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Der Pharmacia Lettre, 2014, 6 (6):76-81
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

A new validated RP-HPLC method for simultaneous estimation of Itopride and Metformin HCl in bulk formulation

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ABSTRACT

An accurate & precise liquid chromatographic method was developed for the simultaneous estimation of Itopride (ITP) and Metformin (MET) in API matrix. The chromatographic analysis was performed on Shimadzu HPLC equipped with UV-Visible and Diode Array detectors, Column used was Waters XTerra RP C_{18} column, 5 µm particle size, 250 mm × 4.6 mm i.d, at ambient temperature. The mobile phase consisting of Phosphate Buffer: Methanol (40:60 v/v), and the pH of phosphate buffer was adjusted to 3.5, using Ortho Phosphoric acid (OPA). The flow rate was maintained at 0.8 ml/min and the eluent was monitored at 238 nm. The calibration curves of peak area versus concentration, which was linear from 1-20 µg /mL for ITP and 16-240 µg/mL for MET respectively. The retention times of ITP and MET were found to be 3.47 and 7.34 min respectively. The method had the requisite accuracy, precision and robustness for simultaneous determination of ITP & MET in API matrix. The percentage assays of ITP & MET in API were found out to be 99.27 % and 98.55 % respectively. The method was validated by determining its accuracy, precision and system suitability. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of ITP & MET in bulk drug and in its pharmaceutical dosage forms.

Keywords: Itopride (ITP), Metformin (MET), RP-HPLC, Estimation

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INTRODUCTION

Itopride is a prokinetic bezamide derivative unlike metoclopromide or domperidone. These drugs inhibit dopamine and have a gastrokinetic effect [1]. Itopride is indicated for the treatment of functional dyspepsia and other gastrointestinal conditions [2]. Itopride, (2(*S*), 4(*S*), 5(*S*), 7(*S*)- *N*-(2-carbamoyl-2-methylpropyl)- 5-amino- 4 hydroxy 2, 7 diisopropyl -8- [4-methoxy-3- (3-methoxypropoxy) phenyl] octanamide hemifumarate) **(Figure.1).** The first oral direct renin inhibitor approved for clinical use, exhibits a novel and advantageous pharmacokinetic and pharmacodynamic profile for the long-term treatment of hypertension. Itopride blocks the renin system at its rate-limiting step by directly inhibiting the catalytic activity of renin, thereby reducing generation [3, 4] of angiotensin I and angiotensin II. Itopride is indicated in the treatment of GI symptoms caused by reduced GI motility: dyspepsia of a non-ulcer/dysmotility type (gastric "fullness", discomfort, and possible pain) and delayed gastric emptying [5].

Metformin hydrochloride chemically, N, N-dimethylimidodicarbonimidic diamide hydrochloride **(Figure 2),** is an antidiabetic agent [6-9]. Surveys of pertinent literature revealed few LC-MS/MS[10, 11], HPLC [9-12] & Spectrophotometric methods [13-16] have been reported individually for the estimation of ITP & MET in pharmaceutical dosage forms at the time of commencement of these investigations. Detailed accounts of all analytical methods existing for the drug are made to avoid duplication of the method developed. The authors had made some humble attempts, hoping to fulfill and bridge this gap, in succeeding the developing analytical methods, by using HPLC system. The results of this labor of love are set forth by developing a simple, precise and accurate RP-HPLC method for the estimation of ITP & MET in bulk matrix.

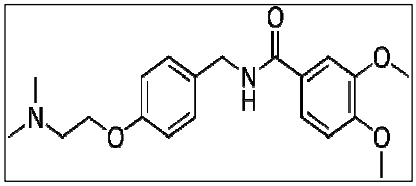

Figure 1: Itopride

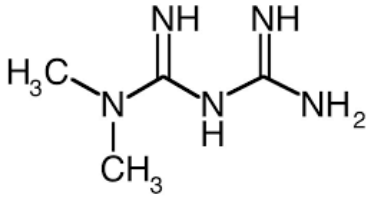

Figure 2: Metformin

MATERIALS AND METHODS

Materials

ITP and MET Working standards were gifted by Lupin Pharma, Pune, India. The reference sample of ITP & MET was supplied by Torrent Pharmaceutical Industries Ltd., Ahmadabad. HPLC grade water and Methanol were purchased from E. Merck (India) Ltd., Mumbai. Potassium Dihydrogen phosphate, Triethylamine and Orthophosphoric acid of AR Grade were obtained from S.D. Fine Chemicals Ltd., Mumbai.

