).[4] Use of verapamil, a calcium channel blocker, significantly reduced the risk of developing diabetes.[5] Similarly, diabetic patients often take antihypertensive medications along with antidiabetic drugs.[6] The treatment of patients with hypertension and diabetes using ARBs has improved both macro- and microvascular alterations.[7]

Diverse classes of antihypertensive prescriptions may be used for blood pressure management in diabetes; among these, calcium channel blockers, ARBs, thiazide diuretics, and ACE inhibitors are common. Collective pharmacological treatments

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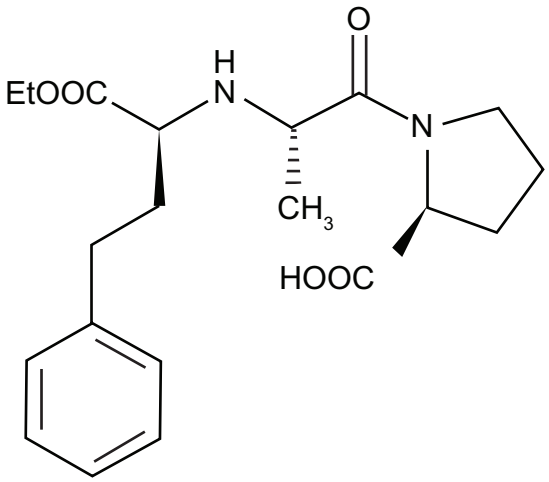

Figure 1 Chemical structure of enalapril.

generally aim to manage type 2 diabetes mellitus in order to attain satisfactory glucose management and also aim to deal with concomitant pathologies. Drug–drug interactions must be cautiously considered with antihypertensive and hypoglycemic drugs.[8] A literature survey revealed that the quantification of gliquidone is achieved by ultraviolet (UV) spectrophotometry,[9] as well as high-performance liquid chromatography (HPLC).[10,11] Our research group earlier reported methods for the simultaneous determination of rosiglitazone and glimepiride by HPLC, and now this method has been developed and used for interaction studies,[12] while enalapril and statins have been used as commercial tablets and human serums.[13] Earlier we reported on the interaction of losartan with gliquidone, as well as pioglitazone and enalapril with H_2 receptor antagonist in combined pharmaceutical dosage forms.[14,15] Combinations of metformin and either glipizide, gliclazide, or glibenclamide are available commercially as single dosage forms. A combination tablet formulation is beneficial in terms of its convenience and rate of patient compliance. The most important purpose of this study was to assess the in vitro drug interaction of enalapril (Figure 1), with commonly prescribed antidiabetic drugs (metformin, glimepiride, and glibenclamide) by utilizing HPLC for the routine monitoring of diabetic patients who take a combination of these medications, and to study the pharmacokinetics of the combined dosage forms.

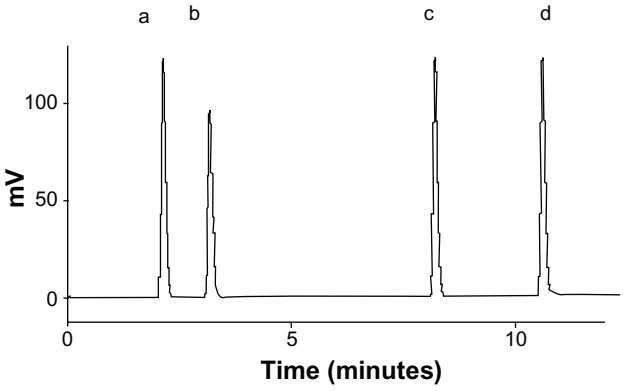

Figure 2 Resolution of metformin, enalapril, glibenclamide, and glimepiride.

Aim of study

Simultaneous determination of these analyses is important for the routine monitoring of diabetic patients who take a combination of medications and for studying the pharmacokinetics of the combined dosage forms.

Methods

Instrumentation

A Shimadzu HPLC system (Shimadzu Corporation, Kyoto, Japan) equipped with a liquid chromatography (LC)-10 AT VP pump and an SPD-10 A VP ultraviolet-visible spectroscopy (UV-VIS) detector, and another HPLC system (Shimadzu Corporation) equipped with an LC-20AT and SPD-20A UV-VIS detector were utilized. The chromatographic system was integrated via a Shimadzu model CBM-102 to an Intel® Pentium® 4 computer (Intel Corporation, Santa Clara, CA, USA) loaded with Shimadzu CLASS-VP software (version 5.03, Shimadzu Corporation) for data acquisition and mathematical calculations. A Rheodyne® manual injector (model 7725; Chrom Tech, Inc, Apple Valley, MN, USA) was fitted with a 20 µL loop, Hypersil ODS C18 column (150 mm × 4.6 mm, 5 microns; Thermo Fisher Scientific, Waltham, MA, USA), a Purospher® STAR RP-18 column

