RESEARCH ARTICLE

Analytical Method Development and Validation for Stability Indicating HPTLC Method for Assay of Stiripentol In Bulk and Dosage Form

Santosh Kumar Kashid*, Amit Tapkir, Pravin Choudhari

Abstract

This work is concerned with the stability-indicating method development and validation of Stiripentol in a bulk drug and formulation by high-performance thin-layer chromatographic method (HPTLC). The pre-coated silica gel 60 F_{254} aluminum plate was selected as the stationary phase, and the solvent system consisted of Ethyl acetate: Dichloromethane: Toluene (2:2:6 v/v) used as developing solvents. Analysis of Stiripentol was carried out at 301 nm with Stiripentol being detected at an R(f) of 0.63. The developed method was validated for linearity, accuracy, precision, limit of detection (LoD), limit of quantitation (LoQ), robustness parameters, and stability are determined by force degradation study. The correlation coefficient of Stiripentol was 0.994 observed. The calibration plot was linear between 50–300 ng/band, respectively. The average percentage recovery of Stiripentol was found to be 100.25 %. Intra and inter-day precision measured as %RSD was less than 2%. Hence stability study of Stiripentol, it was found to degrade in acidic condition(8.52% - 0.1N HCL for 30 minutes at room temperature), alkali condition(7.47%- 0.1 N NaOH for 30min at room temperature), Hydrolytic condition(4.73%– dist. Water for 30min at room temperature), thermal condition(7.69%-40°C for 30min), oxidative condition(7.55% - 3% H_2O_2 for 30min at room temperature) and photolytic UV condition(7.54% -24hr UV radiation) respectively. Stiripentol was unstable in acidic condition and stable in normal dist. Water hydrolytic condition. The proposed method was found to be very sensitive and accurate for the determination of Stiripentol in bulk and formulation.

Keywords: High-Performance Thin Layer Chromatography, Stiripentol, Stress degradation, Validation.

Journal of Applied Pharmaceutical Sciences and Research, (2020); DOI: 10.31069/japsr.v3i4.5

Introduction

Stiripentol is a newly approved antiepileptic drug[1,2] (Figure 1). The racemic mixture of Stiripentol has two enantiomers R (+) Stiripentol and S (-).[3] Stiripentol of which R (+) has 2.4-fold greater anticonvulsant potency and 3-fold faster elimination rate then S (-) Stiripentol.[4] Its clinical development was delayed due to its inhibitory effect on hepatic cytochrome P450 (CYP).[5] By suppressing GABA uptake, Stiripentol increases γ- GABA concentration in brain tissues and reduces GABA transaminase activity.[6] Stiripentol has no affinity for $GABA_A$ and $GABA_B$ receptors.[7] The European marketing authorization has approved Stiripentol for the treatment of Dravet syndrome or severe myoclonic epilepsy in infancy SMEI in combination with sodium valproate and clobazam.[8,9]

Pharmaceutical dosage form of Stiripentol is imperative for the optimum and efficient delivery of its therapeutic activity to the patients by stability testing. The motive of stability testing is to look into changes in the quality of a drug substance or a drug product with time due to environmental factors, such as temperature, humidity, and light. Furthermore, stability testing includes storage conditions and shelf life for the drug product.[10,11] In certain, pharmaceuticals are fundamentally sensitive to environmental conditions, which are usually varied during the different stages (i.e., manufacturing, transportation and storage) of the finished products. Based on the earlier mentioned importance of stability testing, it is essential to investigate degradation pathways and stability of the drug. Recently, the need to establish a stability-indicating assay method for stability testing has become more clearly mandated in the official guidelines at the International Conference on Harmonization (ICH)[11] and United States Pharmacopeia (USP). A detailed literature survey disclosed that only few high-performance liquid chromatography (HPLC) methods were reported to determine STP to study its

