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METHOD DEVELOPMENT FOR SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF *TETRABENAZINE* AND *AMLODIPINE BESYLATE* DRUG

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ABSTRACT

The present study describes the development and validation of a simple, accurate, and cost-effective UV–visible spectrophotometric method for the simultaneous estimation of Tetrabenazine and Amlodipine besylate in bulk and pharmaceutical dosage forms. Comprehensive pre-formulation studies were performed to evaluate the physicochemical characteristics of both drugs, including organoleptic properties, solubility, pH, melting point, and spectral behavior. Methanol was selected as the solvent based on solubility studies. Distinct absorption maxima were observed at 285.0 nm for Tetrabenazine and 360.0 nm for Amlodipine besylate, with an overlapping wavelength at 299.5 nm enabling simultaneous estimation. The method showed linearity in the concentration range of 10–60 µg/mL for Tetrabenazine and 5–30 µg/mL for Amlodipine besylate, with correlation coefficients greater than 0.98. The developed method was validated in accordance with ICH guidelines, demonstrating satisfactory specificity, precision, accuracy, ruggedness, robustness, and sensitivity. Low limits of detection and quantification confirmed the suitability of the method for routine quality control analysis. Overall, the validated UV spectrophotometric method is reliable, economical, and appropriate for simultaneous estimation of Tetrabenazine and Amlodipine besylate in pharmaceutical formulations.

KEYWORDS: Tetrabenazine; Amlodipine besylate; UV spectrophotometry; Method development; Simultaneous estimation; ICH validation.

1. INTRODUCTION

Tetrabenazine is a vesicular monoamine transporter-2 (VMAT-2) inhibitor widely used in the management of hyperkinetic movement disorders such as Huntington's chorea and tardive dyskinesia. By depleting presynaptic dopamine stores, Tetrabenazine effectively reduces involuntary motor activity **(Frank, 2010).**

Amlodipine besylate, a long-acting dihydropyridine calcium channel blocker, is extensively prescribed for the treatment of hypertension and angina pectoris due to its sustained antihypertensive effect and favorable safety profile **(Sweetman, 2011).**

The combination of drugs with distinct therapeutic actions often necessitates reliable analytical techniques for simultaneous estimation during formulation development and quality control. UV–visible spectrophotometry remains one of the most widely employed analytical tools in pharmaceutical analysis owing to its simplicity, rapidity, cost-effectiveness, and acceptable sensitivity **(Beckett & Stenlake, 2007).** Simultaneous spectrophotometric estimation techniques, particularly those based on overlapping absorption spectra, are advantageous for routine analysis where sophisticated instrumentation may not be readily available.

Although individual analytical methods for the estimation of Tetrabenazine and Amlodipine besylate have been reported, limited literature is available on the development of a validated UV spectrophotometric

method for their simultaneous estimation. Pre-formulation studies play a critical role in understanding drug properties such as solubility, stability, and spectral behavior, which are essential for selecting appropriate analytical conditions **(Aulton & Taylor, 2018).**

Therefore, the present study was undertaken to perform physicochemical characterization of Tetrabenazine and Amlodipine besylate and to develop and validate a simple UV spectrophotometric method for their simultaneous estimation in accordance with ICH guidelines. The developed method aims to provide a reliable and economical approach suitable for routine pharmaceutical quality control.

2. MATERIALS AND METHODS

Pre-formulation Studies

Pre-formulation studies were conducted to evaluate the physicochemical properties of the drugs and to provide a rational basis for formulation development.

Organoleptic Evaluation

The organoleptic properties of Tetrabenazine and Amlodipine besylate, including colour, odour, appearance, and physical state, were assessed by visual inspection.

Solubility Studies

Qualitative solubility of the drugs was determined in various solvents (methanol, ethanol, DMSO, acetone, and water) as per USP–NF guidelines. Accurately weighed drug (1 mg) was added to 1 mL of each solvent, and solubility was assessed visually. **(Jain and Verma 2020).**

Melting Point Determination

The melting point of the drugs was determined using the open capillary method. Drug samples were filled in sealed capillary tubes and heated gradually; the temperature range at which melting occurred was recorded. **(Chowk, M. I. 2020).**

pH Determination

The pH of Tetrabenazine and Amlodipine besylate solutions was measured using a calibrated digital pH meter. Measurements were performed in triplicate, and readings within acceptable limits were recorded.

