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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR QUANTITATIVE ANALYSIS TOLBUTAMIDE IN PURE AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A validated simple, sensitive, specific and precise RP-HPLC method was developed for the determination of Tolbutamide in pure and pharmaceutical formulations. Analysis was carried on Zodiac C_{18} column (250 mm × 4.6 mm × 5 µ particle size) using Methanol : 0.1% Orthophosphoric acid : Acetonitrile (10 : 30 : 60) as mobile phase. Detection was carried out by U.V at 231 nm. The proposed method obeyed linearity in the range of 20-120 µg/mL and met all specifications as per ICH guidelines. Statistical analysis revealed that this method can be used in routine quality control studies of Tolbutamide in pure and its formulations.

Key words: Tolbutamide, C_{18} column, Reverse phase, Validation.

INTRODUCTION

Tolbutamide, is chemically-N-(butyl amino) carbonyl-4-methylbenzene sulfonamide. It is used in the treatment of type-II Diabetis mellitus. It is official in I.P[2], B.P[3] and Martindale[1]. In literature, a few methods have been described for the determination of Tolbutamide[4-8]. In the present study, a new RP-HPLC method was developed, which shows high reproducibility and sensitivity. The developed method was validated as per ICH guidelines.

EXPERIMENTAL

Instrumentation

To develop a high pressure liquid chromatographic method for quantitative estimation

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of Tolbutamide, an isocratic peak HPLC instrument with Zodiac C-18 column (250 mm x 4.6 mm, 5 µ) was used. The instrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable LC 7000 UV-detector. A 20 µL Rheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software.

Standards and chemicals used

Tolbutamide was provided by Ranbaxy laboratories, Guargon. All the chemicals acetonitrile, methanol,water were HPLC grade, Merck Specialties Private Limited, Mumbai, India. LR grade orthophosphoric acid was purchased from Quligens Private Limited, Hyderabad. Commercial tablets of Tolbutamide were purchased from local market.

Preparation of the mobile phase

Into a 1000 mL cleaned volumetric flask, HPLC grade methanol (100 mL), 0.1% orthophophoric acid (300 mL) and acetonitrile (600 mL) (which are filtered through 0.25 mm membrane filters by vacuum filtration) were slowly added, mixed well and sonicated upto 20 min. The above solution was cooled and pH was adjusted to 5.9 with orthophosphoric acid. This solution was again sonicated to 10 min. The solution was cooled to room temperature and used for chromatography method.

Preparation of standard drug solutions

100 mg of Tolbutamide was accurately weighed and dissolved in few mL of the mobile phase and sonicated for few min. to dissolve the drug completely. Then it was filtered through 0.25 µm membrane filter paper and the volume was made upto 100 mL with mobile phase to get a concentration of 1 mg/mL (free base) stock solution. This solution was further diluted with same solvent to obtain required working standard concentrations.

Sample preparation

20 Commercial tablets (Rastinone-500 mg) of Tolbutamide were finely powdered and the powder equivalent to 10 mg of Tolbutamide was accurately weighed. Then it was dissolved in few mL of mobile phase in 50 mL volumetric flask. The above solution was subjected to sonication for 15 min. After getting clear solution, it was filtered through 0.25 µm membrane filter and the solution was made upto 50 mL with mobile phase resulting in preparation of 1 mg/mL solution. This was further diluted so as to obtain required concentration of Tolbutamide pharmaceutical dosage form.

Methodology

The HPLC system was stabilized for thirty min. by passing mobile phase, detector

was set at 231 nm and flow rate of 1.0 mL/min was maintained to get a stable base line. One blank followed by six replicates of a single standard solution was injected to check the system suitability. Eight replicates of each standard solutions 20, 40, 60, 80, 100 and 120 µg/mL were injected. Calibration graph was plotted by concentration of Tolbutamide on X-axis and peak area on Y-axis. The amount of drug present in sample was computed in calibration graph.

Pharmaceutical formulations

Prepared dilution of pharmaceutical formulation was injected and the procedure described under bulk samples was followed. The amount of drug present in sample was computed in calibration graph. Chromatographic conditions for estimation of Tolbutamide a described in Table 1.

Table 1: Optimized chromatographic conditions for Tolbutamide

Api concentration	80 µg/mL
Mobile phase	Methanol : 1% Orthophosphoric acid : Acetonitrile in the ratio of 10 : 30 : 60 v/v
Wavelength	231 nm
Column	C_{18} Column
pH	5.9
Concentration	80 µg/mL
Retention time	4.60 min
Run time	10 min
Area	240056
Th. Plates	9814
Tailing factor	1.32
Pump Pressure	13.0 MPa

RESULTS AND DISCUSSION

The objective of the present work is to develop simple, precise and reliable HPLC method for the analysis of Tolbutamide in bulk and pharmaceutical dosage form. This was achieved by using the most commonly employed column C_{18} with U.V. detection at 231 nm. The representative chromatogram indicating Tolbutamide is shown in Fig. 1.

