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Research Article

BIO-ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF PYRAZINAMIDE IN RABBIT PLASMA BY RP-HPLC

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A R T I C L E I N F O

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A B S T R A C T

Pyrazinamide (PYZ), is a first line drug used in treating tuberculosis. it is largely bacteriostatic but sometimes bactericidal on actively replicating tuberculosis bacteria. It is the least understood TB drugs. A rapid, sensitive, simple and cost-effective high performance liquid chromatographic method for the determination of PYZ by UV detection in rabbit plasma which includes extraction process using protein precipitation using a mixture of heptane-1-sulphonoic acid solution with water and acetonitrile in a ratio of 50:50 v/v. PYZ was eluted under isocratic mode using a Luna C18 column. A mixture of heptane-1-sulphonoic acid solution with water and acetonitrile in a ratio of 50:50 v/v was proved to be the most suitable of all the combinations since the chromatography peaks obtained were well defined and resolved and free from tailing. A mobile phase flow rate of 1.0 mL / min. was found to be suitable in the study range of 0.5-1.5 mL/min with a wavelength of detection at 268 nm. The injection volume was 10 µL. The run time of the method was 12 min. The method exhibited good linearity in the range 100-1500 ng/mL. The correlation coefficient (r2) value was found to be 0.999. Precision study showed the % CV value less than 2% in all selected concentrations. The lower limit of quantification (LLOQ) was found to be 100 ng/mL. While, accuracy of LLOQ was found to be 99.9% and %RSD was 0.04%, while the intra- and inter-day accuracies were around accuracies were 98-102% respectively. A rapid, sensitive, simple and cost-effective method for the estimation of pyrazinamide in rabbit plasma was developed and validated according ICH guidelines.

INTRODUCTION

Pyrazinamide is, chemically Pyrazine-2-Carboxamide. It is a first line drug to treat tuberculosis which is largely bacteriostatic and sometimes bactericidal on actively replicating tuberculosis bacteria. Pyrazinamide is the least understood drug among the TB drugs but it improves the TB treatment when used with other drugs. There is less work done on pyrazinamide[1,2,3] It has a half life 10-12 hours. Pyrazinamide is used in the first two months of treatment to reduce the duration of treatment required to six months. Regimen not containing pyrazinamide must be taken for nine months or more[4]. Its dose is also high generally taken as 15-30 mg/kg.

MATERIALS AND METHODS

Pyrazinamide was purchased from Sigma Aldrich. HPLC grade Methanol was procured from Fisher Scientific and others chemicals are of AR grade was purchased from Loba Chemie, India.

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Instrumentation

Chromatographic separation was performed on a Peak chromatographic system using Isocratic waters alliance 2695 HPLC system with UV-Visible detector. The chromatography studies were performed using Luna C18, 250 mm × 4.6mm, 5µm at ambient temperature[5]. Data acquisition was done by using empower version 2.0 software. Sonicator (Power sonic 510, Hwashin technology) Ultrasonicator was used to sonicate the mobile phase and samples. Standard and sample drugs were weighed by using Sartorius electronic analytical balance (SI-234) and pH of the mobile phase was adjusted by using Orion digital pH meter.

Preparation of stock and standard solutions

A stock solution of mg/mL (1000µg/mL) was prepared by accurately weighing 25 mg of the standard drug dissolved in 25mL of methanol. A standard concentration of 1000µg /mL was obtained. The solution was filtered and was used as standard stock solution. The solution was filtered through 0.45 µg/mL nylon membrane filter paper and was used as standard stock solution. The stock solutions were preserved safely and were used when it required. Further dilutions were prepared as per the standard procedure.

Preparation of Buffer

An accurately weighed quantity of 2.5 gm of heptane-1-sulphonic acid taken into 1lt beaker and 1lt water was added, sonicated for 5 mins to get dissolved. pH was adjusted to pH 2.5 with ortho phosphoric acid.

Method development

Mobile phase and the flow rate

In order to get sharp peaks and baseline separation of the components, a number of experiments were carried out by varying the commonly used solvents, and their compositions and flow rate were evaluated. To effect ideal separation of the drug under isocratic conditions, mixtures of commonly used solvents like water, methanol and acetonitrile with or without buffers in different combinations were tested as mobile phases on a C18 stationary phase. A mixture of heptane-1-sulphonoic acid solution with water and acetonitrile in a ratio of 50:50 v/v was proved to be the most suitable of all the combinations since the chromatography peaks obtained were well defined and resolved and free from tailing. A mobile phase flow rate of 1.0 mL / min. was found to be suitable in the study range of 0.5-1.5 mL/min.