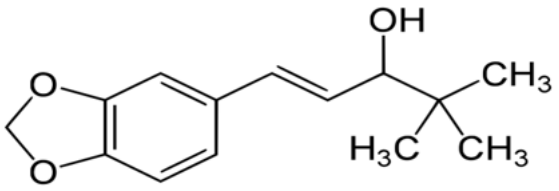

Figure 1: Structure of Stiripentol

PE's Modern College of Pharmacy, Nigdi, Pune, Maharashtra, India

Corresponding Author: Santosh Kumar Kashid, PE's Modern College of Pharmacy, Nigdi, Pune, Maharashtra, India, Email: santoshkashid963@gmail.com

How to cite this article: Kashid SK, Tapkir A, Choudhari P. Analytical Method Development and Validation for Stability Indicating HPTLC Method for Assay of Stiripentol In Bulk and Dosage Form. Journal of Applied Pharmaceutical Sciences and Research. 2020; 3(4):26-30

Source of support: Nil

Conflict of interest: None

pharmacokinetics.[12] It is important for the determination of Stiripentol in bulk and dosage form by a stability-indicating method by high-performance thin-layer chromatography (HPTLC).

Previously there is no research paper published on the stability-indicating assay method by HPTLC. So, it is necessary to validate and study degradation of stiripentol by stability-indicating assay method by HPTLC.

Material and Method

Chemicals and Reagents

Stiripentol working standard of the pharmaceutical grade was gifted from NURAY CHEMICALS PVT. LTD, TAMILNADU and all chemicals and reagents such as methanol, toluene, ethyl acetate, dichloromethane, conc. hydrochloric acid, hydrogen peroxide, sodium hydroxide pellets of analytical grade were provided by the college.

Instrumentation

Chromatography was performed on 10 × 10 cm Aluminum TLC plates $60F_{254}$ precoated with 250 µm layers of silica gel. Samples were applied in the form of bands, under a continuous flow of nitrogen, by means of a Camag Linomat V (Switzerland) sample applicator fitted with 100 µL applicator syringe. A constant application rate of 0.1 µL per second was used and the distance between the adjacent bands were also optimized. The plates were then conditioned for 10 minutes in a presaturated twin-trough glass chamber (10 x 10 cm^2). The spotted plate was then dipped in mobile phase (Toluene: Dichloromethane: Ethyl acetate (6:2:2 v/v) and ascending development was performed to a distance of around 80 mm from the point of application at ambient temperature. Subsequently, plates were dried in a current of air with the help of an air dryer, and spots were visualized in Camag UV cabinet copper formed at 301 nm with Camag TLC scanner III operated in reflectance-absorbance mode and controlled by Win Cats software. The slit dimensions (4 × 0.2 mm) were also optimized and kept constant throughout the analysis.

Preparation of Standard and Construction of Calibration Curve

The stock solution of Stiripentol was prepared by dissolving accurately about 5 mg of Stiripentol with 10 mL methanol. Aliquots of this solution were suitability diluted with methanol to get working standard solutions of Stiripentol with a 500µg/mL concentration. A calibration curve was plotted between concentrations against their respective area for Stiripentol. The calibration curve found that Stiripentol has a linearity range between 50-300 ng/spot.

Results and Discussion

Method Development

The HPTLC method was optimized for validation and method development of Stiripentol. The mobile phase Toluene: Dichloromethane: Ethyl acetate (6:2:2 v/v) resulted in good resolution and sharp peaks of RF 0.63 for Stiripentol. It was observed that prewashing of TLC plates with methanol (followed by drying and activation) and pre-saturation of TLC chamber with mobile phase for 30 min (optimum chamber saturation time) ensured good repeatability (Figure 2).

Method Validation

The method was validated for linearity, accuracy, precision, LoD and LoQ, robustness study and stress degradation study.

Linearity and Range

For establishment of linearity of Stiripentol by proposed method, the calibration curve was obtained at five levels in the concentration range of 50-300ng/spot. The different increasing amounts of Stiripentol working standard (0.4 mg/mL) were spotted three times on individual plates and analyzed as described. The observed peak area and concentrations were subjected to least square regression analysis to calculate calibration equation and correlation coefficient for evaluation of linearity. The observed linearity confirming adherence of the system to Beer's law. The regression analysis equation was y =345.7+16.03*X with correlation coefficient (r) was 0.99419 shown in Table 1.

