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Research Article

Validated UV-Spectrophotometric Method for the Ethionamide Estimation in Bulk, Tablet and Nanoparticles

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Abstract

The objective of present study was to develop a simple, specific and economic UV-spectrophotometric method for estimation of Ethionamide in bulk and marketed formulations. pH 7.4 phosphate buffer was used to prepare the stock solution of Ethionamide followed by determination of wavelength in the absorption spectrum where the absorbance was maximum. Different working standard solutions were prepared from the above solution by diluting with same diluents to obtain the standard curve. Further this method was validated as per ICH guidelines. Ethionamide showed the maximum absorptivity at 288 nm and linearity range found in 6-18 µg/ml (r^2=0.999). Limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.076 and 0.2301 µg/ml respectively. All other validated parameters were found to be satisfactory. The proposed method is simple and no hazard chemicals were used for the estimation of Ethionamide in pure form, marketed formulations and nanoparticles.

Keywords: Ethionamide; Method validation; Nanoparticles; PLGA

Introduction

Tuberculosis continues to be a major worldwide epidemic with approximately one-third of the world population infected with *Mycrobacterium tuberculosis* [1]. According to the WHO South-East Asia Region (SEAR) report in 2015, one fourth of the world population accounts for 38% morbidity and 39% mortality of the global burden of tuberculosis, with an estimated 4.5 million prevalent and 3.4 million incident cases and 440,000 deaths in 2013 [2]. This organism contains lipid-rich cell wall, which restrict the permeability of many agents. Along with this species are intracellular pathogens, and reside within macrophages, which is difficult to access using anti-tubercular drugs due to poor cells permeability. As a result they develop drug resistance [3]. This increasing drug resistance in tuberculosis represents a continuous challenge to the clinician and researcher since tuberculosis first detected [4]. In the treatment of tuberculosis, first line drugs are normally used with large and repeated dose for several months. Due to patient's non-compliance, these drugs fail- results drug resistance, multi drug resistance & extreme multi drug resistance. In that situation, 2nd line anti tubercular drug is used. Ethionamide (ETH) belongs to this category, has good clinical efficacy against *Mycobacterium tuberculosis*, but poor tolerable because of considerable gastrointestinal adverse effect, such as nausea, vomiting, anorexia, a metallic taste and abdominal pain. ETH is mainly metabolized by the liver, only 5% are excreted in unchanged form through urine [5]. Although it acts on similar fashion like Isoniazide, the side effects prohibit this drug from being used as a first line therapy [6]. ETH is administered repeatedly due to its half life of 2-3 hr [7]. A drug and drug product are stable when its physical, chemical, therapeutics and toxicological properties are unchanged according to official monographs [8]. Some HPLC estimation methods [1,9-11] & column chromatographic-mass spectrometric technique [7], Voltammetric Determination [5], catalytic kinetic spectrophotometric method [12] were reported by different authors. But most of the cases, methods neither validated nor provide sufficient information for estimation of Ethionamide. Some of the cases used hazed chemicals.

So, efforts were made to develop an economical, simple, secure UV-spectrophotmetric method using pH 7.4 Phosphate buffer. The developed method was validated and checked its suitability in the estimation of ETH entrapment in its nanoparticles form.

Materials and Methods

Material used

Ethionamide was procured from Shiro Pharma Chem. Pvt. Ltd., Navi Mumbai. Ethomid (Ethionamide tablet IP 250 mg, Macleods Pharmaceuticals Ltd.) was purchased from local market. PLGA (Resomer RG 755 S) was obtained as gift sample from Evonik Industries, Darmstadt, Germany. All other ingredients used were of AR grade. In every stage HPLC grade water was used.

Apparatus

A SHIMADZU (Model: UV-1800) double beam spectrophotometer with UV-probe software version 2.31 was used for all absorbance measurements.

Determination of wavelength of maximum absorbance (λ_{max})

A standard stock solution of Ethionamide (1000 µg/ml) was prepared using pH 7.4 phosphate buffer. Five different concentrations (6, 9, 12, 15 & 18 µg/ml) were prepared after withdrawing five different aliquots from the stock solution and diluted up to 10ml with same diluents. An UV spectroscopic scanning (200-400 nm) was carried out with the prepared diluted solution to determine the λ_{max} for the detection of Ethionamide using pH 7.4 Phosphate buffer as blank (Table 1,2).

