ORIGINAL RESEARCH ARTICLE

Development and validation of UV-spectrophotometric methods for quantitative estimation of Prothionamide in pure and pharmaceutical dosage forms

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ABSTRACT
UV Spectrophotometric method was developed and validated for the quantitative determination of Prothionamide in bulk drug and in pharmaceutical formulations. Prothionamide shows the maximum absorbance at 288 nm in phosphate buffer (pH 7.4). Prothionamide follows Beer's law in the concentration range of 4-20 µg/ml ($r^2 = 0.999$). The detection limit (DL) and quantitation limit (QL) were 0.406 and 1.229 µg/ml respectively. Accuracy and precision were found to be satisfactory. The developed methods were validated according to ICH guidelines. All the validation parameters were found to be satisfactory accordance with the standard values. Therefore, the proposed method can be used for routine practice for the determination of Prothionamide in assay of bulk drug and pharmaceutical formulations.

Key Words: Prothionamide, validation, UV-Spectrophometry, antitubercular drug, content determination.

INTRODUCTION

Prothionamide is chemically 2-Propylpyridine-4-carbothionamide with molecular weight of 180.3. It has been proposed as second line Tuberculostatics as it has activity against Mycobacterial species including *M. lepae* and *M. avium* by inhibiting peptide synthesis (Bijev and Georgieva, 2010). The absorption of Prothionamide following oral administration appears to be rapid and completely absorbed from gut wall. First pass metabolism was absent with an elimination of half-life 2 hr (Venkatesan, 1989). UV Spectroscopy method is the mostly interesting instrumental analytical methods that are widely used in for the assay of pharmaceutical products, because for its simple, easy, less time consuming and an economical method (Nijhu *et al.*, 2011). Prothionamide is official in IP (Indian Pharmacopoeia, 2010, JP (Japan Pharmacopoeia, 2011). Previous literature survey suggested the existence of HPLC method using NS-4000 (Lee *et al.*, 2012), Eurobond RP18 (IP, 2007), Kromasil 100 C4 column (Bartels and Bartels, 1998). Kumar and Sreeramulu (2011) reported stability indicating RP-HPLC with Shimazdu LC-20AT series. Thin layer chromatography was reported by GPHF-Minilab (2010). No UV spectrophotometric method is available for the estimation of Prothionamide formulation at pH of 7.4. So, it was felt necessary to develop a UV spectrophotometric method where no complexation agent, and no baleful chemicals, no extraction, derivatization, or evaporation step, are involved.

MATERIALS AND METHODS

Chemicals and Reagents
Prothionamide was procured from Yarrow Chem products, Mumbai. Protomid (Prothionamide tablets IP 250mg, batch no: APB304A Macleods Pharmaceuticals Ltd.) was purchased from local market. All other reagents used in this study were of AR grade. Distilled water was used for making the solution.

Instrumentation
The UV method was performed on Shimadzu (Model: UV-1800) double beam spectrophotometer with UV-probe software version 2.31. The absorption spectra of all the solution were carried out in the range of 200-400 nm.

Preparation of stock solutions and test solutions & wavelength of maximum absorbance (λ_{max}) determination
Stock solution (1000 µg/ml) of Prothionamide was prepared in pH 7.4 phosphate buffer and sonicated for 20 min. The absorption maximum was determined using 10 µg/ml solution by scanning at 200 to 400 nm in UV-Double beam spectrophotometer (**table 1**).

Preparation of working standard solution for calibration curve
The standard solution (1000 µg/ml) was further diluted with pH 7.4 phosphate. The diluted solution were vortexed and then used for further analysis. The calibration curve was plotted between concentrations and absorbances (**table 2**).

Method Validation
The methods were validated according to ICH guidelines of Q2B (2005) in terms of linearity, sensitivity, precision and accuracy for each analyte.

Linearity
Five point linear curve was prepared by plotting concentration (µg/ml) vs. absorbance and correlation co-efficient was calculated (**figure 1**).

Limit of detection (LOD) and limit of quantification (LOQ)
The limit of detection (LOD) is defined as is the lowest concentration of a substance that an analytical process can reliably distinguished from the absence of that substance. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured

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Table 1: Summary of the UV method validation.

Parameters	UV-method
Working λ_{max}	288 nm
Beer's law limit	4-20 μg/ml
Regression equation	y = 0.049x + 0.004 (without zero interpretation) y = 0.050x (with zero interpretation)
Regression coefficient (r²)	0.999
SD of slope	0.0016
SD of intercept	0.0059
LOD	0.397
LOQ	1.204

Table 2: Standard curve of Prothionamide at pH 7.4.

Concentration (μg/ml)	Absorbance
4	0.207
8	0.399
12	0.599
16	0.810
20	0.999

Figure 1: Calibration curve of Prothionamide at pH 7.4.

with acceptable accuracy, precision and variability (ICH guideline Q2B, 2005) (**table 1**). The LOD and LOQ were calculated as:

$$LOD = 3.3 \times \frac{SD}{S}$$

$$LOQ = 10 \times \frac{SD}{S}$$

Where, S = slope of the linearity curve (0.049), SD = standard deviation of y-intercept

Analysis of tablet formulation

20 marketed tablets of Prothionamide (Protomid, Macleods Pharmaceuticals Ltd.) were weighed and ground to fine powder; amount equal to 10 mg of Prothionamide was taken in 100 ml of volumetric flask and the volume was made up to mark with pH 7.4 phosphate buffer. The flask was sonicated further for 20 min to solubilize the drug present in tablet powder. After sonication, filtration was done through Whatman filter paper No. 41. Filtrate was collected and further diluted with pH 7.4 phosphate buffer to get the final concentrations of drug in the working range. The absorbance of final diluted solution was observed at 288 nm (**table 3**).