Determination of λmax

Standard stock solutions (1000 µg/mL) of Tetrabenazine and Amlodipine besylate were prepared separately in methanol. These were further diluted to obtain working solutions of 30 µg/mL. The solutions were scanned using a UV–Visible spectrophotometer in the range of 200–800 nm against methanol as blank to determine the wavelength of maximum absorbance (λmax). **(Kumbhar and Salunkhe 2013).**

Linearity Studies

Calibration curves were prepared using working standard solutions of Tetrabenazine (10–60 µg/mL) and Amlodipine besylate (5–30 µg/mL). Absorbance was measured at 285.0 nm for Tetrabenazine and 360.0 nm for Amlodipine besylate. Linearity was evaluated by plotting absorbance versus concentration, and regression equations with correlation coefficients were calculated. **(Behera et al., 2012).**

Fourier Transform Infrared (FT-IR) Spectroscopy

FT-IR spectra of the drugs were recorded using the KBr pellet method in the range of 4000–400 cm^{-1} to confirm drug identity and characteristic functional groups. **(Chowk, M. I. 2020).**

UV–Visible Spectroscopic Method Development

Methanol was selected as the solvent based on solubility studies. For analysis of oral dosage forms, powdered tablet samples equivalent to 10 mg of each drug were dissolved in methanol, sonicated for 5 min, and diluted to obtain stock solutions (1 mg/mL). Further dilutions were prepared to obtain concentrations within the linearity range and scanned between 200–800 nm.

Method Validation

The developed UV–Visible spectroscopic method was validated according to ICH guidelines for the simultaneous estimation of Tetrabenazine and Amlodipine besylate.

Specificity

Specificity was confirmed by comparing the spectra of standard drug solutions with no interference observed.

Linearity

Linearity was evaluated over concentration ranges of 10–60 µg/mL for Tetrabenazine and 5–30 µg/mL for Amlodipine besylate, complying with Beer–Lambert's law.

Precision

Precision was assessed as:

- **Intraday precision:** Analysis of 30 µg/mL solutions thrice on the same day
- **Interday precision:** Analysis on three consecutive days
- **Repeatability:** Analysis of independently prepared 30 µg/mL solutions Results were expressed as %RSD.

Ruggedness

Ruggedness was evaluated by performing the assay using two different analysts under identical conditions.

Robustness

Robustness was assessed by introducing minor variations in experimental conditions and observing the effect on absorbance.

Limit of Detection (LOD) and Limit of Quantification (LOQ) (kumar *et al.* 2012)

LOD and LOQ were calculated using the equations:

$LOD = 3.3 \times (\sigma/S)$

$LOQ = 10 \times (\sigma/S)$

where σ is the standard deviation of the response and S is the slope of the calibration curve.

Simultaneous Estimation of Tetrabenazine and Amlodipine Besylate

Standard solutions of both drugs were scanned to determine λmax values, which were found to be 285.0 nm for Tetrabenazine and 360.0 nm for Amlodipine besylate. Calibration curves were constructed within the specified concentration ranges, and absorptivity values were calculated to confirm compliance with Beer's law for simultaneous estimation.

3. RESULT AND DISCUSSION

Physical Appearance

The drug sample was analyzed physical appearance and the parameter given below in table 1.

Table 1: Physical characterization of Tetrabenazine Amlodipine besylate.

S. No	Physical parameter	Observation Tetrabenazine	Observation Amlodipine besylate
1	Color	White color	white to off-white
2	Odor	Odorless	fruit-like breath odor
3	State	Solid powder	Solid powder
4	Texture	crystalline powder	crystalline powder

Tetrabenazine was discovered to have a white powder to it when tested. Tetrabenazine has an Odor less and has a solid state and powder form, according to research conducted on it. Tetrabenazine exhibited the same Texture, color, odor, and state as the I.P. requirements for these characteristics. An evaluation of the API's organoleptic qualities, including Texture, color, odor, and state, was conducted. Amlodipine besylate was discovered to have a white to off-white powder to it when tested. Amlodipine besylate has fruit-like breath odor and has a solid state and powder form, according to research conducted on it. Amlodipine besylate exhibited the same Texture, color, odor, and state as the I.P. requirements for these characteristics. Result show in Table 1.

Solubility study

Table 2: Solubility study of Tetrabenazine and Amlodipine.