Linearity and range

For linearity study, the obtained data of five different prepared concentrations were used for the construction of calibration curve (Figure 1). The LOD and LOQ were also calculated as previous described (Table 1) [13].

Assay of Ethionamide in marketed tablet

One Ethionamide marketed tablet (Ethomid, Macleods Pharmaceuticals Ltd.) was analyzed using the newly developed and validated method. Initially weighed 20 tablets and ground to form fine powder. 10 mg of Ethionamide equivalent powder was taken in 100

Parameters	UV Method
Working λ_{max}	288 nm
Beer's law limit	6-18 µg/ml
Regression equation	y=0.052x+0.000 (with/without zero interpretation)
Regression coefficient (r^2)	0.999
Slope of linear curve	0.052
SD of slope	0.0006
SD of intercept	0.0012
LOD	0.076
LOQ	0.2301

Table 1: Summary of the UV method validation.

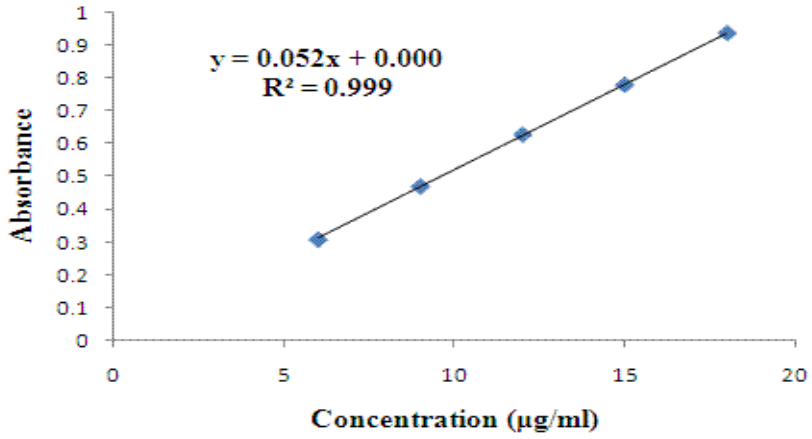

Figure 1: Calibration curve of Ethionamide at pH 7.4.

Concentration (µg/ml)	Absorbance
6	0.310 ± 0.003
9	0.471 ± 0.001
12	0.629 ± 0.001
15	0.780 ± 0.001
18	0.937 ± 0.004

Table 2: Linear curve of Ethionamide at pH 7.4.

Prepared conc.	Absorbance	Conc. recovered (µg/ml)	Dilution factor	Amount recovered in mg	% Label claim	Mean ± SD (N=3)	RSD
8	0.413	7.942	1000	7.942	99.28	99.20 ± 0.14	0.140
	0.412	7.923	1000	7.923	99.04		
	0.413	7.9423	1000	7.942	99.28		
12	0.621	11.942	1000	11.942	99.52	99.36 ± 0.16	0.161
	0.619	11.904	1000	11.904	99.20		
	0.62	11.923	1000	11.923	99.36		
16	0.831	15.981	1000	15.981	99.88	99.84 ± 0.07	0.070
	0.831	15.981	1000	15.981	99.88		
	0.83	15.962	1000	15.962	99.76		
Mean						99.47 ± 0.12	0.123
SD=Standard deviation, RSD=Relative standard deviation, N=Average determination.							

Table 3: Estimation of Ethionamide.

ml of volumetric flask and the volume was made up to 100ml with pH 7.4 phosphate buffer. This content was stirred under magnetic stirrer followed by sonication for 20 min to solubilize the drug. After filtration (Whatman filter paper No. 41), suitable dilution was made to estimate Ethionamide at 288 nm (Table 3).

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100% and 120% [14]. For this measurement, nine determinations [15] was carried out at lower, intermediate and higher concentration level (Table 4).

Precision

Intermediate precision was checked by assay the sample solution on same day and three different days (interday precision). Three different working stock solution in triplicates (5, 10, 15 µg/ml) were prepared from standard stock solution. These solutions were studied for three different times in a day (intraday; n=9) and continued up to

Conc. Level	Sample No	Drug solution	Formulation solution	Amount Added (µg/ml)	Abs	Amt Recovered	% recovery	Mean % Recovered ± SD (N=3)	% RSD
80%	1	10 ml of 15 µg/ml	10 ml of 12 µg/ml	13.5	0.7	13.46	99.72	99.76 ± 0.22	0.22
	2				0.699	13.44	99.57		
	3				0.702	13.5o	100		
100%	1		10 ml of 15 µg/ml	15	0.776	14.92	99.49	99.79 ± 0.27	0.27
	2				0.78	15	100		
	3				0.779	14.98	99.87		
120%	1		10 ml of 18 µg/ml	16.5	0.858	16.50	100	99.85 ± 0.18	0.18
	2				0.855	16.44	99.65		
	3				0.857	16.48	99.88		
Mean Recovery=99.80 ± 0.53									

Table 4: Recovery studies on marketed formulations.