Precision

Precision of the method was verified by repeatability and intermediate precision studies.

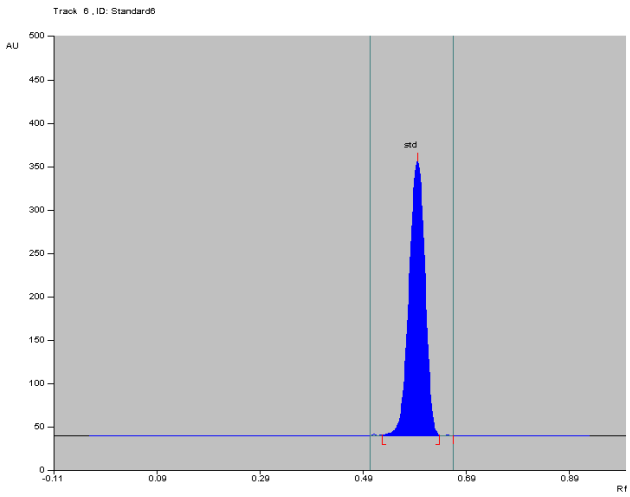

Figure 2: HPTLC densitogram of stiripentol

Table 1: Calibration curve of stiripentol

Sr. No.	*Concentration (ng/band)*	*Rf*	*Area*
1	50	0.64	1145.88
2	100	0.63	2050.08
3	150	0.63	2810.89
4	200	0.63	3348.40
5	250	0.63	4180.49
6	300	0.63	5370.97

Table 2: Repeatability result of stiripentol

Drug	*Amount of drug taken*	*% mean estimated*	*S.D.*	*%R.S. D*
Stiripentol	4mg	99.2	0.624	0.627
	5 mg	99.5	0.493	0.494
	6 mg	99.6	0.416	0.419

Table 3: Intermediate precision of stiripentol

Drug	*Amount of drug taken*	*% Mean estimated*	*S.D.*	*%R.S. D*
Stiripentol	4 mg	99.4	0.493	0.494
	5 mg	99.6	0.435	0.438
	6 mg	99.7	0.416	0.417

Table 4: Accuracy results of stiripentol by HPTLC

Level of recovery (%)	*Amount of drug added (mg)*	*Amount of drug recovered (mg)*	*% Recovery*	*% Recovery Mean*	*SD*	*% RSD*
	4	4.01	100.25			
80	4	4	100	100.33	10.17	0.37
	4	4.03	100.75			
	5	5.01	100.2			
100	5	4.99	99.94	100.23	10.59	0.31
	5	5.02	100.56			
	6	6.01	100.26			
120	6	5.99	99.87	100.21	13.11	0.32
	6	6.03	100.52			

Table 5: Robustness results of stiripentol by HPTLC

Chromatographic changes		
Factor	*Level*	*Rf values*
Mobile phase composition *Ethyl acetate: Dichloromethane: toluene (2:2:6)*		*Stiripentol*
2:3:5	±0.1	0.59
2:2:6	0	0.63
2:1:7	±0.1	0.64
Amount of mobile phase (±1ml)		*Stiripentol*
09	-1	0.62
10	0	0.63
11	+1	0.62
Duration of chamber (±1 min)		*Stiripentol*
5 min	-5 min	0.64
10 min	0 min	0.63
15 min	+5 min	0.59

Repeatability

In the repeatability studies, six replicates of one concentration of Stiripentol were prepared and spotted on HPTLC plate. From the obtained data, %RSD of Stiripentol were found to be less than 2%. The results of repeatability studies for Stiripentol shown in Table 2.

Intermediate Precision

In the intermediate precision studies, six replicates of one concentration were prepared and spotted on HPTLC plate for 3 consecutive days. From the obtained data, %RSD of stiripentol was found to be less than 2%. The intermediate precision results of Stiripentol are shown in Table 3.