Extraction process of plasma samples and their drying

Prior to sample analysis, to about 250 µL of sample, 250 µL of diluent was added and mixed well. Further added 500 µL of Acetonitrile to precipitate all the proteins and mixed in vortex cyclo-mixture with subsequent Centrifugation at 4000 RPM for 15 – 20 min. The supernatant was finally collected and directly injected for analysis. This procedure was followed for all samples of calibration curve plasma spiked dilutions and plasma spiked samples.

The system suitability of the method was done by working stock standard of individual drugs which were injected HPLC to determine the individual retention times of drugs. Then working standard solution was injected five times and we considered relative standard deviation (RSD) for five consecutive injections ≤2, resolution between two adjacent peaks ≥2 and tailing factor <2 acceptable values. Resolution (R), relative standard deviation from five replicate injections of working standard mixture solution, tailing factor (T) and retention time drug was presented in **(Table. 1)**. System suitability test confirmed that the chromatographic system was adequate for the analysis planned to be done. Then, the method was validated for various validation parameters according to the US FDA guidelines.

Method validation

Selectivity and Specificity

Validating specificity of the method is the first and foremost task to rule out any interference from the components of formulation[6]. To establish the specificity of the assay method of pyrazinamide, the standard blank (internal standard levofloxacin) and sample were injected in HPLC and the recorded chromatograms are depicted in **Figures 1 and 2** as standard and sample chromatograms, respectively. From these chromatograms, it can be observed that there are no interfering peaks are within the retention time range. Further, these **Figures** show and confirm that the selected drug is evidently allotted and hence the proposed HPLC method is selected.

Carryover effect

The carryover effect of the present method was established by using six injections of plasma blank and an upper limit of quantification (ULOQ) of PYZ. These samples were analyzed alternately to check any carryover in the blank sample. In this study there were no such effects observed.

Linearity

To establish the linearity of the assay method of pyrazinamide, six standard concentrations in the range of 100 - 1500 ng/mL were evaluated. A calibration chart was made for concentration against peak area. A good linear relationship was observed. (Coefficient of determination $R^2 = 0.999$). The calibration curve is represented by the regression equation of $y = 22467x + 788.18$; where "y" is the peak area and "x" is the concentration of pyrazinamide. The concentration and peak area data are compiled in **Table 2** while the calibration curve is depicted in **Figure 3.**

Extraction recovery

The extraction recovery was determined at three concentration levels (LQC, MQC and HQC) by spiking the solution of pyrazinamide in 100-500 ng/mL concentration range. Very good recoveries ranging from 50% to150% were observed with % RSD typically less than 2.0% satisfying the acceptance criteria **Table 3.**

Intra-day and Inter-day Precision

The precision or repeatability of the assay method was established in accordance with ICH recommendations. For this, low concentrations of drug (each six times) were evaluated on intra and inter day variations at the lowest concentration level(100 ng/mL for pyrazinamide) on the same and on three different days respectively. The results obtained were statistically constructed and it was found that the acceptance criteria are fully met as shown in **Table 4.**

Robustness

Robustness of the method was done by changing slight variation in the parameters like mobile phase composition, flow rate and wavelength. Present method didn't show any significant change when the critical parameters were modified. The tailing factor of drug was always less than 2.0 and the components were well separated under all the changes carried out. Considering the modifications in the system suitability parameters and the specificity of the method, as well as carrying the experiment at room temperature may conclude that the method conditions were robust.

Ruggedness

Ruggedness was studied along with precision and accuracy of batches where effect of column change and analyst change were observed. The observed value for column variation and results obtained for precision and accuracy were within the acceptance criteria (i.e. there were no changes in the retention time, recovery and precision of the drug) according to US-FDA and ICH.

CONCLUSION

The developed method is rapid, sensitive, rugged and reproducible with high recovery. Each sample requires less than 5 min of analysis time. Drug and IS were extracted with the simplest protein precipitation method with less matrix

effect. The developed method was successfully applied in the pharmacokinetic study to evaluate plasma concentration of PYZ in healthy rabbits.