Accuracy

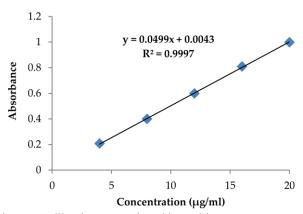Accuracy is the percent of recovered amount by assay from a known added amount. For the measurement of accuracy data, nine determinations over three concentration levels (Kavathia and Misra, 2013) were carried out as lower, intermediate and higher concentration from stock solutions and analyzed. The accuracy of the proposed methods was assessed by recovery studies at three different levels *i.e.*, 80%, 100% and 120% (Jain *et al.*, 2013). Result of recovery studies are reported in **table 4**.

Precision

Repeatability was calculated by taking different levels of concentrations, prepared from pure drug stock solution and analyzed. Intermediate precision was calculated by taking the variations of inter-day and intra-day response. Respective concentrations from stock solution in triplicates were prepared three different times in a day and studied for intraday (n=9) and inter-day variation (n=15). The relative standard deviation (% RSD) of the estimated concentrations from the regression equation was taken as precision (**table 5**).

RESULTS AND DISCUSSION

UV-Spectrophotometric method was developed for Prothionamide which can be conveniently employed for routine analysis in pharmaceutical dosage forms and will eliminate unnecessary tedious sample preparations. The standard error, standard deviation, and coefficient of variance were obtained for Prothionamide was satisfactorily low. Prothionamide shows λ_{max} at 288nm. 5 Point calibration curve data was constructed in the range of 4 to 20 μg/ml, where the Beer's law was obeyed. The correlation co-efficient was found 0.999, which is within the limit (r² > 0.990). The limit of detection (LOD) and limit of quantification (LOQ) for Prothionamide is found to be

Table 3: Estimation of Prothionamide in tablet formulations.

Label claim (mg/tab)	Conc. prepared (μg/ml)	Conc. recovered (μg/ml)	Amount found (mg/tab)	% Label claim	Mean ± SD (n=3)	% RSD
		9.82	245.5	98.2		
	10	9.74	243.5	97.4	98.40 ± 0.91	0.925
		9.96	249.0	99.6		
		14.82	247.0	98.8		
250	15	15.3	255.0	102.0	99.80 ± 1.56	1.563
		14.78	246.5	98.6		
		19.64	245.5	98.2		
	20	19.74	246.75	98.7	98.87 ± 0.62	0.627
		19.94	249.25	99.7		

SD = Standard deviation, RSD = Relative standard deviation, N= Average determination.

Table 4: Recovery studies on marketed formulations.

Conc. level	Sample no.	Drug	Formulation	Amount added (µg/ml)	Abs.	Amount recovered	% recovery	Mean % recovered ± SD (N=3)	% RSD
80%	1		10 ml of 8µg/ml	9	0.437	8.74	97.11	97.55 ± 0.36	0.39
	2				0.441	8.82	98.0		
	3				0.439	8.78	97.55		
100%	1	10ml of 10µg/ml	11 ml of 10µg/ml	10	0.488	9.76	97.6	98.00 ± 0.33	0.34
	2				0.490	9.80	98.0		
	3				0.492	9.84	98.40		
120%	1		12 ml of 12µg/ml	11	0.536	10.72	97.46	98.60 ± 0.839	0.85
	2				0.547	10.94	99.45		
	3				0.544	10.88	98.90		

Table 5: Results of validation (Mean ± SD).

Conc. (µg/ml)	Intra Day						Inter Day	
	DAY-1		DAY-2		DAY-3			
	Repeatability ± SD (N=9)	% RSD	Repeatability ± SD (N=3)	% RSD	Repeatability ± SD (N=3)	% RSD	Repeatability ± SD (N=15)	% RSD
5	0.2464±0.002	0.8130	0.2550±0.0016	0.6275	0.255 ± 0.0008	0.3137	0.2497 ± 0.0047	1.8823
10	0.4858±0.0059	1.2145	0.4887±0.0056	1.1459	0.4977 ± 0.0052	1.0448	0.4887 ± 0.0073	1.4938
15	0.7474±0.0081	1.0838	0.7533±0.0082	1.0885	0.7453 ± 0.0095	1.2747	0.7482 ± 0.0088	1.1762

0.397µg/ml and 1.204 respectively, indicating that the proposed UV method is highly sensitive. Assay value of Prothionamide in tablet formulations ranged from 98.40 ± 0.91 to 99.80 ± 1.56 with relative standard deviation not more than 1.56%. Assay values of formulation were same as label claim; this indicate that no interference of excipients in the determination of Prothionamide by the proposed method. The standard deviation, coefficient of variance and standard error were obtained for Prothionamide was significantly low. Result of precision at different levels was found to be within acceptable limits (RSD < 2).

CONCLUSION

Simple, rapid, accurate and precise UV- spectrophotometric methods have been developed and validated for route analysis of Prothionamide in API and tablet dosage forms. There is no interference of phosphate buffer pH 7.4 solution in the estimation of Prothionamide. This proposed method, thereby decreasing the time and the error in the quantization.

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