ORIGINAL ARTICLE

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ANALYSIS OF METFORMIN

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ABSTRACT

The reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed to quantify metformin hydrochloride (MfCl) in raw material and pharmaceutical formulations using C_{18} analytical reverse-phase column. Diazepam was used as an internal standard. Mobile phase consisted of methanol-water (30:70 v/v), pumped at a flow rate of 0.5 ml/min at ambient temperature and the retention time was about 4.4 min with symmetrical peaks. (MfCl) was detected by ultraviolet absorbance at 233 nm with no interference of commonly used excipients. The method was linear over the concentration range 0.312–5 µg/mL (R^2 = 0.9995). The limit of detection of metformin was 0.1 µg/mL and the limit of quantitation was 0.3 µg/mL. The results obtained showed a good agreement with the declared contents in case of pharmaceutical formulations. The proposed method is rapid, accurate, economical and selective and it may be used for the quantitative analysis of metformin in Neodipar tablets because of its sensitivity and reproducibility.

Keywords: RP-HPLC; metformin; neodipar tablets.

INTRODUCTION

Metformin, N,N-dimethylimidodicarbonimidic diamide hydrochloride (fig. 1) is a biguanide derivative, known to posses antihyperglycemic activity because it does not normally cause hypoglycemia. It lowers blood glucose level in NIDDM patients by suppressing hepatic glucose output and enhances peripheral glucose uptake and insulin sensitivity. The mechanism of action involves binding of the a-polar biguanide hydrocarbon side-chain to membrane phospholipids, evoking a change in the electrostatic surface potential (Hermann, 1979). Subsequently, various metabolic effects are elicited, depending on the target cell, tissue, organ, species (Bailey, 1985) and metabolic regulation (Hermann, 1981).

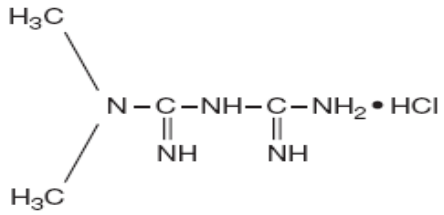

Fig. 1. Structure of Metfomrin N,N-dimethylimidodi-carbonimidic diamide hydrochloride.

Several methods have been reported for the determination of metformin by gas chromatography (Tache *et al*., 2001; Lin *et al*., 2001), capillary electrophoresis (El-Khateeb *et al*., 1988; Song *et al*., 1998), NMR spectrometry (El-Khateeb *et al*., 1988), fluorimetry (Hassan *et al*., 1999), UV-visible spectrophotometer (Hassan *et al*., 1999; Ashour *et al*., 2003; El-Bardicy *et al*., 1989), potentiometry (Hassan *et al*., 1999), PVC membrane sensor (Calatayud *et al*., 1985; Rizk 1995), conductometry (Calatayud *et al*., 1985; Aboudan *et al*., 2001), voltammetry (Liu *et al*., 2001) by RP-HPLC in multi component dosage forms (Vasudevan *et al*., 2001; Schaffer, 1983) in combined dosage forms (Kolte *et al*., 2004; Khanolkar *et al*., 1999; Kolte *et al*., 2004) in human serum (Zarghi *et al*., 2003; Yuen *et al*., 2002; Yuen *et al*., 1998; Klip *et al*., 1990; Tache *et al*., 2001). All these methods are time consuming, expensive, complex in nature and damage the susceptibly of the column. In these methods mobile phases used are mostly expensive and hazardous for the column health and efficiency (theoretical plates). Consequently, the presently developed method is simple, efficient and economical for the determination of metformin in reference drug and in dosage formulations.

EXPERIMENTAL

Instrumentation

The liquid chromatographic system consisted of Shimadzu LC-10AT VP gradient pump, a Shimadzu model SPD-10ADT VP variable wavelength UV-visible detector (Shimadzu Corporation, Kyoto, Japan). Chromatographic system was integrated via Shimadzu model CBM-102

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communication Bus Module. Analysis was conducted on a OD-5-100, C_{18} μ-bondapack column (0.4 x 25cm) with 0.5 μm particle size at ambient temperature. Sample was introduced in described chromatographic system by means of Rheodyne injector valve with a 20 μL sample loop.