Drug	Solvents	Observation/Inference of Tetrabenazine	Observation/Inference of Amlodipine
Tetrabenazine and Amlodipine	Water	Low solubility	Partial soluble
	Ethanol	Soluble	Sparingly soluble
	Methanol	Fully soluble	Freely soluble
	Acetone	soluble	Partial solubility
	DMSO	Sparingly Soluble	Soluble

The solubility of Tetrabenazine and Amlodipine besylate were determined in various non-volatile or volatile liquid vehicles such as Dimethyl sulfoxide, methanol, ethanol, acetone, and water shown in Table 2. From the results, it was observed that Tetrabenazine is fully soluble in Acetone, in methanol Tetrabenazine is sparingly soluble, in Ethanol, DMSO Tetrabenazineis only Soluble and low solubility in water. And Amlodipine besylate is freely soluble in Methanol, partial soluble in water and Acetone, sparingly soluble in Ethanol and soluble in DMSO.

Determination of pH

Table 3: pH determination.

S. No.	Drug	Observed
1	Tetrabenazine	7.8
2	Amlodipine besylate	8.6

The digital pH meter used to determination the pH of the drugs. The pHs of the Tetrabenazine and Amlodipine besylate were found to be 7.8 and 8.6. This is well within the limits of the drug specification range.

Melting Point

Table 4: Melting Point of Tetrabenazine and Amlodipine besylate.

Drugs	Observed	Reference
Tetrabenazine	127 ºC	127-129 ºC
Amlodipine besylate	134°C	134-136°C

The capillary method is used to determine the melting point of a substance. The melting point of the Tetrabenazine and Amlodipine besylate were found to be 127 ºC and 134°C, which is well within the limits of the drug specification.

Determination of λ max by UV spectroscopy

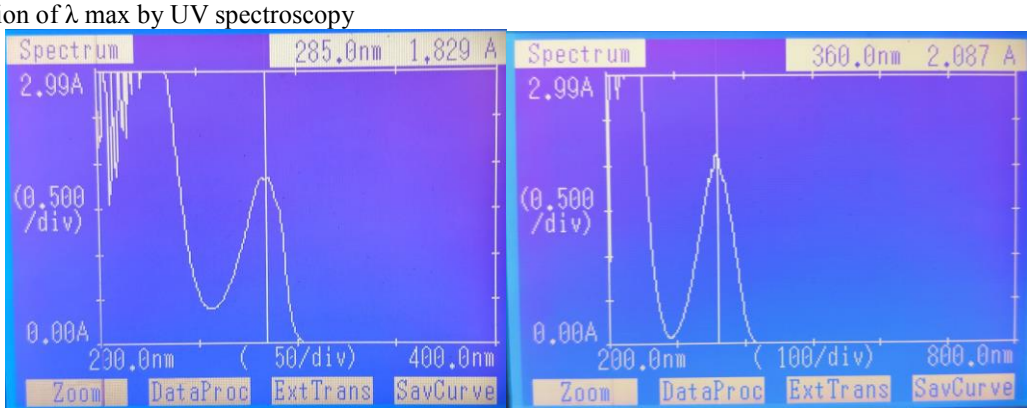

Figure 1: UV graph of Tetrabenazine and Amlodipine besylate.

Table 5: Lambda max.

S. No.	Drug Name	Lambda max
1	Tetrabenazine	285.0 nm
2	Amlodipine besylate	360.0 nm
3	Combination of both drugs	299.5 nm

Development of Calibration Curve

The calibration curve of Tetrabenazine and Amlodipine besylate both were prepared in methanol. Accurately weighed 10 mg drug and dissolved in a small number of respective solvent in a 10 ml volumetric flask. The volume was made up to 10 ml with solvent (1000ug/ml). 1 ml of stock solution was diluted up to 10 ml with respective solvent (100ug/ml). Further dilutions were made in the concentration range of 10-60 and 5-30 ug/ml from the secondary stock solution. Absorbance was taken by UV spectroscopy and was plotted against the corresponding concentration to obtain both calibration curve Tetrabenazine and Amlodipine besylate (**Akshay *et al.*, 2020).**

Standard calibration curve

Table 6: Calibration Curve of Tetrabenazine in Methanol.