Instrumentation

The present work was carried out on Shimadzu HPLC equipped with UV-Visible and Diode Array detectors.. Glassware's used were of 'A' grade and were soaked overnight in a mixture of chromic acid and sulphuric acid, rinsed thoroughly with double distilled water and dried in hot air oven. A Bandline Sonerex sonicator was used for enhancing the dissolution of the compounds. A Digisum DI 707 digital pH meter was used for the adjustments.

Optimized Chromatographic Conditions

The analysis of the drug was carried out on a Shimadzu HPLC system equipped with a column of Waters XTerra RP C_{18} column, 5 µm, 250 mm × 4.6 mm, the binary pump, a 20 µL injection loop and a dual absorbance detector and running on Shimadzu. The UV spectrum of the drugs was taken using ELICO SL-159 UV-Visible spectrophotometer. A mixture of phosphate buffer and Methanol in the ratio of 60:40 v/v was found to be the most suitable mobile phase for ideal separation of ITP & MET, at a flow rate of 0.8 ml/min with a runtime of 20 minutes. Prior to the use the mobile phase was degassed by ultrasonic bath and filtered by Millipore vacuum filter system equipped with a 0.45 µm high vacuum filter. Both the drugs were detected and quantified at 238 nm.

Preparation of standard solutions

ITP & MET Stock and working solution

About 100 mg of ITP and 100 mg of MET was weighed accurately, transferred into a 100 ml volumetric flask and dissolved in 25 ml of a 60:40 v/v mixture of phosphate buffer and Methanol. The solution was sonicated for 15 min and the volume made up to the mark with a further quantity of the diluent to get a 1000 µg/mL solution. From this, a working standard solution of the drugs was prepared by diluting the above solution to 10 ml in a volumetric flask. Further, the final concentrations obtained ranges from 1-20 µg/mL for ITP and 16-240 µg/mL for MET respectively.

Method Validation

The method was validated in accordance with ICH guidelines[17, 18]. The parameters assessed were Linearity, Accuracy, Limit of detection (LOD), Limit of Quantification (LOQ), Precision, Reproducibility, Robustness and System Suitability tests.

Linearity

Aliquots of standard ITP & MET stock solutions were taken in different 10 ml volumetric flasks and diluted up to the mark with the mobile phase such that the final concentrations of ITP & MET were in the range of 1-20 µg/mL & 16 – 240 µg/mL respectively. Each of these drug solutions (20 µL) was injected three times into the column, and the peak areas and retention times were recorded. Evaluation was performed with UV-730D detector at 238 nm and the calibration graph was obtained by plotting peak area versus concentration of ITP & MET. The plot of peak areas of each sample against respective concentration of ITP & MET was found to be linear in the range of 1-20 µg/mL & 16-240 µg/mL with correlation coefficient of 0.999 for both the API matrix. Linear regression least square fit data obtained from the measurements are given in **Table I**. The respective linear regression equation being Y= 60153.876x + 9366.2787 for ITP & Y = 88430.983x + 126513.1959 for MET. The regression characteristics, such as slope, intercept, and %RSD were calculated for this method and given in **Table I.**

Accuracy

Accuracy was evaluated in triplicate by addition of three different amounts of ITP & MET to a previously analyzed samples and comparing the amounts of analytes recovered with the amounts added. The amounts added were equivalent to 80, 100, and 120% of the amount originally present. From the data obtained, it is obvious that the method is remarkably accurate, which ensures that this method produces reliable results as depicted in **Table: II**. The accuracy was expressed as percent analyte recovered by the proposed method.

Precision

The precision of an analytical method is the degree of agreement among the individual test results, when the method is applied repeatedly to multiple sampling of homologous samples. The precision of the method was checked by repeatability of injection, repeatability (intra-assay), intermediate precision (inter-assay) and reproducibility. Injection repeatability was studied by calculating the percentage relative standard deviation (% RSD) for ten determinations of peak areas of ITP (10 µg/mL) and MET (64 µg/mL), performed on the same day. For both intra- and inter-assay variation, standard solutions of ITP (5, 10 and 15 µg/mL) and MET (32, 64 and 128 µg/mL) were injected in triplicate. The % Relative Standard Deviation (RSD) and % range of error (at 0.05 and 0.01 confidence levels) were calculated and presented in **Table: I** respectively.