Parameters	Conditions
Method	Reversed phase high performance liquid chromatography
Mobile phase	Gradient elution water-methanol (70:30)
Column	OD-5-100, C_{18} μ-bondapack column (0.4 x 25cm) with 0.5 μm particle size
Flow rate	0.5 ml/min
Detection	UV-detector, 233 nm
	200 – 800 nm for peak purity testing
Column temp	Ambient temperature
Injection	20μl for assay and compatibility
Volume	Samples 50 μl for dissolution

Materials

All chemicals used in this method were HPLC grade from Merck. Reference standard of metformin was a gift from Merck Marker Pakistan Ltd. and Neodipar® tablets (500mg) were purchased from the market manufactured by Sanophy Aventis.

Preparation of stock solution

Samples of different concentrations of metformin reference were prepared from stock solution which was prepared by accurately weighing about 10 mg metformin and transferring to 100 ml volumetric flask, then methanol/water (50:50 v/v) added as a diluent, the sample was dissolved completely by shaking on an ultrasonic bath, the volume made up to the mark with diluents, mixed well, filtered and serially diluted to make 5, 2.5, 1.25, 0.625 and 0.312 μg/ml concentrations**.** This was then filtered through 0.45 micron membrane filters.

Twenty Neodipar tablets (each containing 500 mg active) were accurately weighed and finely powdered. An amount equivalent to 10 mg metformin was dissolved in ultrasonic bath for 20 minutes and diluted to 100 ml with methanol: water (50:50 v/v). This was further diluted ten folds and dilutions of known concentrations in the range 0.312 - 5μg/ml were prepared. Diazepam was added as an internal standard (40μg/ml). Resulting solutions were injected in the column.

RESULTS AND DISCUSSION

In the present investigation optimum results were achieved using the described column specifications and solvent system. Diazepam did not interfere in our analysis and was used as an internal standard. Under same experimental conditions, duration of separation was shorter and observed peak symmetry was better. Optimum retention times (metformin = 4.4 min. and diazepam = 6.1 min) were achieved.

Assay procedure and validation

The mobile phase consisted of methanol: water (30:70 v/v) which was delivered gradiently at 0.5 ml/min, having retention time 4.4 minutes with insignificant (n = 80) relative standard deviation values. To obtain the optimum retention time, the composition of mobile phase was varied and best results were achieved in the above stated composition. A representative chromatogram showing separation of metformin and internal standard is given in figure 2. The results given in table 1 showed that the precision of this method was acceptable. For the assessment of inter-day and intra-day precision, complete set of metformin reference standards in mobile phase were injected (n = 80) at 4 different times over 12 hour period for four days.

Accuracy and precision

Accuracy and precision calculated for the QC samples during the intra- and inter-day run are given in table 1. The intra-day (day 1) accuracy ranged from −3.86% to −0.01%. The results obtained from intermediate precision (inter-day) also indicated a good method precision. All the data were within the acceptance criteria.

System suitability

The system suitability was assessed by five replicate analyses of the drug at a concentration of 5 μg/ml. The percent coefficient of variation (%CV) of peak area and retention time for both drug and internal standard were within ± 2% indicating the suitability of the system (table 2).

Limit of detection and quantitation

The lowest amount of analyte in a sample that can be detected but not necessarily quantitated or LOD was expressed as a concentration that gives a signal to noise ratio of 2:1 or 3:1 (Aboudan *et al*., 2001). The lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions or quantitation limit LOQ was measured in terms of signal to noise ratio of 10:1 (Aboudan *et al*., 2001). Accordingly, the LOD and LOQ were calculated by the following:

$$LOD = \frac{3.3\ \sigma}{S} \quad \text{and} \quad LOQ = \frac{10\ \sigma}{S}$$

Where, σ and S are the standard deviation and slope respectively. The lower limits of detection and quantitation for MfCl were found to be 0.1 and 0.3 µg/ml, respectively.

Linearity (calibration curve)

The calibration curve was constructed with five concentrations including the LOQ ranging from 0.312 to 5 µg/ml (figure 3). The peak area of the drug to the concentration was considered for plotting the linearity graph. Standard deviations of the slope and intercept for the calibration curves generated on four different days were 0.002 and 0.012, respectively. The correlation coefficient (r^2) of all the calibration curves were consistently greater than 0.9995.

