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Research Article

NOVEL RPHPLC & UV SPECTROSCOPIC METHOD FOR ESTIMATION OF INVITRO DRUG RELEASE THROUGH DISSOLUTION STUDIES OF DEUTETRABENAZINE

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ABSTRACT

In this study, UV-Spectrophotometric and RP-HPLC, were developed and validated for the quantitative analysis of Deutetrabenazine in tablet formulations. UV-Spectrophotometry utilized 0.1 N HCL as the solvent, demonstrating simplicity, precision, accuracy, and cost-effectiveness, with a sensitivity range of 10-30 µg/ml and maximum absorbance at 282 nm. RP-HPLC employed a phenomenex C18 column and an isocratic mobile phase (acetonitrile , 80:20% v/v), offering rapid analysis with a sensitivity range of 10-30 µg/ml and maximum absorbance at 284 nm. Both methods were validated per USP guidelines, ensuring reliability and suitability. Specificity tests confirmed accurate measurement of Deutetrabenazine amidst sample matrix components. Precision studies showed consistent results (system and method precision), indicating reproducibility. Linearity assessments verified a proportional relationship between analyte concentration and response. Robustness testing demonstrated method reliability under varied conditions. Dissolution studies complemented the methods, providing insights into tablet dissolution profiles with satisfactory performance criteria met. Overall, these validated methods provide robust tools for routine Deutetrabenazine analysis in pharmaceutical formulations, ensuring accurate dosage assessment and quality control in manufacturing. Future validation for other compounds would broaden their applicability in diverse analytical contexts.

Keywords:- Deutetrabenazine, UV-Spectrophotometry, RP-HPLC, Pharmaceutical Analysis, Method Validation.

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INTRODUCTION

Deutetrabenazine, a monoamine depletory and adrenergic uptake inhibitor, is utilized for the symptomatic treatment of chorea associated with Huntington's disease. [1] This compound acts as a reversible inhibitor of the human vesicular monoamine transporter type 2 within the basal ganglia [2], leading to the depletion of monoamine neurotransmitters such as serotonin, norepinephrine, and dopamine from their synaptic stores [3]. By reducing their uptake into synaptic vesicles and inhibiting their transmission, deutetrabenazine effectively modulates hyperkinetic movement disorders associated with dopamine dysregulation.

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The analytical methods employed to assess the drug release characteristics of deutetrabenazine include RP-HPLC (Reverse Phase High Performance Liquid Chromatography) and UV Spectroscopy [4,5]. RP-HPLC utilizes a chromatographic column filled with stationary phase material, a mobile phase pumped through the column, and a detector that measures retention times of analytes [6]. This method is crucial for determining the dissolution profiles and ensuring the consistency of drug release from pharmaceutical formulations. Validation of these analytical methods according to Good Manufacturing Practices (GMP) regulations ensures that the documented evidence supports the reliability and reproducibility of the analytical results [7]. This validation process establishes confidence that the methods consistently produce accurate data, essential for maintaining product quality and meeting regulatory requirements in pharmaceutical development and manufacturing [8]. The present study aims to conduct in vitro studies of deutetrabenazine using these validated analytical techniques, providing essential data on its dissolution behavior from dosage forms. This research contributes to the understanding and optimization of deutetrabenazine formulations, crucial for its therapeutic efficacy and safety in clinical applications.

METHODOLOGY

Determination of Melting Point– Method I (IP 2010)

Procedure

A small quantity of dry sample (0.1 g) was placed inside a capillary tube with one end closed. The capillary tube with the sample was tapped until the length of tightly packed material is 3- 5 mm. The capillary tube was then attached to the lower end of calibrated thermometer and immersed in the center of liquid paraffin bath. The start and completion of melting of the sample were carefully observed and recorded.

Infra- Red Spectral Analysis

A blank pellet was prepared using KBr as reference sample (KBr Pellet technique). The sample to be analyzed was then prepared and FT- IR spectrum was recorded.

Method Development and Validation by Ultraviolet Spectrophotometry-Calibration Graph Method

Determination of λmax

By trial and error, the λmax of Deutetrabenazine in 0.1M Hydrochloric acid by UV spectrophotometer was found to be 282nm.

Preparation of standard solutions

Preparation of 0.1M Hydrochloric acid

8.65ml of concentrated hydrochloric acid was accurately transferred using a pipette into a 1000ml volumetric flask and made upto the volume with freshly prepared double distilled water to obtain 0.1M strength.