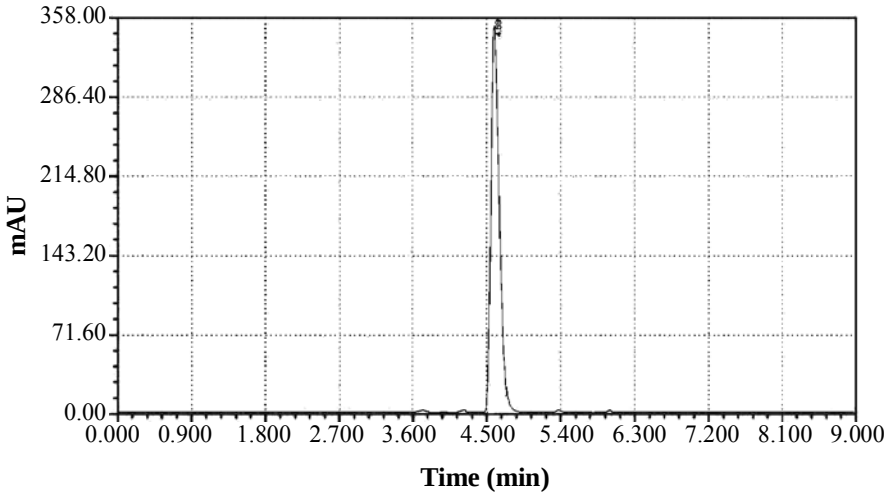

Fig. 1: Chromatogram of Tolbutamide

Parameter fixation

In developing this method, a systemic study of effects of various parameters was undertaken by varying one parameter at a time and controlling all other parameters. The following studies were conducted for this purpose.

Stationary phase characteristics

Based on nature and solubility characteristics of Tolbutamide, reverse phase mode of HPLC was selected for chromatography. Among different RP-HPLC stationary phases tried, C_{18} column was found to be optimum.

Mobile phase characteristics

In order to get sharp peak with base line separation from interfering peaks, a number of experiments were carried out by varying the composition of solvents and mobile phase flow rate. To have an ideal separation of the drug under isocratic conditions, mixtures of solvents like methanol, aceertonitrile with or without different buffers in different combinations were tested as mobile phase. A mixture of methanol : 0.1% orthophophoric acid: acetonitrile (10 : 30 : 60) was proved to be the most suitable of all the combinations, since the chromatographic peak obtained was better defined, resolved and almost free from tailing.

Validation of the proposed method

As an integral part of analytical method development is validation, the proposed method was validated as per ICH guidelines.

Linearity

It is the ability of the method to Elict test results directly propotional to analyate concentration within a given range. Linearity was performed by preparing standard solutions of Tolbutamide at different concentration levels. Twenty micro liters of each concentration was injected into the HPLC system. The peak responses were read at 231 nm and the corresponding chromatograms were recorded. Linearity plots of concentration over areas were constructed individually. Linearity results were obtained in the concentration range of 20-120 µg/mL. The results are presented in Table 2.

Table 2: Linearity results of tolbutamide

Concentration (µg/mL)	Area
20	57867
40	110648
60	179396
80	240056
100	305209
120	369780

Precision

Precision is the degree of repeatability of an analytical method under normal operational conditions. Precision of the method was performed as intraday precision and inter day precision.

Intraday precision

To study the intraday precision, six replicate standard solutions (60 ppm) of Tolbutamide were injected. The percent relative standard deviation (% RSD) was calculated and it was found to be 1.61, which are well within the acceptable criteria of not more than 2.

Interday precision

To study the interday precision, six replicate standard solutions (60 ppm) of Tolbutamide was injected on third day of sample preparation. The percent relative standard

deviation (% RSD) was calculated and it was found to be 1.79, which are well within the acceptable criteria of not more than 2.0.

Specificity

The effect of wide range of excipients and other additives usually present in the formulation of Tolbutamide in the determinations under optimum conditions were investigated. infact, may have no observation at this UV maximum chromatographic parameters maintained are specific for Tolbutamide.

Ruggedness

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC (LC2010 A HT), Aglient HPLC and water's Breeze HPLC by different operators using different columns of similar type like Hypersil C_{18}, Phenomenexy Gemini C_{18} and Hichron$_{C18}$. They didn't show any significant change.

Limit of detection and limit of quantification

A calibration curve was prepared using concentrations in the range of 20-120 µg/mL (expected detection limit range). The standard deviation of Y-intercepts of regression line was determined and kept in following equation for the determination of detection limit and quantitation limit.

The results are reported in Table 3.

Table 3: Limit of detection and limit of quantification of tolbutamide

LOD	5 µg/mL
LOQ	1.54 µg/mL

Accuracy

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed sample solution. The standard addition method was performed at 50%, 100% and 150% level of 40 ppm. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery was calculated and the results are presented in Table 4. Satisfactory recoveries ranging from 99% to 101% were obtained by the proposed method. This indicates that the proposed method was accurate.

Table 4: Recovery results

% Recovery	Tolbutamide				
	Target conc. (µg/mL)	**Spiked conc. (µg/mL)**	**Final conc. (µg/mL)**	**Conc. obtained**	**% of Recovery**
50%	40	20	60	59.9	99.9
	40	20	60	59.5	99.2
	40	20	60	60.08	100.1
100%	40	40	80	80.4	100.5
	40	40	80	80.6	100.7
	40	40	80	80.2	100.2
150%	40	60	100	99.4	99.4
	40	60	100	100.8	100.8
	40	60	100	100.8	100.8

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which means that the RP-HPLC method developed is robust. The results of robustness are given in Table 5.

Table 5: Robustness results

Parameter	Change	Area	% of change
Standard	……	240057	……
MP	Methanol: 1% OP : Acetonitrile		0.02
	5 : 30 : 65	240121	
	15 : 30 : 55	241323	0.52
pH	4.9	242904	1.18
	4.7	243222	1.01
WL	233 nm	241073	0.42
	229 nm	243045	1.24

Formulation

Formulation	Brand name	Prepared conc.	Area	% Assay	Amount found
Tolbutamide	Rastinone (Tab-500 mg)	80 µg/mL	235400	98.05	78.44

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