Accuracy

The method's accuracy was determined by calculating the recovery of Stiripentol by the standard addition method at three concentration levels (80%, 100% and 120%). The percentage recoveries of Stiripentol were found to be in the range of 98-102%. The Accuracy results of stiripentol are shown in Table 4. The weight of the capsule powder taken is 6 mg.

Limits of Detection and Quantitation

The LoD was found to be 0.0174 and LoQ was found to be 0.053 ng/spot for Stiripentol.

Robustness

To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters such as a change in chamber saturation time, change in the composition of the mobile phase. This was studied to find out the robustness of the proposed method %RSD was found to be less than 2%. The Robustness result of change in saturation time (±5min) of Stiripentol. Change in Mobile phase composition (± 1 mL) of Stiripentol is shown in Table 5.

Forced Degradation Study of Stiripentol

5 mg Stiripentol was separately transferred to six different 10.0 ml volumetric flasks (Flask No. 1, 2, 3, 4,5 and 6) shown

Table 6: The results of the stress degradation studies of stiripentol by HPTLC

Sr. No.	*Stress Condition*	*Temp and Time*	*Percent degradation*	*Rf Value of degraded product*
1.	Acid (0.1 N HCl)	Room temp for 30 min	8.52%	0.65,0.43
2.	Alkali (0.1 N NaOH)	Room temp for 30min	7.47%	0.64,0.41
3.	Neutral (H_2O)	Room temp for 30 min	4.73%	0.62,0.41
4.	Thermal	40°C for 30 min	7.69%	0.64,0.42
5.	Oxide (3 % H_2O_2)	Room temp for 30min	7.55%	0.63,0.40
6.	Photolytic Degradation	24 hr.	7.54%	0.63,0.42

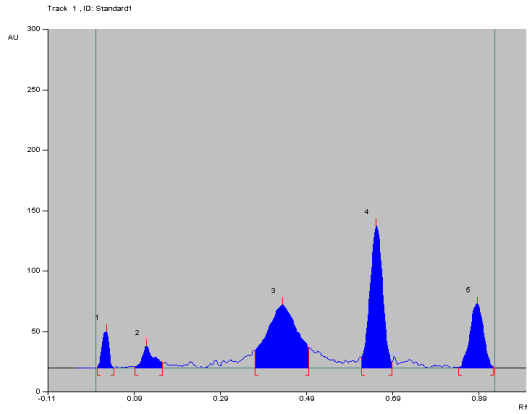

Figure 3: HPTLC Densitogram of acid degradation of stiripentol in 0.1N HCl at room temperature after 30 min.

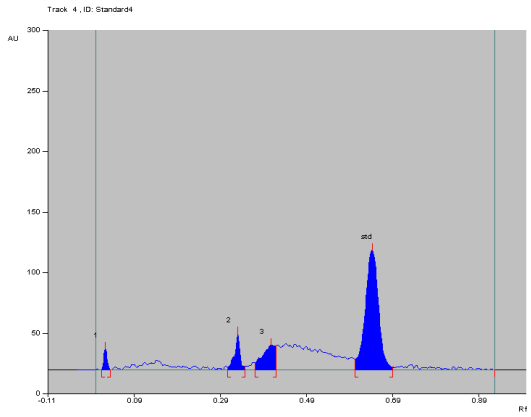

Figure 4: - HPTLC Densitogram of alkaline degradation of stiripentol in 0.1N NaOH at room temperature after 30min.

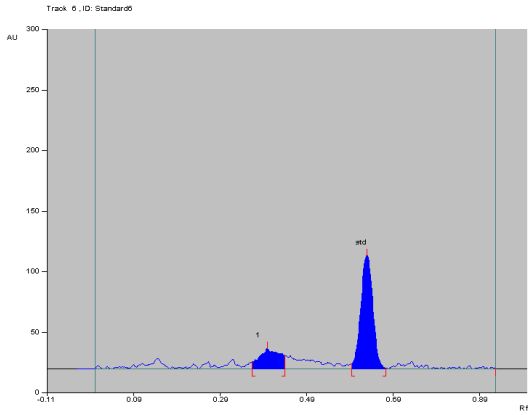

Figure 5: HPTLC Densitogram of oxidative degradation of Stiripentol in 3% H2O2 at room temperature after 30mins.