S. No	Concentration (µg/ml)	Mean Absorbance
1	10	0.172
2	20	0.369
3	30	0.789
4	40	0.921
5	50	1.261
6	60	1.425
Mean	0.822833	
SD	0.488861	
%RSD	59.41	

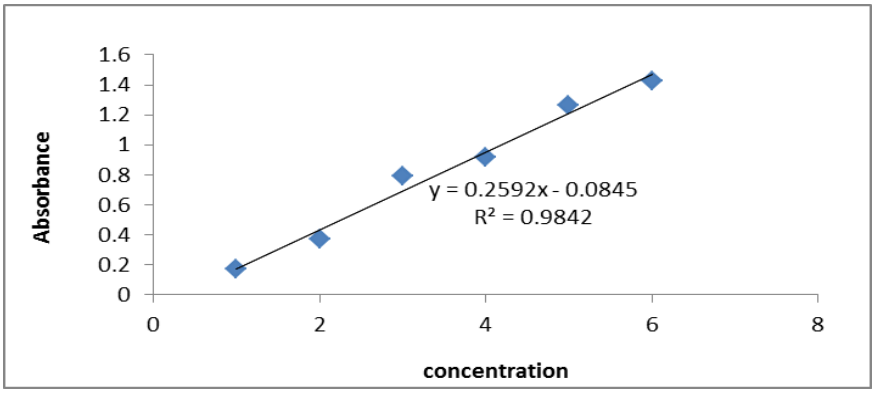

Figure 5: Calibration curve of Tetrabenazine.

Table 7: Calibration Curve of Amlodipine besylate in Methanol.

S. No	Concentration (µg/ml)	Mean Absorbance
1	5	0.191
2	10	0.383
3	15	0.522
4	20	0.724
5	25	0.896
6	30	1.171
Mean	0.647833	
SD	0.356583	
%RSD	55.04	

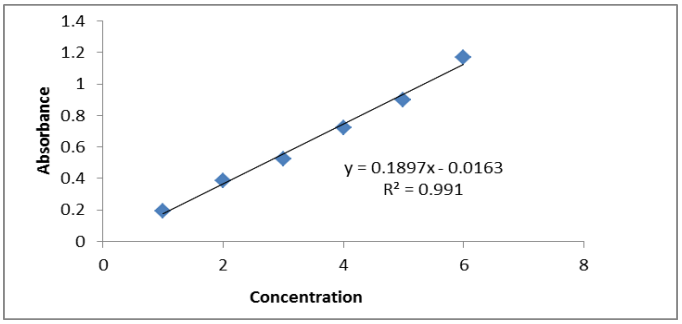

Figure 6: Calibration curve of Amlodipine besylate.

Functional group identified by Infra-Red spectroscopy

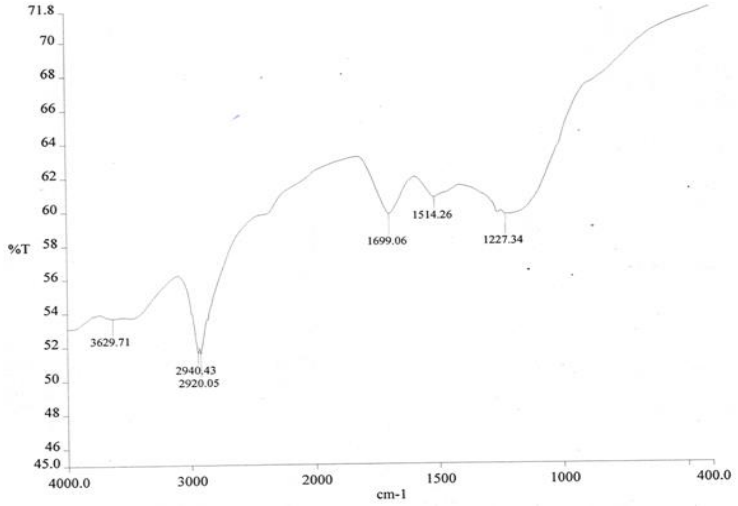

Figure 7: IR study of Tetrabenazine.