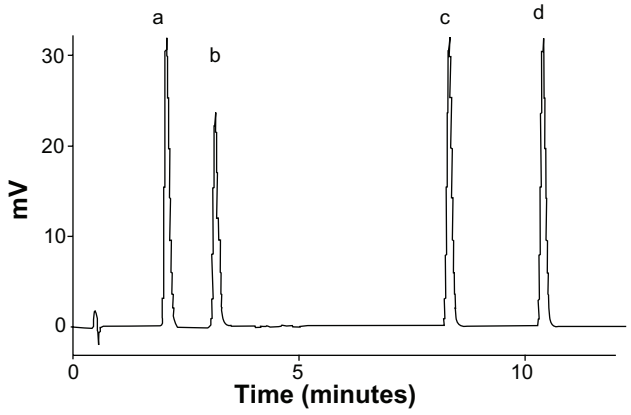

Figure 3 Representative chromatogram of meformin, enalapril, glibenclamide, and glimepiride.

Notes: Representative chromatogram of (**a**) metformin (2.4 minutes); (**b**) enalapril (3.7 minutes); (**c**) glibenclamide (8.3 minutes); and (**d**) glimepiride (11 minutes) in mobile phase methanol:water (70:30) and flow rate of 1 mL/minute^{-1} at 230 nm in formulations.

Table 1 Regression equation

Drugs	Concentration (μg mL^{-1})	Regression equation	r^2	LOD (ng mL^{-1})	LOQ (ng mL^{-1})
ENP	2.5–100	y = 2489.4x + 255.5	0.9996	1.53	4.6
MET	2.5–100	y = 10406x + 24139	0.9993	0.317	0.96
GLB	2.5–100	y = 14651x + 33832	0.9998	0.19	0.58
GMP	2.5–100	y = 15438x + 39969	0.9996	0.1	0.32

Abbreviations: ENP, enalapril; MET, metformin; GLB, glibenclamide; GMP, glimepiride; LOD, limit of detection; LOQ, limit of quantification.

(Merck Millipore, Billerica, MA, USA), and a DGU-14 AM online degasser (Shimadzu Corporation). In addition, a Mettler Toledo electronic balance, microliter syringe, and micropore filtration assembly (Mettler Toledo AG, Greifensee, Switzerland) were used in this study.

Materials and reagents

Enalapril maleate was a kind gift from Merck & Co, Inc (Whitehouse Station, NJ, USA); the antidiabetic drugs used in this study were metformin, glimepiride, and glibenclamide, which were from Sanofi-aventis Pakistan Ltd (Karachi, Pakistan), Parke-Davis and Co, Ltd, and Safe Pharmaceutical (Pvt) Ltd (Karachi, Pakistan). All of these drugs had an expiry date of not less than 1 year at the time of the study. All reagents used were of HPLC grade. Acetonitrile, methanol, and phosphoric acid 85% (Merck, Darmstadt, Germany), and HPLC-grade deionized filtered water were used to prepare the mobile phase. Stock solutions of enalapril and antidiabetic drugs were prepared in the mobile phase. Fresh working solutions were prepared daily. All solutions were filtered through 0.45 μm and degassed using a sonicator.

Preparation of solutions

Standard solutions of enalapril and antidiabetic drugs were prepared by dissolving appropriate amounts of each in mobile phase methanol:water (70:30 v/v, pH 2.5) to obtain the 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 contents of 20 tablets were powdered, and a weighed portion of the powder equivalent to the suitable amount of drug (according to the claims on the labels) was transferred into a 50 mL volumetric flask. The drug was fully dissolved in the mobile phase, diluted appropriately, and seven dilutions of each drug were prepared – a portion of which was filtered through a disposable 0.45 μm filter and then injected to Rheodyne® injector. In case of oral dosage forms, 20 tablets of each drug were crushed, finely powdered, and amounts equivalent to 20 mg of each drug were transferred to separate 100 mL volumetric flasks, and made up to the mark with respective buffer solutions.

Plasma samples, obtained from healthy volunteers, were collected and stored at −20°C. To an aliquot of 1.0 mL of plasma, 9 mL of acetonitrile was added and the mixture was vortexed for 1 minute, and centrifuged at 10,000 rpm for 10 minutes. The supernatant was then filtered through a (0.45 μ pore size membrane filter). An aliquot serum sample was fortified with enalapril and a hypoglycemic agent to get the final concentrations.

Equal volumes of metformin, glimepiride, and glibenclamide were mixed with enalapril to produce final concentrations of 100 μg mL^{-1} in reaction flasks. These flasks were kept in a water bath at a constant temperature (37°C) with constant stirring. A total of 2 mL of aliquot was drawn from the reaction flask at 0 minutes, and periodically after 15-minute time intervals for 2 hours. Aliquots that were withdrawn were diluted to 10 mL with methanol, filtered through a Millipore filter (0.45 μM; Merck Millipore), and chromatographed.

