Development and validation of UV spectrophotometric method for estimation of stiripentol

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Abstract: A rapid, specific and economic UV spectrophotometric method has been developed using a solvent, pH 1.2 buffer for the estimation of Stiripentol at a predetermined λ_{max} of 264 nm. Validation was conducted according to ICH guidelines. This method shows linearity between the ranges 5-25µg/ml with correlation coefficient (R^2), 0.9988. The % recovery was found to be in the range of 98-102%. Sensitivity was also confirmed with a limit of detection (LOD) of 2.4322 µg/ml and a limit of quantitation (LOQ) of 7.3703 µg/ml, indicating that even small quantities of stiripentol can be accurately measured. The low values of % RSD are indicative of the reproducibility of the method. The method can be employed for the routine analysis of stiripentol in the bulk and in the pharmaceutical dosage form.

Keywords: Stiripentol, UV Spectrophotometry Analysis, Validation, Method Development.

1. Introduction:

Stiripentol is an aromatic allylic alcohol with the chemical formula 4,4-dimethyl-1-[3,4-(methylenedioxy)-phenyl]-1-penten-3-ol.[1]. It's a white to pale yellow crystalline powder. Stiripentol is used to treat seizures. Stiripentol is second generation antiepileptic drug belongs to GABAA allosteric modulator.[2] Stiripentol is used as adjunctive therapy with clobazam and valproic acid in treating Severe Myoclonic Epilepsy Infancy SMEI.[6] Stiripentol exhibits its mechanism of action in two way: positive modulation of the Gamma-GABAergic system; (direct effect) and hepatic clearance isozyme inhibition (indirect effect).

Gamma aminobutyric acid (GABA) is the major inhibitory transmitter controlling synaptic transmission and neuronal excitability. STP produced positive allosteric modulation effect at GABAA receptors with strong potentiation effect at 3 receptors and weak potentiation at receptors

containing ß1 and subunits. Also, STP induces sensitivity of the receptor to GABA, without increasing the maximum response to saturating GABA concentrations. STP enhances the activity of the GABA neurotransmitter by potentiating GABA , STP increases the inhibitory effects on neurons, leading to a reduction in excitability and seizure activity. It may also modulate voltage gated calcium channels, which can affect neuronal excitability. STP inhibits the CYP3A4, CYP1A2 and CYP2C19 enzyme, which is involved in metabolism of other antiepileptic drugs. [7]The drug is official in United States Pharmacopoeia. A few analytical methods have been reported for determination of the stiripentol drug. The reported methods for the estimation of stiripentol were HPLC[12, 13, 14, 16,17, 18] and HP-TLC[15]. There is no UV spectrophotometric method for quantification of stiripentol. Therefore the present study was aimed to develop new UV spectrophotometric method for the estimation of stiripentol.[3]

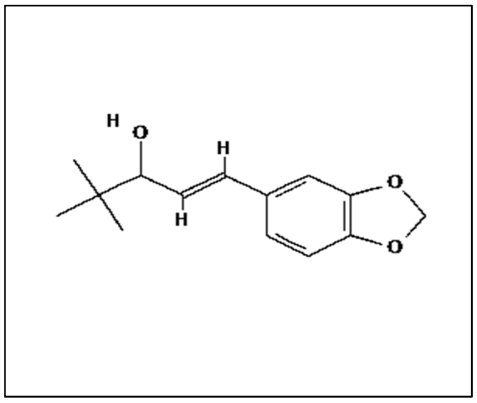

Figure 1. Structure of Stiripentol

2. Materials and Methods

2.1 Material

The analysis utilized a Shimadzu UV 1800 UV-Visible Double Beam Spectrophotometer paired with 1-cm quartz cells. Samples were precisely weighed with a Shimadzu digital balance, and an ultrasonic cleaner was employed to sonicate the sample solution.

All reagents and chemicals employed in the study were of analytical reagent (AR) grade. The reference standard for pure Stiripentol was obtained as gift samples from Metrochem API Pvt. Ltd. India. Methanol used which was AR grade procured from Merk Life Sciences.

