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ION-pair liquid chromatography technique for the estimation of metformin in its multicomponent dosage forms

M. Vasudevan *, J. Ravi, S. Ravisankar, B. Suresh

Centre for Advanced Drug Research and Testing, J.S.S. College of Pharmacy, Ootacamund, Tamilnadu, India

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Abstract

A simple, precise and accurate high performance liquid chromatography (HPLC) method was developed for the simultaneous estimation of metformin with gliclazide and glipizide present in multicomponent dosage forms. The method was carried out on Inertsil® C_{18} column. A mobile phase composed of acetonitrile–water containing camphor sulphonic acid (adjusted to pH 7 using 0.1 N sodium hydroxide; 75 mM) at a flow rate of 1 ml min^{-1} was used for the separation. Detection was carried out at 225 nm. Tolbutamide was used as internal standard. Validation of the developed HPLC method was carried out. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Metformin HCl; Gliclazide; Glipizide; Anti-diabetic dosage forms; Simultaneous estimation; Reversed phase ion-pair chromatography

1. Introduction

Metformin HCl is chemically 1,1-dimethyl biguanide hydrochloride. Gliclazide is 1-(3-azabicyclo [3.3.0] oct-3-yl)-3-p-tolylsulphonylurea. Glipizide is chemically 1-cyclo-hexyl-3-[4-[2-(5-methylpyrazine-2-carboxamido) ethyl]-benzene-sulphonyl] urea. These three drugs are oral hypoglycemic agents. A combination of 500 mg of metformin and 80 mg of gliclazide (combination-I), 500 mg of metformin and 5 mg of glipizide (combination-II) are available commercially as tablets [1]. These two combinations are used in the treatment of non-insulin dependent diabetes mellitus (NIDDM).

Many methods have been reported in the literature for the estimation of metformin, gliclazide and glipizide, individually [2–9]. However, there is no method reported for the simultaneous estimation of metformin with gliclazide and glipizide. The complexity of the multicomponent dosage forms includes multiple entities and excipients poses considerable challenge to the analytical chemist during the development of assay procedure. Estimation of the individual drugs in these multicomponent dosage forms becomes difficult

* Corresponding author. Tel.: + 91-423-43393(43847); fax: + 91-423-42937.
E-mail address: jayapalravi@hotmail.com (M. Vasudevan).

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due to cumbersome extraction or isolation procedures. In the early part of this century, colorimetric and spectrophotometric methods were used for drug analysis due to reasons of economy and easy availability. These methods, however, are used to a lesser extent today because they lack specificity, sensitivity and accuracy. For the simultaneous estimation of the drugs present in multicomponent dosage forms, HPLC method is considered to be most suitable since this is a powerful and rugged method. It is also extremely specific, linear, precise, accurate, sensitive and rapid. The present work describes a simple, precise, accurate and validated HPLC method for the simultaneous estimation of metformin with glipizide and metformin with gliclazide in tablets. Validation of the proposed HPLC method was also carried out [10].

2. Experimental

2.1. Reagents and chemicals

Camphor sulphonic acid AR grade obtained from E. Merck (India) Ltd, sodium hydroxide AR grade and acetonitrile of HPLC grade supplied by S.d. Fine Chemicals, India, water HPLC grade obtained from Milli-Q RO system, were used for the study. Reference standards of metformin, glipizide, gliclazide and tolbutamide were obtained as gift samples from the manufacturers of the formulations.

2.2. Optimisation of the chromatographic conditions

A gradient run was performed for the initial separation. From the gradient run the 35:65, v/v of acetonitrile in phosphate buffer (adjusted to pH 2.5 with *ortho*phosphoric acid) was selected as mobile phase. When isocratic run was performed, the peak of metformin was unretained but gliclazide and glipizide were eluted at 15.32 and 17.54 min respectively with symmetric peak shape. To improve the retention of metformin the organic content of the mobile phase was reduced and different pH ranges of buffers in acetonitrile were tried. When the organic content of the mobile phase was reduced to 25%, peak of metformin was not retained but the peak of gliclazide and glipizide were eluted at 19.26 and 22.45 min with asymmetric peak shape. Different pH of buffers in acetonitrile mobile phase did not make significant changes in the elution pattern of the metformin. Hence a reversed phase ion-pair method was used to improve the retention of the metformin. Further optimization studies such as effect of ionic strength, solvent strength, nature of stationary phase and other variables were also studied.