Results and discussion

Method validation

This newly developed method has been validated and holds well for the determination of these drugs in raw materials,

Table 2 Accuracy in formulations

Concentration (μg mL^{-1})	ENP		MET		GLP		GMP	
	% RSD	% rec	% RSD	% rec	% RSD	% rec	% RSD	% rec
80	0.011	101	0.002	100.9	0.002	99.9	0.08	100
100	0.326	100.3	0.001	100.5	0.001	100	0.002	100.2
120	0.001	100	0.008	99.7	0.008	99.7	0.001	100.1

Abbreviations: ENP, enalapril; MET, metformin; GLP, glibenclamide; GMP, glimepiride; RSD, relative standard deviation; rec, recovery.

Table 3 Intraday and interday precision of the method

Concentration	Intraday (% recovery)				Interday (% recovery)			
	ENP	MET	GLB	GMP	ENP	MET	GLB	GMP
Bulk material								
2.5	100.9	100.9	100	98.85	97.44	97.4	97.6	99.2
5	101.1	101.1	101	99.5	100.5	102	100.8	102
10	99.49	100.9	101	100.55	99.87	99.5	100	100
25	101.2	100.5	101	101.19	99.2	101	102	102
50	98.94	99.45	99.1	98.14	100.8	101	100.2	100.1
100	101.1	100.6	99.6	99.58	99.92	100	101.8	101.6
Serum								
2.5	100.8	99.9	99.9	100.2	98.5	98.4	97.6	100
5	101	100.2	99.8	100.02	100.5	102	101.2	101.2
10	100.4	100.2	99.8	100.55	99.87	99.5	100	98.7
25	101.2	101.2	99.9	101.19	100.2	101	100.3	98.8
50	99.94	98.9	99.1	99.8	100.8	101	100.2	98.9
100	100.1	101.2	99.6	99.58	100.3	100	100.2	99.9

Abbreviations: ENP, enalapril; MET, metformin; GLB, glibenclamide; GMP, glimepiride.

dosage formulations, and human serum. For validation of the analytical method, the guidelines of the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use were followed, where they recommended the accomplishment of selectivity, specificity, linearity, accuracy of tests, precision, sensitivity, limit of detection, and quantification of the method.

Selectivity and specificity

The selectivity and specificity of the method was established through the study of the resolution factor at the peak of enalapril from medications used in noninsulin-dependent diabetes mellitus. The method demonstrated good resolutions and was found to be free of interference from excipients (Figure 2) used in formulation products, and thus the method is specific for enalapril and noninsulin-dependent diabetes mellitus drugs (Figure 3).

Range and linearity

Linearity is generally reported as the variance of the slope of the regression line. Linearity was tested with known concentrations of enalapril, metformin, glibenclamide, and glimepiride (ie, 2.5, 5, 10, 25, 50, and

Table 4 Robustness of the method (n = 6)

	Level	K′	T	(R_s)
A: pH of mobile phase				
2.6	–0.2	4.8	1.39	2.4
2.8	0	4.5	1.43	2.3
3	0.2	4.2	1.4	2.2
Mean ± SD (n = 6)		4.5 ± 0.3	1.43 ± 0.020	2.3 ± 0.1
B: flow rate (mL min^{-1})				
0.8	–0.2	4.1	1.45	2.32
1	0	4.3	1.44	2.36
1.2	0.2	4.4	1.42	2.37
Mean ± SD (n = 6)		4.3 ± 0.212	1.44 ± 0.015	2.36 ± 0.026
C: percentage of water in mobile phase (V/V)				
25	–5	4.6	1.42	2.38
30	0	4.3	1.43	2.36
35	5	4.5	1.46	2.33
Mean ± SD (n = 6)		4.36 ± 0.070	2.36 ± 0.025	2.36 ± 0.025
D: wavelength (nm)				
225	–5	4.5	1.42	2.38
230	0	4.3	1.43	2.36
235	5	4.4	1.45	2.32
Mean ± SD (n = 6)		4.3 ± 0.070	1.43 ± 0.015	2.36 ± 0.030

Abbreviations: n, theoretical plates; K′, capacity factors; T, tailing factor; R, resolution; SD, standard deviation; V, volume.