2.2 Method

Preparation of Buffer pH 1.2

Weigh 2.982 grams of potassium chloride that has been dissolved in 100 milliliters of distilled water gives 0.2 M potassium chloride solution. Prepare 0.2 M hydrochloric acid by dissolving 18 ml of concentrated HCl in 1000ml volumetric flask and top it up with distilled water. 50 ml of 0.2M potassium chloride solution was transferred to 200 volumetric flask then 80 ml of 0.2M hydrochloric acid was added and volume was adjusted by distilled water. [4, 5]

Preparation of standard solutions

10 mg of the STP was weighed, dissolved, and diluted to 10 ml with methanol in a volumetric flask, resulting in a concentration of 1000 µg/ml. 1 ml of this solution was diluted to 10 ml with pH 1.2 buffer solution, yielding a concentration of 100 µg/ml.

Selection of wavelength of maximum absorption

A 10 µg/ml solution of stiripentol scanned from 400-200 nm using the UV spectrophotometer and λmax was determined from the spectrum.

Linearity

0.5, 1, 1.5, 2, 2.5 ml of standard solution were diluted to 10 ml with buffer pH 1.2 in 10ml volumetric flasks to get 5, 10, 15, 20, and 25 µg/ml of STP solutions. The absorbance of each solution was then measured using a UV-visible Spectrophotometer at a wavelength 264 nm, with buffer pH 1.2 serving as the blank reference.

Precision

The precision of an analytical method analyzed by repeatability determined by measuring how closely the results match when the same sample is tested multiple times under set conditions. [10] Absorbance of 15 µg/ml STP solution was recorded and repeated for six times.

Accuracy

Accuracy is closeness of the agreement between the result of a measurement and a true value of the measured. By adding sample solution to the standard solutions at concentrations of 80%, 100%, and 120%, the recovery was estimated. [9]

Test Solution: Dissolve 10 mg of stiripentol in 10 ml of volumetric flask and top it up with methanol to create a 1000 µg/ml methanolic solution. From this 1000 µg/ml solution pipette out 1 ml and dilute up to 20 ml with pH 1.2 buffer to yield 50 µg/ml sample solution. 5 ml of standard solution diluted to 10 ml with pH 1.2 buffer to yield 50 µg/ml of stiripentol. To the preanalyzed sample solution, a known amount of standard STP solution was added at different levels and absorbance was recorded(table no. 2). The drug content of preparation was calculated using standard calibration curve equation.

Limit of Detection

The detection limit of an analytical method is the smallest amount of a substance in a sample that can be detected, although it may not be necessarily quantitated in given experimental conditions. The formula for calculating detection limit is;

DL =3.3 x SD/Slope

Limit of Quantification

The quantitation limit of an analytical procedure is the smallest amount of a substance in a sample that can be measured with acceptable precision and accuracy. It is particularly important for determining low levels of compounds, such as impurities or degradation products, in different sample matrices. The formula for computing limit of quantitation; [11]

QL =10 x SD/Slope

Robustness

The robustness of an analytical method measures its ability to remain consistent despite small, intentional changes in method parameters. This indicates its reliability during regular use. The 10 µg/ml STP solution is prepared from 100 µg/ml stock solution of stiripentol this solutions' absorbances were recorded and confirmed at least six times at 263nm, 264nm and 265nm wavelength. The % relative standard deviation (%RSD) was calculated and robustness of developed method was determined.

3. Results

3.1 Selection of wavelength of maximum absorption

The absorption spectrum revealed a prominent peak at 264 nm. Selecting this wavelength ensures maximum absorbance, (λ_{max}) enhancing the sensitivity and accuracy for quantifying Stiripentol in various pharmaceutical formulations.