2.3. Chromatographic conditions

A Shimadzu® HPLC system was used for the study. The method was carried out on an Inertsil® C_{18} (15 cm × 4.6 mm i.d., 5 μ) column as a stationary phase. Mobile phase consisting of acetonitrile–water containing camphor sulphonic acid (adjusted to pH 7 using 0.1 N sodium hydroxide; 75 mM; 35:65, v/v for combination-I and II) at a flow rate of 1 ml min^{-1} was used for the separation. Tolbutamide was used as internal standard in both experiments. A Rheodyne 7725i injector with a 20 μl loop was used for the injection of samples. An SPD-M10Avp Photodiode array detector equipped with CLASS VP data station was used for the processing of peaks. The peaks were integrated at 225 nm. The mobile phase was filtered through a 0.45 μ membrane filter and degassed. The separation was carried out at the room temperature of about 20°C.

2.4. Preparation of mixed standard solutions

Standard stock solution of 1 mg ml^{-1} of metformin, gliclazide, glipizide and tolbutamide were prepared separately using a mixture of 0.05 N sodium hydroxide–acetonitrile (1:1, v/v). From the standard stock solutions, mixed standard solution was prepared using the mobile phase to contain 125 μg ml^{-1} of metformin, 20 μg ml^{-1} of gliclazide and 50 μg ml^{-1} of tolbutamide as internal standard (for combination-I) and 125 μg ml^{-1} of metformin, 1.25 μg ml^{-1} of glipizide and 50 μg ml^{-1} of tolbutamide as internal standard (for combination-II).

2.5. Preparation of sample solutions

Twenty tablets were weighed and finely powdered. A quantity of powder equivalent to 125 mg of metformin and 20 mg of gliclazide was taken into a sintered glass crucible. To this 50 mg of tolbutamide was added. The drugs were extracted with three quantities, each of 20 ml of the mixture of acetonitrile–0.05 N sodium hydroxide (1:1, v/v), and the combined extracts were made upto 100 ml with mobile phase. The resulting solution was further diluted using the mobile phase to get a concentration 125 μg ml^{-1} of metformin, 20 μg ml^{-1} of gliclazide and 50 μg ml^{-1} of tolbutamide (theoretical value). This solution was used for the estimation (for combination-I).

For the combination-II, a quantity of powder equivalent to 125 mg of metformin and 1.25 mg of glipizide was taken and drugs were extracted using the above procedure. The resulting solution was further diluted using the mobile phase to get a concentration 125 μg ml^{-1} of metformin, 1.25 μg ml^{-1} of glipizide and 50 μg ml^{-1} of tolbutamide (theoretical value).

2.6. Procedure

With the above chromatographic conditions, the mixed standard solutions and sample solutions were injected in triplicate and the chromatograms were recorded (Figs. 1 and 2). The retention time of metformin, tolbutamide, gliclazide and glipizide were 2.14, 5.89, 8.33 and 10.03 min, respectively. The response factor of the standard solution (peak area ratio of standard peak area and the internal standard peak area) and the sample solution were calculated.

3. Validation of the method

The specificity of the method was carried out by peak purity test method using the diode array detector. The first derivative spectra were recorded for the sample peaks and these were compared with the first derivative spectra of standard drug peaks. Accuracy of the method was studied by recovery experiments. To the powdered tablets (125 mg of metformin and 20 mg of gliclazide for combination-I and 125 mg of metformin and 1.25 mg of glipizide for combination-II), 50 mg of tolbutamide and the standard drugs at the level of 50 and 100% of the assay level were added. The extraction of drugs was followed using the procedure adopted for the preparation of sample solution. The solutions were then analyzed and the percentage recoveries were calculated.

Precision of the method was demonstrated by repeatability studies. This was done by injecting consecutively the standard solution for 10 times and passing them through the assay procedure. From the response factor of the drug peaks, mean and percentage RSD of the response factor of the peaks were calculated.

Linearity and range of the method were determined by analyzing mixed standard solutions containing 62.50–187.50 μg ml^{-1} of metformin, 10–30 μg ml^{-1} of gliclazide and 0.625–1.875 μg

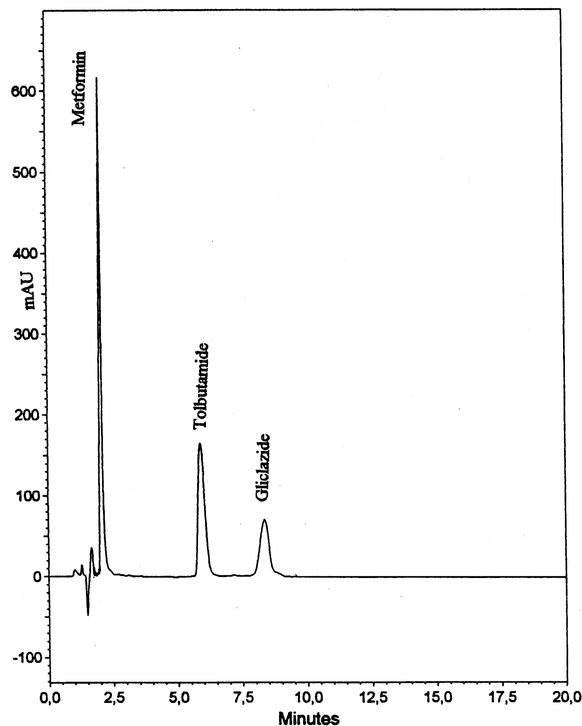

Fig. 1. Chromatogram of metformin and gliclazide in sample solution.