Table 5 Ruggedness of the method

HPLC system			LC 10		LC 20	
Drugs	**Columns**	**Concentration (μg mL^{-1})**	**Found (μg mL^{-1})**	**Recovery (%)**	**Found (μg mL^{-1})**	**Recovery (%)**
ENP	Hypersil ODS	8	8.03	100.3	8.06	100.9
		10	9.99	99.9	9.98	99.99
		12	12	99.98	12	99.89
	Purospher STAR	8	7.99	100	8.3	101
		10	9.83	100	10.3	101.3
		12	12	100.3	12.22	102
MET	Hypersil ODS	8	8.4	100.9	8.3	100.9
		10	9	100.5	9.98	99.99
		12	11.9	99.7	11.9	99.89
	Purospher STAR	8	7.99	100	8.6	101
		10	10	101	10.3	100
		12	12.3	100.3	12.3	102
GLB	Hypersil ODS	8	7.98	99.9	8.06	100.9
		10	9.99	100	9.99	100
		12	11.98	99.7	11.9	99.8
	Purospher STAR	8	7.99	100.9	8.9	101
		10	10.36	101.1	9.99	100
		12	11.39	99.49	12.9	102
GMP	Hypersil ODS	8	7.98	99.7	8.9	102
		10	9.99	100.2	9.98	100
		12	12	100.1	11.9	99.9
	Purospher STAR	8	7.99	100.9	8.9	101
		10	10.3	101.1	10.9	101
		12	11.9	99.49	12.22	101

Notes: Hypersil ODS: Thermo Fisher Scientific, Waltham, MA, USA. Purospher® STAR: Merck Millipore, Billerica, MA, USA.
Abbreviations: HPLC, high-performance liquid chromatography; LC, liquid chromatography; ENP, enalapril; MET, metformin; GLB, glibenclamide; GMP, glimepiride.

100 μg mL^{-1}, respectively). Five runs were performed for every concentration. Injected concentrations versus peak area were plotted, and the correlation coefficients were calculated, which are shown in Table 1.

Accuracy and recovery

The accuracy or closeness levels of the measured values were evaluated and compared to the true as the percentage of relative error between the measured mean concentrations and the taken concentrations in order to ascertain whether the method is capable of measuring all the taken amount or not. The difference in these is accounted for in relative standard deviation (RSD). A minimum of three concentration levels covering the specified ranges were selected and three runs were performed for every concentration; the peak area was then calculated, as given in Table 2.

Precision

The intra- and interday precision was evaluated by assaying the samples (Table 3). In this assay, the intraday precision and the interday precision recovery was 98%–102% in bulk materials and in human serum. Intra- and interday precision was performed and the percentage of RSD was found to be less than 2%, which indicates that the method was sufficiently accurate and precise.

Robustness

Robustness of the method was accomplished by design modifications made to the method parameters such as composition, flow rate, pH of the mobile phase, detection wavelength, injection volume, and column temperature

Table 6 Percentage availability of enalapril and antidiabetic drugs

Time (minutes)	ENP	MET	ENP	GLB	ENP	GMP
0	99.89	100.01	100.34	100.34	102	99.99
30	99.65	99.02	99.54	99.54	101.3	100
60	100.23	95.31	98.12	98.99	102.3	101
90	101.61	105.56	99.69	99.69	102.3	102.3
120	100.2	98.3	98.46	98.46	101.2	102
150	101.98	98.88	100.3	100.63	102.3	103
180	106.46	99.99	100.36	100.36	102.3	104.3

Abbreviations: ENP, enalapril; MET, metformin; GLB, glibenclamide; GMP, glimepiride.

(Table 4), and it was found that the percentage of RSD values did not exceed more than 2%.

Ruggedness

The ruggedness of the study was established by determining enalapril, metformin, glibenclamide, and glimepiride using the same and different chromatographic systems by using two different columns. The assay results indicated that the method was capable with high precision (Table 5).

Limit of detection and quantification

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected, but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The limit of detection and limit of quantification are calculated and given in Table 1.

Interaction studies using HPLC

Results of these interactions are summarized in Table 6. The percentage of availability of enalapril and metformin was found to be between 98%–106%, indicating that there was no reaction between the two drugs. These results clearly indicated that enalapril could be safely coadministered with metformin. The two drugs did not inhibit or disturb the absorption of each other. Similar behavior was observed with glibenclamide and glimepiride; the availability of enalapril was found to be between 102% and 103% with glibenclamide and glimepiride, and the availability of glibenclamide and glimepiride remained almost unchanged. No remarkable change in the area under the curve and drift in retention time were observed; however, the results showed that no interaction occurred as the percent of recovery remained almost unchanged.

Conclusion

A liquid chromatographic method for the simultaneous quantitation of enalapril with metformin, as well as glibenclamide and glimepiride was developed and utilized for the monitoring of in vitro interaction studies of enalapril with these hypoglycemic drugs. It has been concluded that the current method is fast and easy to perform, has low limit of detection and quantification values, shows a high percentage of recoveries, and is linear up to a wide range of concentrations. Moreover, on the basis of the interaction results obtained from HPLC technique, it is concluded that no interaction occurred between metformin, glibenclamide, and glimepiride with enalapril.

Disclosure

The authors report no conflicts of interest in this work.

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