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	4.99 ± 0.017	0.334	4.93 ± 0.017	0.344	4.79 ± 0.032	0.668	4.91 ± 0.083	1.703
10	9.99 ± 0.013	0.128	9.95 ± 0.014	0.137	9.79 ± 0.029	0.295	9.91 ± 0.089	0.899
15	14.97 ± 0.017	0.0167	14.88 ± 0.029	0.194	14.75 ± 0.055	0.371	14.87 ± 0.099	0.666

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

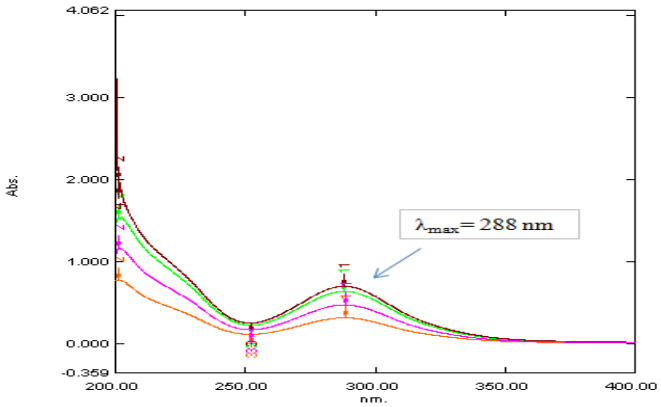

Figure 2: λ_{max} determination of Ethionamide nanoparticles.

3rd day (inter-day; n=15). The relative standard deviation (RSD) was taken as precision.

Preparation of Ethionamide Nanoparticles

PLGA nanoparticles were prepared by solvent evaporation technique. PLGA incorporated dichloromethane solution of ETH was prepared and further transferred to 10ml PVA solution for 10 min vortexing. A micro-emulsion was formed when sonicated for 5min over the ice bath. The prepared emulsion kept on magnetic stirrer at room temperature for 3 hr to evaporate Dichloromethane. The nanoparticles were recovered by ultracentrifugation at 18000 rpm for 25 minutes followed by one wash with distilled water. Supernatant contained the un-entrapped ETH was estimated with this validated method and also checked the influences of other ingredients on the λ_{max}.

Results and Discussions

UV-Spectroscopic methods were developed with commonly known chemicals, which can be used for the routine analysis of Ethionamide in pharmaceutical dosage forms. At λ_{max} of 288 nm, Ethionamide proved linear in the range of 6 to 18 µg/ml (Table 2) and exhibited good correlation co-efficient (r^2=0.999). LOD and LOQ for Ethionamide were found to be 0.076 µg/ml and 0.2301 µg/ml respectively, indicating that the proposed UV method is highly sensitive (Table 1). Assay values of Ethionamide tablet were found to be 99.47 ± 0.12% (Table 3). No interference of excipients was detected in the estimation of Ethionamide. This method demonstrated excellent mean recovery of 99.80 ± 0.53 % (Table 4). All the estimated parameters were found to be statistically significant due to low RSD values (RSD< 2) (Table 5). Supernatant containing un-entrapped drug was estimated by present proposed method and drug content was found to be 11.20%. Similarly the nanoparticles containing the entrapped drug were subjected to methanol treatment for removing the coating and to bring the entrapped drug. This solution was made up with buffer and tested at λmax 288nm again for reagent blank containing methanol buffer. The drug content found by the same proposed method as 88.80%. Although different chemical was used in the formulation of ETH nanoparticles, no significant shifting of λ_{max} was detected (Figure 2). So, this validated method can precisely estimate the ETH in bulk and formulation without the interference of solvent effect.

Conclusion

The results and statistical parameters signify that the validated UV- spectrophotometric method is simple, accurate and precise. This method can be used for estimation of Ethionamide in bulk, marketed formulation and nanoparticles without the interference of commonly used chemicals or solvents.

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