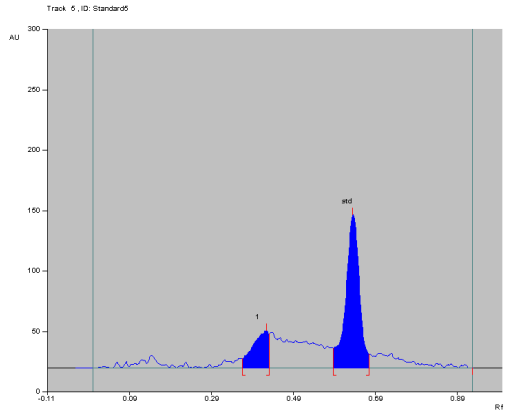

Figure 6: - HPTLC Densitogram of photolytic degradation of stiripentol on exposure to UV light for 24 hrs.

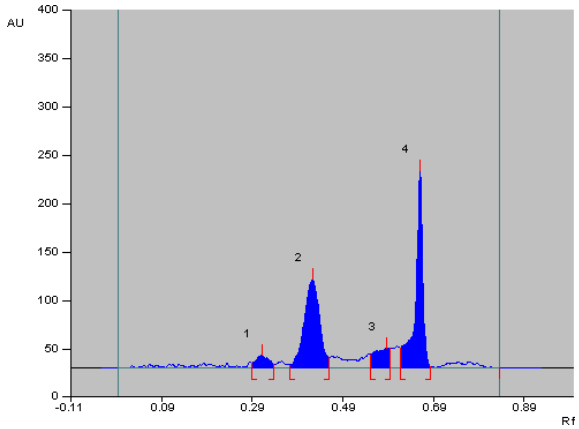

Figure 7: HPTLC Densitogram of thermal degradation of stiripentol on exposure to 40°C for 30 min.

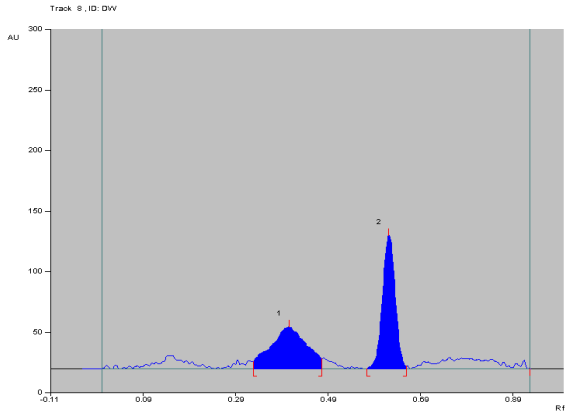

Figure 8: - HPTLC densitogram of Hydrolytic degradation of stiripentol in distilled Water at room temperature after 30 min.

in Table 6, added 3.0 ml of 0.1 N HCl, 0. 1 N NaOH, H_2O to Flask No. 1, 2, 3 respectively. In flask No. 4, 3% H_2O_2 is added and kept at dark for 30 min. Flask No. 1, 2, 3 were kept at room temperature for 30 min. Flask No. 5 containing Stiripentol was kept at 40°C for 30 min to study the effect of heat on drug sample (heat degradation). The forced degradation was performed in the dark to exclude the possible degradative effect of light. Flask No.6 was exposed to ultraviolet radiations at 254 nm for 24 hrs in a UV- chamber. All the flasks were removed, the Stiripentol samples were treated and analyzed in similar manner as described under analysis of pure drug. The typical densitogram for acidic, alkali, oxide, neutral, heat and UV exposure, are shown in Figure 3-8.