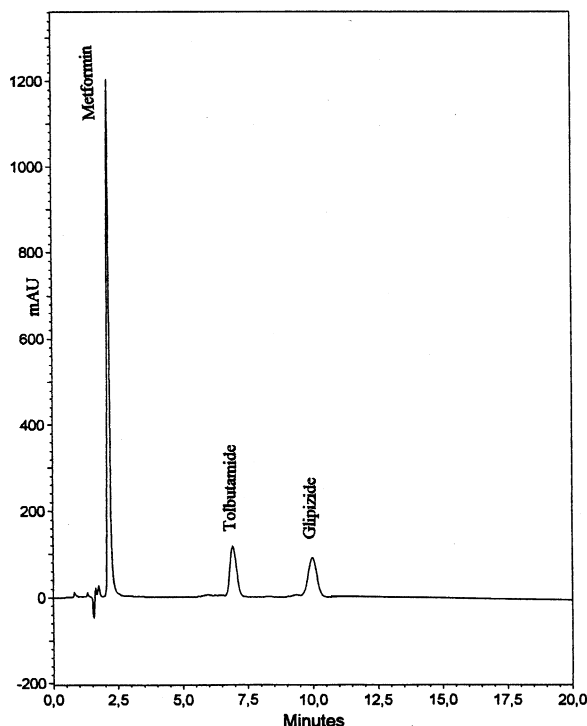

Fig. 2. Chromatogram of metformin and glipizide in sample solution.

ml^{-1} of glipizide (50–150% of targeted level of the assay concentration) containing 50 μg ml^{-1} of tolbutamide as internal standard, respectively. These solutions were analyzed and the response factors were calculated. The calibration curves were plotted by using response factor *V*s concentration of the standard solutions. The limit of detection (LOD) and limit of quantification (LOQ) of the method were determined by injecting progressively low concentrations of the standard solutions and the methodology as described in the ICH guidelines was followed [10].

The ruggedness of the method was carried out by changing the experimental conditions such as, using different source of reagents and solvents (different manufacturers), changing to another stationary phase of similar type (Kromasil C_{18}, Hypersil C_{18}, μBondapak C_{18} and Hichrom C_{18}), using other makes of HPLC instruments (Waters HPLC and Spectraphysics HPLC), slightly changing the pH of the buffer solution (7.0 ± 0.2), ionic strength of the buffer solution (75 ± 5 mM), ratio of the mobile phase ($\pm 1\%$) and flow rate of the mobile phase (1.0 ± 0.2 ml) has been made and the chromatographic patterns were studied.

The stability of these solutions (mobile phase, standard and sample solutions) was studied by performing the experiment after 3 days of storage and looking for the change in the chromatographic pattern when compared with that of the freshly prepared solutions. System suitability studies were also carried out and the parameters like column efficiency, resolution and peak asymmetry were calculated.

4. Results and discussion

The results of the proposed HPLC method showed that the amounts of drugs present are consistent with the label claim of the formulation (Table 1). The specificity of the method was carried out by peak purity tests using diode array detector and the first derivative spectrum of standard and sample peaks were recorded and compared (Fig. 3). The first derivative spectrum of the sample peaks were matching with the corresponding first derivative spectrum of the standard drug peaks, which showed that the peaks of analyte were pure and also formulation excipients and impurities were not interfering with the analyte peaks.

The accuracy of the method was determined by recovery studies. The recovery studies were carried out as described earlier and the percentage recovery was calculated (Table 1). Results of recovery studies showed that the method developed is accurate. The precision method was demonstrated by RSD values of the response factors for metformin and gliclazide (combination-I), metformin and glipizide (combination-II), which were 0.205 and 0.157, 0258 and 0.109 respectively. This shows that the method is precise.

The linearity and range of the assay method were determined by plotting the calibration curves (Table 2). The calibration curve showed linear response over the range of concentration used in the assay procedure, which justifies the use of single point calibration. The slope and intercept

Table 1
Analysis of formulation and recovery studies

Combination	Drug	Labeled amount (mg tab^{-1})	Amount taken for assay (µg ml^{-1})	Amount obtained[a] (mg tab^{-1})	% Label claim[a]	% Recovery[a]
Combination-I	Metformin	500	125	124.88 (1.621)	99.90 (2.014)	100.17 (1.022)
	Gliclazide	80	20	19.85 (6.132)	99.25 (2.041)	99.89 (0.962)
Combination-II	Metformin	500	125	124.92 (2.379)	99.93 (2.021)	100.29 (1.029)
	Glipizide	5	1.25	1.23 (8.142)	98.40 (2.798)	99.24 (0.179)

[a] Mean% R S.D. of 6 observations.