Preparation of Deutetrabenazine standard stock solution- I (1000 µg/ml)

25 mg of Deutetrabenazine API was accurately weighed into 25 ml volumetric flask and dissolved in freshly prepared 0.1M Hydrochloric acid and made upto the volume to get concentration of 1000 µg/ ml.

Preparation of Deutetrabenazine standard stock solution- II (100 µg/ml):

2.5 ml from the stock solution- I was pipetted into 25 ml volumetric flask and made volume with freshly prepared 0.1M Hydrochloric acid to get 100 µg/ ml concentration.

Preparation of standards for calibration curve (10- 30 µg/ ml):

From stock solution- II, 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml and 3.0 ml were accurately transferred to respective 10 ml volumetric flasks and made upto volume with freshly prepared 0.1M Hydrochloric acid which corresponds to concentrations of 10, 15, 20, 25, 30 µg/ ml respectively. The absorbance for the above dilutions was measured at 282nm.

Method Validation

Linearity and Range

Linearity for the concentration range 10-30 µg/ ml was established by plotting concentrations on X- axis and corresponding absorbance on Y- axis. Statistical parameters like correlation coefficient (R2), line equation including slope (m), y- intercept (C) were determined. The specified range was derived from linearity studies by determining the difference between highest and lowest concentrations.

Precision

Interday precision (Repeatability)-Repeatability of the developed method was assessed by 30 determinations covering 5 concentrations each of 6 replicates. % RSD was calculated for the results obtained. Intraday Precision-Variations in the results for the developed method was assessed amidst 3 different days (n= 6). % RSD was calculated for the results obtained.

Limit of Detection and Limit of Quantitation

LOD and LOQ were determined by instrumental methods based on the standard deviation of the response (blank sample) and slope of the calibration curve.

Assay of Formulation

20 tablets were weighed accurately and average weight of tablet was noted that constitutes 12 mg

Deutetrabenazine and was finely powdered. The tablet powder equivalent to 5 mg of Deutetrabenazine was accurately weighed and transferred to 50 ml volumetric flask and dissolved in about 10 ml of the solvent (0.1 M Hydrochloric acid). It was then vortexed for 30 minutes to enhance maximum extraction of the active pharmaceutical ingredient from the dosage form and filtered through Whatmann No 1 filter paper to remove insoluble excipients to the maximum extent. It was then made upto the volume with the same solvent. This constitutes 100 mcg/ ml of Deutetrabenazine. From the stock solution, aliquot corresponding to medium concentration of standard curve (20 µg/ ml) was prepared and made upto the mark with the solvent. The absorbance was noted and the corresponding concentration was then determined from the standard calibration curve.

Dissolution Studies of AUSTEDO Tablets

Dissolution studies were carried out using rotating paddle apparatus (USP type- II) by dissolving each Austedo tablet in a dissolution jar containing 900 ml of 0.1 M Hydrochloric acid (dissolution medium) maintaining the temperature at 37 ± 2^{o}C rotating the paddle at a speed of 50 rpm for 60 minutes. 5 ml of sample was withdrawn at time intervals of 0, 5, 15, 30, 45 and 60 min respectively. Each 5 ml of withdrawn sample is replaced by 5 ml of 0.1 M Hydrochloric acid to maintain sink condition. The samples were then filtered through Whatmann No 1 filter paper to avoid interference of excipients. The absorbance of the resulting solutions was measured at 282 nm by UV Spectrophotometry against 0.1 M Hydrochloric acid as blank.

Table: 1 Linearity findings

Line equation	y= 0.0101x+ 0.0034
correlation coefficient (R2)	0.9999
y- intercept (C)	0.0034
Slope (m)	0.0101

Table: 2 Intraday precision Day-I

DTB (µg/ml)	Absorbance at 282.0 nm					
	1	2	3	Mean	SD	%RSD
10	0.118	0.119	0.120	0.119	0.001	0.84034
15	0.178	0.179	0.176	0.177	0.001	0.8597
20	0.233	0.235	0.234	0.234	0.001	0.42735
25	0.285	0.289	0.285	0.286	0.0023	0.80654
30	0.340	0.344	0.341	0.341	0.0020	0.60927
Average % RSD						0.70865