Limit of Detection and Quantitation

Limit of Detection (LOD) of the method was determined as the lowest concentrations of active pharmaceutical ingredients producing a signal-to-noise (S/N) ratio of about 3. The Limit of Quantitation (LOQ) was determined as the lowest concentrations of active pharmaceutical ingredients capable of being quantified with acceptable accuracy and precision producing signal-to-noise (S/N) ratio of about 10.

Method Applicability & System Suitability

System suitability is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The present developed method was evaluated by applying to pharmaceutical dosage forms for the estimation of ITP & MET by our research group. To ensure the validity of the analytical procedure, a system suitability tests were established. The following parameters like theoretical plate number (N), tailing factor, retention time, resolution, area and % peak area were analyzed by using

20 µL of the working standard solution containing ITP (10 µg/mL) & MET (64 µg/mL) injecting six times into HPLC system.

Specificity of the Analyzed Method

Specificity was measured as ability of the proposed method to obtain well separated peak for ITP & MET without any interferences from component of the API matrix. The specificity of the method was also checked for the interference in the analysis of a blank solution (without any sample) and then a drug solution (API) was injected into the column, under optimized chromatographic conditions to demonstrate the separation of both ITP & MET from any form of the interference, if present. With the analyzed chromatographs obtained, it clearly depicts there were no interferences and also no change in the retention times, thus enhancing a benchmark that the method found was specific and also confirmed with the results of analysis with the standard. The mean retention time for ITP & MET were found out to be 3.47 and 7.34 minutes respectively.

RESULTS AND DISCUSSION

HPLC Method Development and Optimization

In response to lack of simple, reliable and easy-to-use method for the determination of ITP & MET concentrations in pharmaceutical matrices, an isocratic RP - HPLC method was developed for quantification of above mentioned, API. Nevertheless, there is need to consider the successive steps for the development of the method. In fact, the problems relating to the standardization of sample preparation and selection of mobile phase needs to be emphasized. The authors examined several HPLC method variables with respect to their corresponding effects on the result of analysis. To optimize the chromatographic conditions, different combinations of Methanol-Water, Acetonitrile-Water, and Acetonitrile-Methanol were tested. Phosphate Buffer: Methanol (40.60 v/v), and the pH of phosphate buffer was adjusted to 3.5, using Ortho Phosphoric acid (OPA) which was promisingly preferred, because it resulted in greater resolution of API after several preliminary investigatory runs, compared with other mobile phases. The other parameters in this factorial design were temperature, flow rate, detection wavelength and volume of injection. Buffer molarity was changed and optimum buffer strength was selected as 0.01M on the basis of theoretical plate number. At 238 nm, UV responses of all two active pharmaceutical analytes were good and free form interferences. Under these conditions, the analyte peaks were well defined and free from tailing. Considering the whole body of the data obtained from this extensive study, the set of conditions indicated earlier in this article was selected for further validation. Typical chromatogram of ITP & MET has been shown in **Figure: 3,** the system suitability tests were carried out on freshly prepared standard stock solutions of ITP & MET. Parameters that were studied to evaluate the suitability of the system were discussed and presented in **Table 2**.

Method Validation Test

Recommended method[18] validation characteristics including method precision (RSD, %), method accuracy (Recovery % and RSD, %), linear range (Correlation Coefficient), and LOD & LOQ, were investigated systematically.

Linearity

The plot of peak areas of each API against respective concentrations were found to be linear, in the range of 1 –20 µg/mL & 16-240 µg/mL for ITP & MET with correlation coefficient of 0.999 for ITP & 0.999 for MET (**Table: 1)**. Linear regression least square fit data obtained from the measurements are given in **Table: 1.** The respective linear regression equation being Y= 60153.876x + 9366.2787 for ITP & Y = 88430.983x + 126513.1959 for MET respectively. The regression characteristics, such as slope, intercept, and %RSD were calculated for this method and given in **Table 1.** These results show that there was an excellent correlation between peak areas and analyte concentration.