Discussion and Conclusion

The HPTLC method was developed on precoated silica gel using Toluene: Dichloromethane: Ethyl acetate (6:2:2 v/v) as mobile phase with densitometric detection at 301 nm. This study found that HPTLC method development for determination of Stiripentol in bulk and dosage form is accurate, precise, linear, highly sensitive, and robust. The force degradation study concludes that Stiripentol is most labile to acid hydrolysis followed by thermal degradation. The proposed degradation study is sensitive, precise, accurate, and stability-indicating, resolving all the drug's degradation products. Force degradation studies play an important role in the development of pharmaceuticals. The results of degradation studies help in the development of a stability-indicating method. The ICH Q1A guideline states that the validated stability-indicating test methods must be performed to monitor the shelf life of drug substances that are susceptible to change during storage and are likely to affect the quality, safety, and efficacy of the formulation.

Units of Measurement

Sr. No	*Terms*	*Full forms*
1.	Conc.	Concentration
2.	gm	Gram
3.	lit.	Liter
4.	µl	microliter
5.	m.mol	Milli molar
6.	PPM	Parts per millions
7.	ng	Nanometers
8.	λ	Wavelength
9.	hrs	Hours
10.	ng/band	Nano gram per band
11.	UV	Ultraviolet
12.	HPTLC	High Performance Thin Layer Chromatography.
13.	SD	Standard deviation
14.	RSD	Relative Standard Deviation
15.	LOD	Limit of detection
16.	LOQ	Limit of Quantification
17.	Rf	Retention factor
18.	DP'S	Degradation product

References

1. M. K. Trojnar, K. Wojtal, M. P. Trojnar, and S. J. Czuczwar, "Stiripentol. A novel antiepileptic drug," Pharmacological Reports, vol. 57, no. 2, pp. 154–160, 2005.
2. J. N. Lisgarten and R. A. Palmer, "The structure of Stiripentol: 4,4 dimethyl- 1-(3,4-methylenedioxyphenyl)-1-penten-3-ola novel antiepileptic drug," Acta Crystallographic, Section C: Crystal Structure Communications, vol. 44, pp. 1992–1994, 1988.
3. D. F. Chollet, "Determination of antiepileptic drugs in biological material," Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences, vol. 767, no. 2, pp. 191–233, 2002.
4. D. D. Shen, R. Levy, J. L. Savitch, A. V. Boddy, F. Tombret, and F. Lepage, "Comparative anticonvulsant potency and pharmacokinetics of (+)- and (–)-enantiomers of Stiripentol," Epilepsy Research, vol. 12, no. 1, pp. 29–36, 1992.
5. J. L. Fisher, "The effects of Stiripentol on GABAA receptors," Epilepsia, vol. 52, no. 2, pp. 76–78, 2011.
6. Wegmann R, Ilies A, Aurousseau M, Pharmaco-cellular enzymology of the mechanism of action of Stiripentol in cardiazol-induced epilepsy. III. Protein, nucleoprotein, lipid, and proteoglycan metabolism, Cell Mol Biol Incl Cyto Enzymol, 1978, 23, 455-480.
7. M. Poisson, F. Huguet, A. Savattier, F. Bakri-Logeais, and G. Narcisse, "A new type of anticonvulsant, Stiripentol. Pharmacological profile and neurochemical study," ArzneimittelForschung, vol. 34, no. 2, pp. 199–204, 1984.
8. E. C. Wirrell, L. Laux, D. N. Franz et al., "Stiripentol in Dravet syndrome: results of a retrospective US study," Epilepsia, vol. 54, no. 9, pp. 1595–1604, 2013.
9. European Medicines Agency, "Stiripentol: Scientific Discussion," http://www.ema.europa.eu/docs/en GB/document library/ EPAR Scientific Discussion/ human/000664/WC500036521.pdf.
10. The United States Pharmacopeia (USP) 24th version, United States Pharmacopeial Convention, Rochville, Md, USA, 2000.
11. ICH Guidance for Industry, Q1A (R2): Stability Testing of New Drug Substances and Products (Revision 2), U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), Rockville, Md, USA, 2003.
12. H. S. Lin and R. H. Levy, "Pharmacokinetic profile of a new anticonvulsant, Stiripentol, in the rhesus monkey," Epilepsia, vol. 24, no. 6, pp. 692–702, 1983.