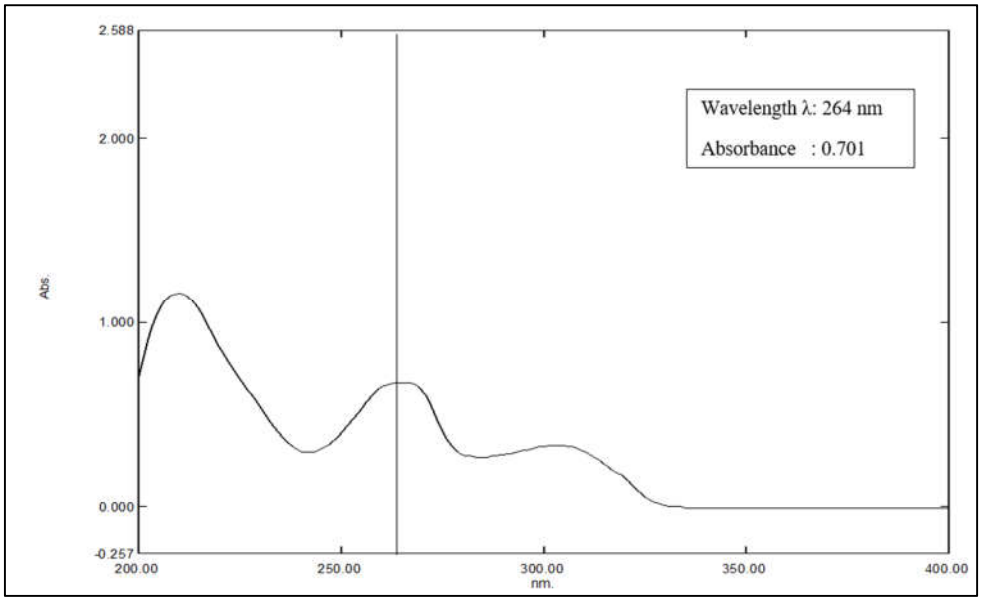

Figure 2. UV Spectra of STP (10 µg/ml)

3.2 Validation of Methods:

The validation of the developed procedures was conducted in compliance with the guidelines set by the International Council for Harmonization (ICH, 2005).[8]

Linearity and range:

The linearity of an analytical procedure means that the test results are directly proportional to the concentration of the substance being measured within a certain range. The linearity of the response of the drug was obtained at 5 to 25 µg/ml concentrations. The calibration curve was obtained by plotting the graph of absorbance Vs. concentration data and was later subjected to linear regression analysis. Table no. x. The equation of standard calibration curve was found to be y = 0.0785*x*-0.0729, the correlation coefficient r^2 of determination was 0.9988. The calibration curve was found to be linear for aforementioned concentrations.