Application of the method to dosage formulation

This developed HPLC method was sensitive and specific for the quantitative determination of metformin from dosage formulation. The method was applied for the estimation of drug in 'Neodipar tablets. The amount of MfCl present in the formulation was evaluated in 250 mg strength tablets manufactured by Sanophy Aventis. Each sample was analyzed in triplicate after sample preparation as mentioned in the experimental section. The recovery of metformin

Table 1

	Nominal concentration				
	0.312 µg/ml	0.625 µg/ml	1.25 µg/ml	2.50 µg/ml	5.00 µg/ml
Day 1					
Mean	0.300	0.623	1.247	2.500	5.011
S.D.	0.008	0.002	0.002	0.000	0.007
CV	2.823	0.246	0.151	0.005	0.148
%Bias	-3.84	-0.346	-0.21	-0.01	-0.21
Day 2					
Mean	0.309	0.603	1.221	2.448	4.936
S.D.	0.002	0.016	0.020	0.037	0.046
CV	0.784	2.600	1.663	1.509	0.918
%Bias	-1.09	-3.55	-2.23	-2.09	-1.28
Day 3					
Mean	0.309	0.619	1.232	2.495	4.997
S.D.	0.002	0.004	0.014	0.003	0.002
CV	0.785	0.637	1.033	0.133	0.047
%Bias	-1.09	-0.89	-1.44	-0.19	-0.07
Day 4					
Mean	0.309	0.625	1.060	2.263	5.033
S.D.	0.002	0.000	0.134	0.164	0.023
CV	0.678	0.046	12.67	7.397	0.463
%Bias	-0.95	-0.07	-15.19	-9.47	-0.66

Each mean value is the result of triplicate analysis.

Table 2

	Metformin (5.0 µg/ml)		Internal standard (40.0 µg/ml)	
	Retention time (min)	Peak area	Retention time(min)	Peak area
Mean (n= 5)	4.4	459411.2	6.1	75035.15
S.D.	0.014	1142.58	0.006	1327.33
% CV	0.32	0.249	0.10	1.785

hydrochloride in 'Neodipar tablets' was found to be 98.88% (table 3). None of the tablet excipients interfered with the analyte peak.

Table 3: Recovery characteristics of metformin in dosage formulations

Conc. µg/ml	Peak area	Found conc. (µg/ml)	Percent Recovery
0.312	28843	0.324	103.90
0.625	57441	0.618	98.88
1.25	106445	1.248	99.87
2.50	225490	2.502	100.07
5.00	455857	5.017	100.34

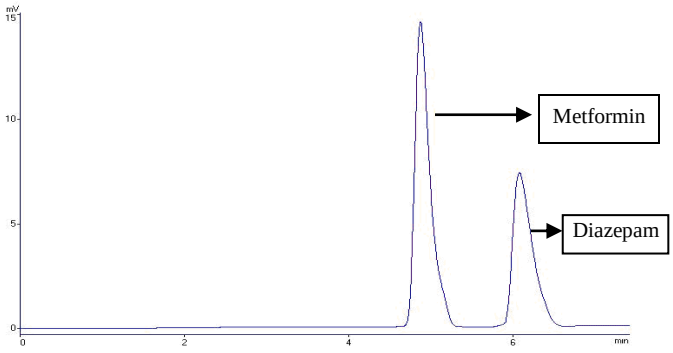

Fig. 2: A representative chromatogram showing separation of metformin and internal standard.

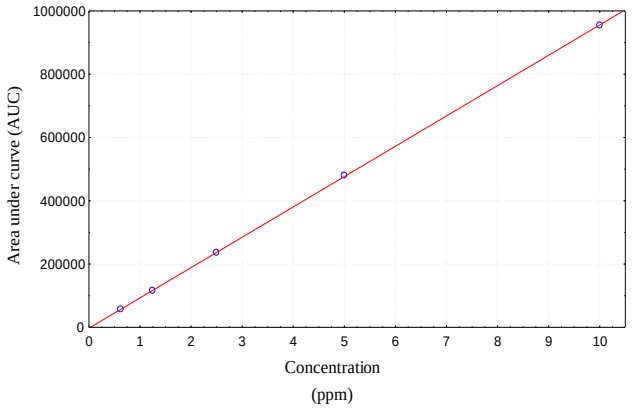

Fig. 3: Linearity of metformin in methanol: water (30: 70 v/v).

CONCLUSIONS

The developed RP-HPLC method is able to determine metformin in raw materials and in all dosage formulations because of a good separation and resolution of the chromatographic peaks with optimum retention time of 4.4 minutes. The method enables the determination of metformin, linearly as low as 0.3 µg with a relative standard deviation up to ±0.1, while this method hold effective for LOD of 0.1 µg. There was no interference of varying parameters observed during inter-personal and inter-operator variations, inter-day and intra-day determination as reported in tables 1. The obtained results were in good agreement with the declared statistical and Pharmacoepial contents. Hence, the precision, reliability, rapidness, simplicity, sensitivity and economical nature of this HPLC method makes it advantageous to the other reported HPLC techniques for the assay of metformin.

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