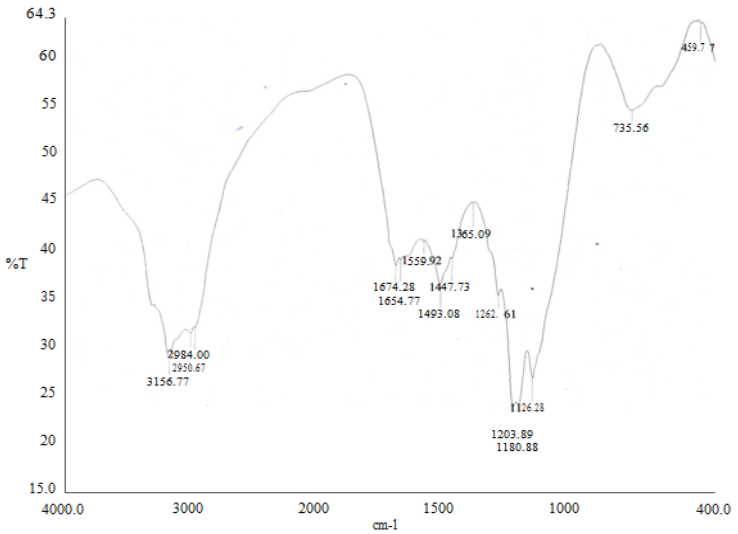

Figure 8: IR study of Amlodipine besylate.

Interpretation of Tetrabenazine FTIR

Table 8: Interpretation of FTIR of D+E.

S. No.	Peak obtained	Reference peak	Functional group	Name of functional group
1	3629.71	3700-3584	O-H stretching	Alcohols
2	2940.43	3000-2800	N-H stretching	amine salt
3	2920.05	3000-2840	C–H stretching	Alkane
4	1699.06	1710-1685	C=Ostretching	conjugated aldehyde
5	1514.26	1550-1500	N-Ostretching	nitro compound
6	1227.34	1250-1020	C-N stretching	Amine

Interpretation of Amlodipine besylate FTIR

Table 9: Interpretation of FTIR of D+E.

S. No.	Peak obtained	Reference peak	Functional group	Name of functional group
1	3156.77	3200-2700	O-H stretching	Alcohols
2	2984.00	3000-2840	C–H stretching	Alkane
3	1674. 28	1685-1666	C=Ostretching	conjugated ketone
4	1559.92	1600–1475	C=Cstretching	Alkene
5	1493.08	1550–1475	N–O asymmetric stretch	nitro compounds
6	1365.09	1370–1350	C–H rock	Alkanes
7	1262.16	1335–1250	C–N stretch	aromatic amines
8	1126.28	1150-1085	C-Ostretching	aliphatic ether
9	735.56	800–600	C–Cl	Chloride

FTIR spectra of Tetrabenazine O-H stretching peak of Alcohol at 3629.71cm-1, C-H bending peak of alkane group at 2920.05cm-1, C-N stretching peak of amine at 1227.34cm-1, C=O stretching peak of conjugated aldehyde at 1699.06 cm-1, N-Ostretching peak of nitro compound at 1514.26cm-1.

FTIR spectra of Amlodipine besylate Alcohol O-H stretching Weak, broad peak appeared at 3156.77cm-1, C-H stretching peaks of Alkane at 2984.00cm-1. The C=O stretching peak of conjugated ketone group at 1674. 28cm-1, C=C stretching peak Alkene at 1559.92 cm-1, C-H rock bending peak of alkanes group at 1365.09cm-1 N–O asymmetric stretch peak, nitro compounds at 1493.08.

Infra-Red (IR) spectroscopy is very important tool for identification of structural changes of the sample at molecular levels. As each and every functional group show its specific absorption peak in the IR region, it is easy to find out interaction of molecules of different materials or components.

Method Validation via UV spectroscopy Tetrabenazine and Amlodipine besylate

Precision study of Tetrabenazine

(A) Intraday Precision

Table 10: Result of Intraday Precision (three times on the same day)

Concentration (µg/mL)	Day 1 Absorbance (1) at 285 nm	Day 1 Absorbance (2) at 285 nm	Day 1 Absorbance (3) at 285 nm
30	0.789	0. 791	0. 793
30	0. 790	0. 792	0. 794
30	0. 792	0. 790	0. 791
Mean	0.789	0.791	0.792667
SD	0.001528	0.001	0.001528
%RSD	0.1936	0.1264	0.1927
AVG % R.S.D	0.17		

(B) Interday Precision

Table 11: Result of Inter day Precision (Three times on the different day).

Concentration (µg/ml)	Day 1 Absorbance at 285 nm	Day 2 Absorbance at 285 nm	Day 3 Absorbance at 285 nm
30	0.789	0.793	0.795
30	0.792	0. 796	0. 797
30	0. 795	0. 794	0. 798
Mean	0.7905	0.793	0.795
SD	0.002121	0.001528	0.001528
%RSD	0.26	0.19	0.19
AVG % R.S.D	0.64		

(C) Repeatability

Table 12: Result of repeatability.