Table: 3 Intraday precision Day-II

DTB (µg/ml)	Absorbance at 282.0 nm					
	1	2	3	Mean	SD	%RSD
10	0.123	0.126	0.127	0.1253	0.0028	1.6609
15	0.180	0.182	0.183	0.1816	0.0015	0.84084
20	0.238	0.239	0.241	0.2393	0.0015	0.63824
25	0.290	0.292	0.290	0.2906	0.0015	0.39726
30	0.345	0.343	0.347	0.345	0.002	0.57971
Average % RSD						0.82339

Table: 4 Intraday precision Day-III

DTB (µg/ml)	**Absorbance at 282.0 nm**					
	1	2	3	Mean	SD	%RSD
10	0.129	0.132	0.129	0.130	0.00173	1.33235
15	0.183	0.186	0.185	0.184	0.00153	0.82718
20	0.235	0.241	0.239	0.2383	0.00306	1.28184
25	0.292	0.290	0.289	0.2903	0.00153	0.52613
30	0.345	0.349	0.347	0.347	0.002	0.57637
Average % RSD						**0.90877**

Table: 5 Interday precision

DTB (µg/ml)	Absorbance at 282.0 nm								
	1	2	3	4	5	6	Mean	SD	%RSD
10	0.123	0.126	0.127	0.129	0.132	0.129	0.1276	0.00308	2.41002
15	0.180	0.182	0.183	0.183	0.186	0.185	0.1831	0.00214	1.16668
20	0.238	0.239	0.241	0.235	0.241	0.239	0.2388	0.00223	0.93312
25	0.290	0.292	0.290	0.292	0.290	0.289	0.2905	0.00122	0.4216
30	0.345	0.343	0.347	0.345	0.349	0.347	0.346	0.0021	0.60625
Average % RSD									**1.10753**

Table: 6 Assay of formulation (Austedo 12 mg tablets)

Formulation	Absorbance	Label claim	Amount found	% Assay ±SD
Austedo	0.199 0.202 0.200	12 mg	11.67	97.30% w/w ±0.51

Table: 7 Dissolution profile of Deutetrabenazine tablets by Calibration graph method

Time (mins)	Absorbance	Cumulative amount of drug dissolved (mg)	% drug Release
0	0.000	0.000	0.000
5	0.185	8.88	64.72
15	0.238	9.08	83.61
30	0.264	10.12	92.88
45	0.298	12.12	101.00
60	0.276	11.89	97.16