Conc. in µg/ml	Absorbance
05	0.343
10	0.701
15	1.080
20	1.490
25	1.912

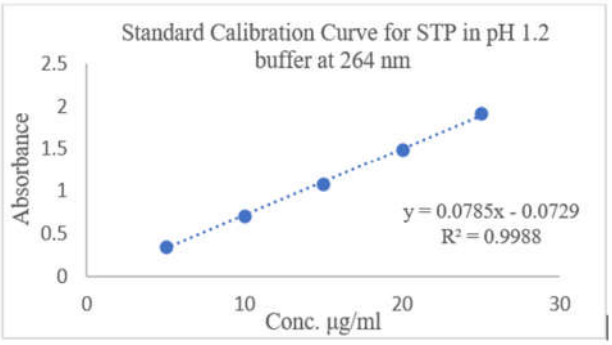

Figure 3 Linearity of responses of stiripentol standard solutions

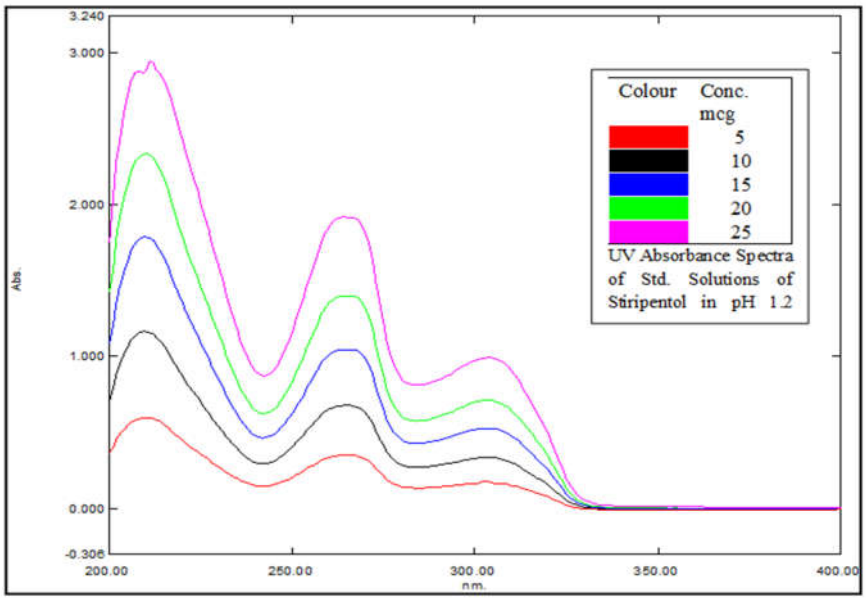

Figure 4. Overlay of UV Spectra of Standard Solutions of STP

Precision Study

The %RSD is very low (0.1786%), which indicates excellent repeatability (intra-day precision). All absorbance values are very close to the mean, showing consistent instrument response and high method precision at 15 µg/ml. This level of precision is well within acceptable limits for analytical methods, typically considered acceptable if %RSD < 2% for UV-Vis methods. The method demonstrates high precision at the tested concentration (15 µg/ml).It is suitable for quantitative analysis under these experimental conditions.

Table 1 Precision data of stiripentol standard solution

Sr. No.	Conc. µg/ml	Wavelength (nm)	Absorbance	Mean ± S. D.	% R. S. D.
1.	15	264	0.980	0.981±0.001751	0.178571
2.		264	0.983		
3.		264	0.980		
4.		264	0.981		
5.		264	0.982		
6.		264	0.978		

Accuracy Study

The % Recovery values obtained are within the acceptable range specified by ICH and USP guidelines, indicating that the analytical method is accurate for the concentration levels tested.

Table 2 Results of accuracy study

Level of Recovery	Volume of Test solution ml	Volume of standard solution ml	Diluted with pH 1.2 Buffer	Amount Recovered (µg/ml)	Amount Added (µg/ml)	% Recovery
80 %	1	0.8	10	3.62	3.52	102.8%
100%	1	1	10	4.32	4.4	98.10%
120%	1	1.2	10	5.18	5.28	98.10%

Limit of Detection:

The limit of detection (LoD) for stiripentol in pH 1.2 Buffer was found to be 2.4322 µg/ml.

Limit of Quantitation:

The limit of quantitation was found to be (LoQ) 7.3703 µg/ml.

Robustness:

In obtained results of robustness study of developed method, **264 nm** exhibits the lowest %RSD (0.21%) and a moderate absorbance value, suggesting high precision and reliable signal strength. **265 nm** shows the highest absorbance (0.8445), which can be beneficial for sensitivity. However, it also has the highest %RSD (0.75%), indicating slightly less precision compared to 264 nm. **263 nm** has acceptable precision (%RSD = 0.44%) but the lowest absorbance, which might impact sensitivity. Based on the balance between precision and absorbance intensity, **264 nm** is the most suitable wavelength for quantitative analysis in this context.

Table 3 Results of robustness data for developed method

Concentration in µg/ml	**Absorbance at 263 nm**	**Absorbance at 264 nm**	**Absorbance at 265 nm**
10	0.612	0.703	0.844
10	0.614	0.704	0.845
10	0.618	0.703	0.851

10	0.614	0.702	0.852
10	0.615	0.706	0.836
10	0.610	0.705	0.839
Mean	0.613828	0.703833	0.8445
Std. Dev.	0.002714	0.001472	0.006348
% R. S. D.	0.442169	0.209135	0.751714

4. Discussion:

The UV spectrophotometric method developed for the estimation of stiripentol is simple, rapid, cost-effective, and reliable. The selected analytical wavelength of 264 nm provided a clear and consistent maximum absorbance (λmax), ensuring sensitive detection. The method demonstrated excellent linearity within the concentration range of 5–25 µg/ml, with a correlation coefficient (R^2 = 0.9988), indicating a strong linear relationship between absorbance and concentration. The accuracy of the method, validated through recovery studies at 80%, 100%, and 120% levels, yielded recoveries in the range of 98.10–102.8%, confirming the method's reliability and reproducibility. Precision results exhibited very low %RSD (0.178%), signifying high repeatability of the assay. Sensitivity was also confirmed with a limit of detection (LOD) of 2.4322 µg/ml and a limit of quantitation (LOQ) of 7.3703 µg/ml, indicating that even small quantities of stiripentol can be accurately measured. Robustness testing across slight variations in wavelength (263 nm, 264 nm, 265 nm) confirmed the method's stability, with acceptable %RSD values across all readings. These results affirm that the developed method can be reliably used under varied conditions without significant loss of performance.