Table 2
Linearity and range*

	Metformin					Gliclazide			Glipizide		
Internal standard peak area (50µg ml^{-1})	Concentration (µg ml^{-1})	Peak area[a]	Peak area[b]	Response factor[a]	Response factor[b]	Concentration (µg ml^{-1})	Peak area	Response factor	Concentration (µg ml^{-1})	Peak area	Response factor
30 772 59[a]	62.50	1 999 396	2 021 396	0.6497	0.6012	10	919 128	0.2986	0.6250	1 203 469	0.3579
33 621 21[b]	93.75	2 999 097	3 045 097	0.9746	0.9057	15	1 378 692	0.4480	0.9375	1 805 211	0.5369
	125.00	3 998 791	4 048 091	1.2994	1.2040	20	1 838 256	0.5973	1.2500	2 406 948	0.7159
	156.25	4 998 479	5 060 113	1.6243	1.5050	25	2 297 817	0.7467	1.5625	3 008 685	0.8948
	187.50	5 998 179	6 064 179	1.9419	1.8036	30	2 757 391	0.8960	1.8750	3 610 422	1.0738

* Metformin: concentration[a] = 96.485 × response factor − 0.1646; concentration[b] = 103.93 × response factor − 0.0712. Gliclazide: concentration = 33.48 × response factor − 0.0018. Glipizide: concentration = 1.75 × response factor + 0.004.

[a] Combination-I.

[b] Combination-II.

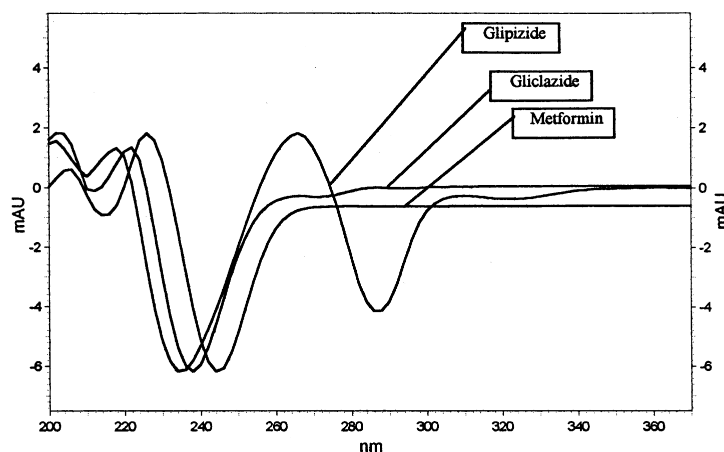

Fig. 3. Overlaid first derivative spectrum of metformin, glipizide and gliclazide in sample solution.

Table 3
System suitability studies

Sample number	Parameters	Metformin	Tolbutamide	Gliclazide	Glipizide
1	Theoretical plate[a]	2681.36[b] 2930.66[c]	1852.39[b] 3325.38[c]	2681.36	6657.84
2	Resolution	–	7.9[b] 10.2[c]	3.2	6.0
3	Asymmetric factor	1.0[b] 1.0[c]	1.03[b] 1.02[c]	0.99	1.01
4	LOD (ng ml^{-1})	10[b] 10[c]	25[b] 25[c]	25	25
5	LOQ (ng ml^{-1})	25[b] 25[c]	100[b] 100[c]	100	100

[a] Per column length.
[b] Combination-I.
[c] Combination-II.

of the regression equations were given in Table 2. The percentage RSD values were 0.216 and 0.127 for metformin and gliclazide (combination-I) and 0.283 and 0.168 for metformin and glipizide (combination-II).

The LOD and LOQ values for metformin, gliclazide, glipizide and tolbutamide are presented in Table 3. The ruggedness of the method was studied by observing the chromatographic pattern when slight changes were made in experimental conditions. A study of the chromatographic pattern showed that the method is rugged. The stability of the solutions were studied and the data obtained showed that the mobile phase, sample and standard solutions were stable up to 3 days when these were stored at about 5°C (in the refrigerator). System suitability studies were also carried out to determine column efficiency, resolution and peak asymmetry (Table 3).

The developed HPLC method is thus simple, accurate, precise, linear and rapid. Hence, this method is suitable for the quality control of raw materials, formulation and can be applied for dissolution studies.

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