Figure: 1 IR Spectrum of Deutetrabenazine

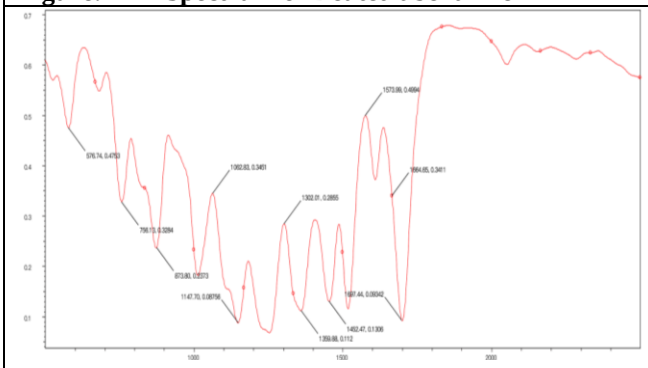


Figure: 2 λmax of Deutetrabenazine

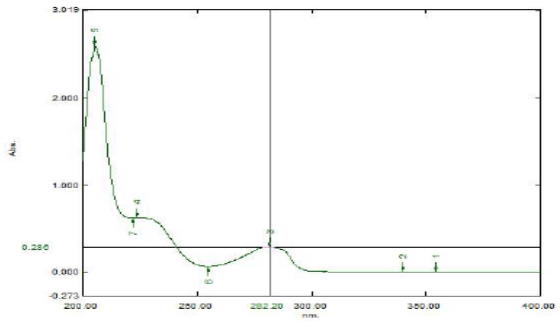


Figure: 3 Standard Calibration Curve for the Linearity Set at 282.00 nm

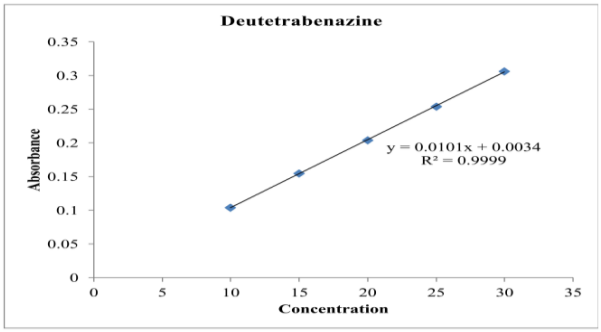


Figure: 4 Overlay Assay Spectra of Deutetrabenazine

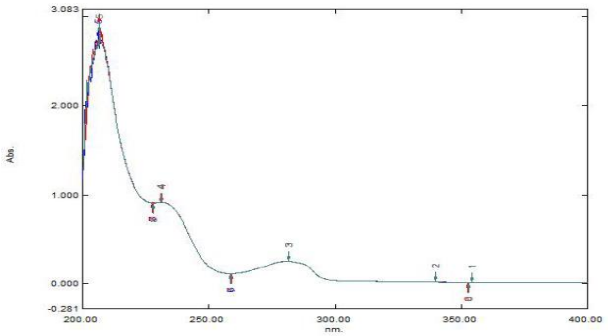
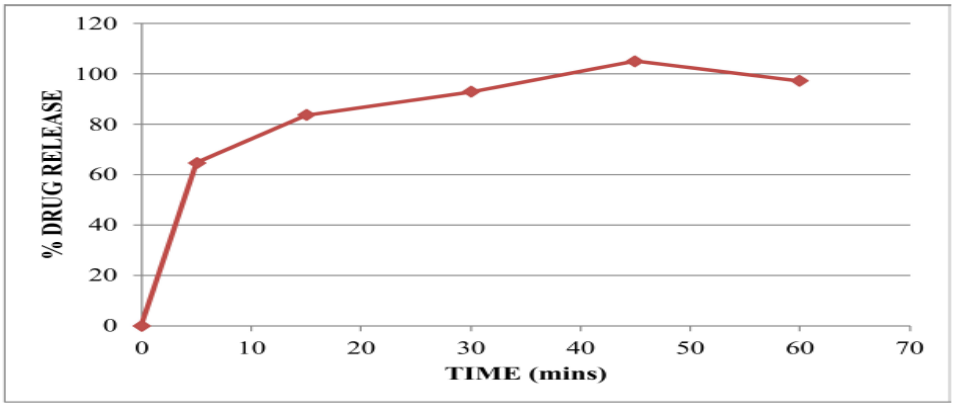


Figure: 5 Graph showing % drug release of Deutetrabenazine with time by Calibration graph method



DISCUSSION

The present study successfully developed and validated two analytical methods, UV-Spectrophotometric and RP-HPLC, for the quantification of Deutetrabenazine in tablet formulations. UV-Spectrophotometry, employing 0.1 N HCL as the solvent due to the drug's solubility characteristics, demonstrated simplicity, precision, accuracy, and cost-effectiveness. The method showed a maximum absorbance at 282 nm, with a sensitivity range of 10-30 µg/ml. Conversely, RP-HPLC, utilizing a phenomenex C18 column and an isocratic mobile phase of acetonitrile and water (80:20% v/v), provided a rapid and less expensive alternative with a sensitivity range also found to be 10-30 µg/ml and a maximum absorbance at 284 nm. Both methods were validated according to USP guidelines, confirming their reliability and suitability for the intended purpose. Specificity tests ensured accurate measurement of Deutetrabenazine amidst potential sample matrix components, yielding results within acceptable limits. Precision studies, including system precision and method precision, demonstrated consistent results across multiple measurements, indicating the method's reproducibility. Linearity assessments confirmed a proportional relationship between analyte concentration and response, essential for quantitative analysis. Robustness testing under varied conditions further validated the methods' reliability, showing results within predefined criteria. Additionally, dissolution studies complemented the analytical methods, providing insights into Deutetrabenazine's release profile from tablets. The methods' performance in dissolution testing was satisfactory, with absorbance and peak areas meeting acceptance criteria. Overall, the validated methods offer robust tools for routine analysis of Deutetrabenazine in pharmaceutical formulations, ensuring accurate dosage assessment and quality control in manufacturing processes. Future validation for other nonpolar compounds would expand the applicability of these methods in broader analytical contexts.

CONCLUSION

RP-HPLC, Calibration Graph, and AUC methods in UV Spectrophotometry are reliable for precise Deutetrabenazine analysis in bulk and tablet forms. These methods are cost-effective, require minimal analysis time, and demonstrate high accuracy, linearity, and precision after thorough validation. They are suitable for routine pharmaceutical quality control due to their simplicity and sensitivity. Additionally, their application in dissolution studies enhances understanding of Deutetrabenazine's pharmacokinetics and biological fate, supporting informed drug development and clinical decisions.

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