Sr. No.	Concentration (µg/ml)	Absorbance	Statistical analysis	
1	30	0.787	**Mean**	0.7968
2	30	0.793	**SD**	0.005762
3	30	0.798	**% RSD**	0.723
4	30	0.801		
5	30	0. 799		
6	30	0.803		

Ruggedness

Table 13: Result of ruggedness.

Analyst-1		Analyst-2	
Concentration (µg/ml)	**Absorbance**	**Concentration (µg/ml)**	**Absorbance**
30	0.788	30	0.789
30	0. 796	30	0. 795
30	0. 799	30	0. 792
Mean	0.789	**Mean**	0.789
SD	0.005132	**SD**	0.003
% RSD	0.65	**% RSD**	0.38

Robustness

Table 14: Results showing robustness.

Temperature 25^{0}C		Temp 30^{0}C	
Concentration (µg/ml)	**Absorbance**	**Concentration (µg/ml)**	**Absorbance**
30	0.785	20	0. 788
30	0.790	20	0. 793
30	0. 797	20	0. 791
Mean	0.7875	**Mean**	0.790667
SD	0.003536	**SD**	0.002517
% RSD	0.449	**% RSD**	0.138

Precision study of Amlodipine besylate

(A) Intraday Precision

Table 15: Result of Intraday Precision (three times on the same day).

Concentration (µg/mL)	Day 1 Absorbance (1) at 360 nm	Day 1 Absorbance (2) at 360 nm	Day 1 Absorbance (3) at 360 nm
30	0.724	0. 726	0. 725
30	0. 723	0. 724	0. 726
30	0. 725	0. 723	0. 724
Mean	0. 724	0.7243	0. 725
SD	0.001	0.001528	0.001
%RSD	0.0026	0.210	0.137
AVG % R.S.D	0.3496		

(B) Interday Precision

Table 16: Result of Inter day Precision (Three times on the different day).

Concentration (µg/ml)	Day 1 Absorbance at 360 nm	Day 2 Absorbance at 360 nm	Day 3 Absorbance at 360 nm
30	0.724	0.726	0.728
30	0.726	0. 725	0.727
30	0. 727	0. 724	0. 726
Mean	0. 725	0. 726	0. 728
SD	0.001414	0.001	0.001
%RSD	0.195	0.137	0.137
AVG % R.S.D	0.469		

(C) Repeatability

Table 17: Result of repeatability.

Sr. No.	Concentration (µg/ml)	Absorbance	Statistical analysis	
1	30	0.724	**Mean**	0. 7262
2	30	0.726	**SD**	0.001862
3	30	0.728	**% RSD**	0. 256
4	30	0.727		
5	30	0. 723		
6	30	0.726		

Ruggedness

Table 18: Result of ruggedness.

Analyst-1		Analyst-2	
Concentration (µg/ml)	**Absorbance**	**Concentration (µg/ml)**	**Absorbance**
30	0.724	30	0.724
30	0. 727	30	0. 726
30	0. 726	30	0. 725
Mean	0.724	**Mean**	0.724
SD	0.001528	**SD**	0.001
% RSD	0.211	**% RSD**	0.138

Robustness

Table 19: Results showing robustness.

Temperature 25^0C		Temp 30^0C	
Concentration (µg/ml)	**Absorbance**	**Concentration (µg/ml)**	**Absorbance**
30	0.724	20	0. 724
30	0.728	20	0. 727
30	0. 725	20	0. 726
Mean	0. 726	**Mean**	0.725667
SD	0.002082	**SD**	0.001528
% RSD	0.286	**% RSD**	0.210

LOD and LOQ

Table 20: Results showing LOD and LOQ.

S. No.	Drug name	Wavelength	LOD (µg/ml)	LOQ (µg/ml)
1	**Tetrabenazine**	285.0	1.99	6.03
2	**Amlodipine**	360.0	1.74	5.30

Table 21: Optical Characteristics and Validation Study of Formulation.