5. Conclusion:

The present study successfully established a validated UV spectrophotometric method for the quantification of Stiripentol in bulk and pharmaceutical formulations. The method is **linear, accurate, precise, sensitive, robust, and economical**, meeting the validation requirements set by **ICH Q2 (R1)** guidelines. Due to its simplicity and reliability, it is well-suited for routine quality control and analytical testing of Stiripentol in pharmaceutical laboratories.

References:

1. PubChem [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004-. PubChem Compound Summary for CID 5311454, Stiripentol; [cited 2025 Feb. 7]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Stiripentol
2. Europa.eu. [cited 2025 Jan 21]. Available from: https://www.ema.europa.eu/en/documents/product-information/diacomit-epar-product-information_en.pdf
3. Borman P, Elder D. Q2 (R1) validation of analytical procedures: text and methodology. ICH quality guidelines: an implementation guide. 2017 Sep 27:127-66.
4. USP. Validation of compendial procedures. 2016.15; 1640-1645. USP Vol 4 2020edition page no. 62223-62224.
5. Thompson M, Ellison SLR, Wood R. Guidelines for single laboratory. Validation of methods of analysis. Pure and Applied Chemistry, 2006. 16; 835–855.
6. Dravet C, Bureau M, Guerrini R, Giraud N, Roger J. Severe myoclonic epilepsy in infants. In: Roger J, Dravet C, Bureau M, Dreifus FE, Perret A, Wolf P, eds. Epileptic syndromes in infancy, childhood and adolescence. 2nd edition. London: John Libbey, 1992: 75–102.
7. Viswas Raja Solomon, Vikram J. Tallapragada, Mary Chebib, G.A.R. Johnston, Jane R. Hanrahan, GABA allosteric modulators: An overview of recent developments in non-benzodiazepine modulators, European Journal of Medicinal Chemistry, Volume 171, 2019, Pages 434-461.
8. Analytical aspects of UV visible spectroscopy. [Internet] [Cited 2019 October 25] Available from: https://www.pharmatutor.org/pharma-analysis/analytical-aspects-of-UV- VIS spectroscopy.
9. Willard H. Merritt L, Dean A. Instrumental Method of Analysis, 7th ed. Delhi: CBS Publishers and Distributors. 2001:1-9.
10. Skoog DA, Holler FJ, Niemen TA. Principles of Instrumental Analysis. Thomson Brooks/Cole.2004; 5th ed.:695-7.
11. Jena AK. HPLC: highly accessible instrument in pharmaceutical industry for effective method development. Pharmaceutical Analytical Act. 2011;3.
12. Darwish HW, Bakheit AH, Attia MI. Highly sensitive synchronous spectrofluorimetric method for determination of stiripentol in capsules and human urine: Application to in-vitro drug release and weight variation test. Dig. J. Nanomater. Biostruct. 2014 Apr 1;9(2):819-29.

13. Takahashi R, Imai K, Yamamoto Y, Takahashi Y, Hamano SI, Yoshida H. Determination of Stiripentol in plasma by high-performance liquid chromatography with fluorescence detection. Iryo Yakugaku (Japanese J. Pharma. Health Care and Sci.). 2015 Sep 10;41(9):643-50.
14. Sara M, Campos G, Rodrigues M, Ana F, Amilcar F, Gilberto A. Salting-out assisted liquid–liquid extraction method optimized by design of experiments for the simultaneous high-performance liquid chromatography analysis of perampanel and stiripentol in mouse matrices. J of Separation Sci. 2020 Sept 25;43(23):4289-4304.
15. O.A. Saleh, M.F. El-Behairy, A.M. Badawey, et al., Analysis of Stiripentol Enantiomers on Several Chiral Stationary Phases: A Comparative Study, Chromatographia. 78 (2015) 267–271.
16. H. Lin, R. Levy, Pharmacokinetic Profile of a New Anticonvulsant, Stiripentol, in the Rhesus Monkey Huey-shin, Epilepsia. 24 (1983) 692–702
17. R. Arends, K. Zhang, R. Levy, et al., Stereoselective pharmacokinetics of stiripentol: an explanation for the development of tolerance to anticonvulsant effect, Epilepsy Res. 18 (1994) 91–96.
18. S. Auvin, C. Lecointe, N. Dupuis, et al., Stiripentol exhibits higher anticonvulsant properties in the immature than in the mature rat brain, Epilepsia. 54 (2013) 2082– 2090.