Accuracy

Recovery of the individual substances (API) at 80%, 100%, and 120% of specified concentrations were between 99.84-100.50%, which proves the accuracy of the method. From these data obtained, RSD was always less than 1%, which indicates it is obvious that the method is remarkably accurate, produces reliable results (**Table: 1**).

Precision

The low value (<1%) of RSD indicates the repeatability of the method. These data indicate a considerable degree of precision and reproducibility for the method both during one analytical run and between different runs (**Table: 1**).

Limit of Detection (LOD) and Limit of Quantification (LOQ):

The Limit of Detection (LOD) & The Limit of Quantification (LOQ) analyzed were found to be 1.2 & 3.7 µg/mL for ITP & 0.7 & 2.3 µg/mL for MET respectively. These values reflect the high sensitivity of the method, which is of great importance in most studies and also indicating the method can be used for detection and quantification of analytes in a very wide concentration range.

Specificity

No evidence of signals, in the corresponding times of the chromatogram were monitored as a sign of potential interfering peaks, were found when the pharmaceutical API formulations were tested. Hence, this method can be used reliably for the estimation of respected active pharmaceutical ingredients in a variety of dosage forms in the nearby future.

TABLE 1: LINEAR REGRESSION DATA OF CALIBRATION CURVE & PROPOSED METHODS

PARAMETER	ITOPRIDE (ITP)	METFORMIN (MET)
Concentration range (µg/mL)	1 - 20	16-240
Slope (m)	60153.876	88430.983
Intercept (Y)	17123.5453	115944.2195
Standard error of estimate (c)	9366.2787	126513.1959
Correlation coefficient (r)	0.998	0.999
Linear regression coefficient (r^2)	0.997	0.998
%RSD	1.05	0.84

Table 2: Validation Summary / System Suitability:

PARAMETER	ITP	MET
Theoretical Plates(N)	6557.842	5892.03
Tailing factor	1.22	1.56
Retention time (min)	3.47	7.34
Resolution	-----	1.325
Area	4796299	50789325
% Peak Area	99.730	96.58
LOD (µg/mL)	1.2	0.7
LOQ (µg/mL)	3.7	2.3

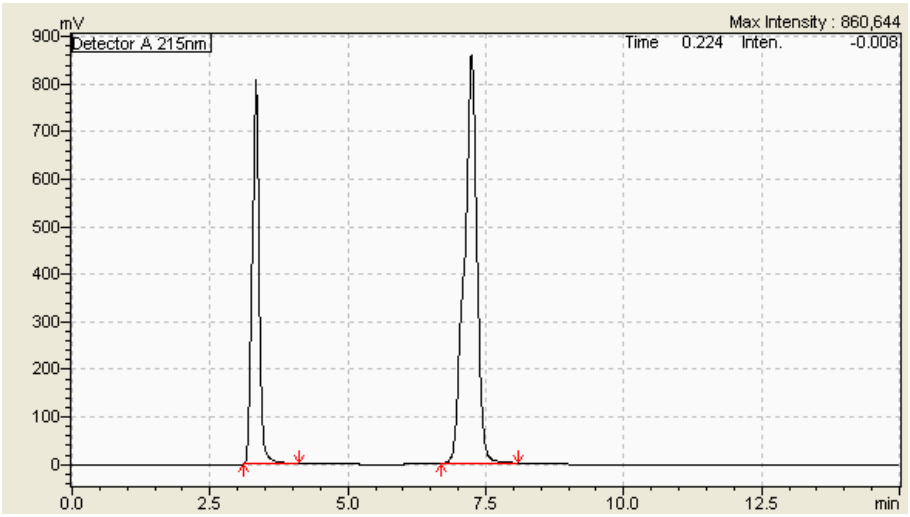

Figure: 3: Typical chromatogram of Itopride and Metformin

CONCLUSION

A simple and easily available HPLC method was developed in this study, for the quantification of ITP & MET in pharmaceutical matrices. The main advantages of this method are its considerably shorter run times, easy-to-use and its simplicity. All of these properties are very important in practice, particularly when a large number of samples are to be analyzed. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the formulation. The results of validation tests were, collectively, indicative for a method with a relatively wide linear range, acceptable precision and accuracy and practically reliable sensitivity. The method enables simple, selective, sensitive, and specific analysis of ITP & MET can be used for routine analysis in pharmaceutical quality control within a short time.

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