Parameters	Tetrabenazine	Amlodipine
Wavelength λ max nm	285.0	360
Beer's law limit µg/ml	10-60	5-30
Correlation coefficient (R2)	0.984	0.991
Slope	0.259	0.189
Intercept	0.084	0.016
SD	0.488861	0.356583
% RSD	59.41	55.04
Precision Intraday (% RSD) Interday (% RSD) Repeatability (% RSD)	 0.17 0.64 0. 723	 0.3496 0.469 0. 256
Ruggedness Analyst 1 (% RSD) Analyst 2 (% RSD)	 0.65 0.38	 0.211 0.138
Robustness Temp.25^0C (% RSD) Temp.30^0C (% RSD)	 0.449 0.138	 0.286 0.210
LOD (µg/ml)	57.98	1.74
LOQ (µg/ml)	175.71	5.30

Simultaneous estimation of Tetrabenazine and Amlodipine besylate

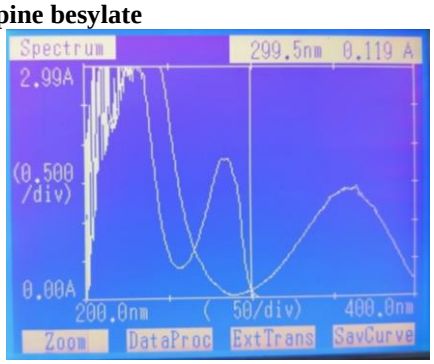

Figure 2: UV graph of Tetrabenazine and Amlodipine besylate (299.5 nm).

In UV-Spectroscopic method, the crossing points of spectra were utilized for developing the equations for simultaneous analysis. After simultaneous estimation the intersecting point of both drugs were found to be on 299.5 nm. The overlain spectra are shown in Figure 18 and analytical data was presented in Table 13 and 24. In contrast, the spectra of each pure drug was found to show crossing point and assisted in their simultaneous estimation. In this method, wavelengths were utilized 285.0 nm for Tetrabenazine and 360 nm for Amlodipine besylate.

4. CONCLUSION

A simple, precise, and validated UV–visible spectrophotometric method for the simultaneous estimation of Tetrabenazine and Amlodipine besylate was successfully developed. Comprehensive pre-formulation studies confirmed that both drugs comply with pharmacopeial standards regarding identity, purity, solubility, and stability. The distinct and overlapping absorption maxima enabled accurate simultaneous estimation without mutual interference. Validation studies performed as per ICH guidelines demonstrated excellent linearity, precision, ruggedness, robustness, and sensitivity, with %RSD values well below acceptable limits. The low LOD and LOQ values further confirmed the sensitivity of the method. Overall, the developed UV spectrophotometric method is economical, reliable, and suitable for routine quality control analysis of Tetrabenazine and Amlodipine besylate in bulk and pharmaceutical dosage forms.

5. REFERENCES

1. ICH. (2005). *Validation of analytical procedures: Text and methodology Q2(R1).* International Conference on Harmonisation.
2. Frank, S. (2010). Tetrabenazine: the first approved drug for the treatment of chorea in US patients with Huntington disease. Neuropsychiatric disease and treatment, 657-665.
3. Sweetman, S. C. (Ed.). (2011). *Martindale: The complete drug reference* (37th ed.). Pharmaceutical Press.
4. Beckett, A. H., & Stenlake, J.B. *Practical pharmaceutical chemistry* (4th ed.). CBS Publishers, 2007.
5. Aulton, M. E., & Taylor, K. M. G. *Aulton's pharmaceutics: The design and manufacture of medicines* (5th ed.). Elsevier, 2018.
6. Jain, N. E. E. L. A. M., & Verma, A. N. U. R. A. G. Preformulation studies of pilocarpine hydrochloride as niosomal gels for ocular drug delivery. Asian J Pharm Clin Res., 2020; 13(6): 149-155.
7. Chowk, M. I. Preformulation study of terbinafine for novel drug delivery system formulation. Tablet, 2020; 250: 500mg.
8. Kumbhar da, nalawade am, kumbhar ss, nalawade ra, salunke gd. Synthesis of co doped zno nanoparticles by sol gel method and their characterizations for solar cell application, 2013.
9. Behera, S., Ghanty, S., Ahmad, F., Santra, S., & Banerjee, S. UV-visible spectrophotometric method development and validation of assay of paracetamol tablet formulation. Journal of Analytical & Bioanalytical Techniques, 2012; 3(6): 1–6. https://doi.org/10.4172/2155-9872.1000151
10. Kumar, R., Ansari, K. M., Saxena, N., Dwivedi, P. D., Jain, S. K., & Das, M. Detection of ochratoxin A in wheat samples in different regions of India. Food control, 2012; 26(1): 63-67.