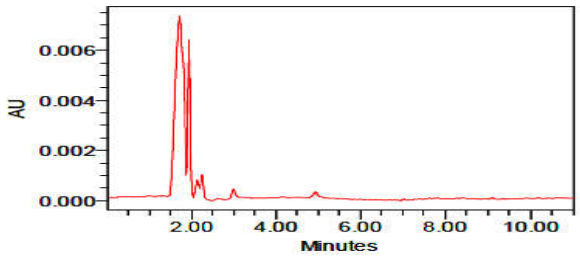

Figure 1 Chromatogram obtained for pyrazinamide with blank plasma.

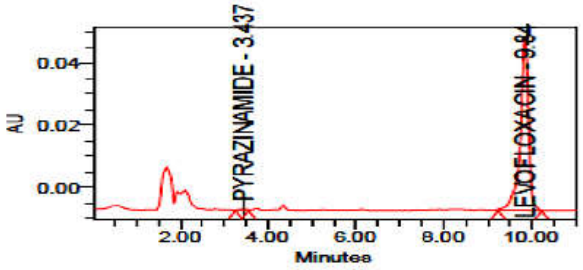

Figure 2 Chromatogram obtained for pyrazinamide with spiked plasma.

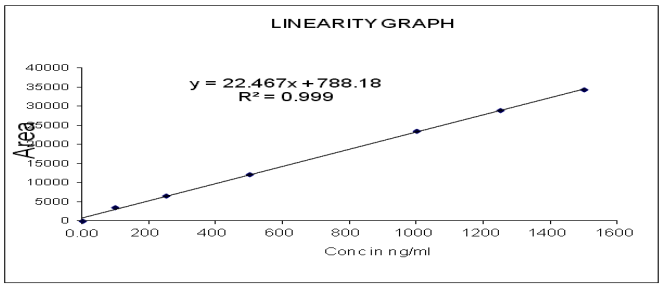

Figure 3 Linearity graph for the estimation of pyrazinamide in plasma samples.

Table 1 Optimized chromatography conditions

Parameter	Value
Flow rate	1.0 mL /min
Run time	12 min
Column oven temperature	Ambient (25±2^{0}C)
Auto sampler temperature	10^{0}C
Volume of injection	10µL
Detection wave length	268 nm
Retention time of pyrazinamide	3.437 min.

Table 2 Linearity range for the estimation of pyrazinamide in plasma

Plasma concentrations of PYZ (ng/mL)	Mean peak area
100	3564
250	6633
500	12099
1000	23428
1250	28845
1500	34298

Table 3 Recovery of pyrazinamide

Level	Concentration	Area	% Mean Recovery	Statistical analysis of % Recovery	
50%	100	1694534	99.8	SD	%RSD
100%	250	2901693	99.9	0.0422	0.04%
150%	500	4212157	99.9		

Table 4 Precision of the proposed HPLC method for pyrazinamide in plasma.

Drug	Sample (ng/mL)	Intra-day precision			Sample (ng/mL)	Inter-day precision		
		Mean Area	SD	% RSD		Mean Area	SD	% RSD
	100	2517262			100	2153388		
	100	2899401			100	2171620		
	100	2926996			100	2143152		
PYZ	100	2886363	5697.12		100	2152336	15169.83	
	100	2918923		0.19	100	2168933		0.704
	100	2919405			100	2131690		

References

1. The Indian Pharmacopoeia Commission, Ghaziabad, Government of India, Ministry of Health & Family Welfare,*Indian Pharmacopoeia*,2010, Volume 1.
2. Felder E, Pitre D. Pyrazinamide.*Analytical Profiles of Drug Substances* 1983;Vol. 12, pp. 433-462. Academic Press.
3. Zhang Y, Mitchison D. The curious characteristics of pyrazinamide: a review. *The international journal of tuberculosis and lung disease*. 2003;7(1:6-21.
4. East and Central African/Medical Research Council Fifth Collaborative study."Controlled Clinical trial of four short- Course regimens of Chemotherapy (Three 6 months and one 9 month) for pulmonary tuberculosis". *Tubercule.,* 1983; 64 (3): 153-166.
5. Mariappan TT, Singh B, Singh S. A Validated Reversed Phase (C18) HPLC Method for Simultaneous Determination of Rifampicin, Isoniazid and Pyrazinamide in USP Dissolution Medium and Simulated Gastric Fluid. *Pharmacy and Pharmacology Communications*. 2000;6(8):345-9.
6. Validation of compendial methods, Pharmacopeial convention, *United States Pharmacopoeia*, Inc., Rockville, MD, 2003; 2439-2442.

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