Development and Validation of Stability Indicating Isocratic Reverse Phase-Liquid Chromatography for Determination of Phenytoin in Bulk and Pharmaceutical Formulation

SURAJ KISHAN GHODKE[1], SAILESH. J. WADHER[2]
[1]*Research Student, School of Pharmacy S.R.T.M University Nanded (MH),*
[2]*Professor, School of Pharmacy S.R.T.M University Nanded (MH), India.*

Abstract— The proposed method involves the utilization of a Shimadzu LC 2010 AT system equipped with a C18 Chromasil column (4.6 x 250mm, 5µm). A systematic optimization of chromatographic conditions, including mobile phase composition [Methanol: Water (0.1% Ortho-phosphoric acid) (70:30], before use, the mobile phase was filtered through 0.45 µm membrane filter and degassed by ultra-sonication. The flow rate was 1.0 mL/min, column temperature 30°C, the injection volume was 20 µl, and detection was performed at 254 nm using a UV detector. The retention time was obtained to be 6.15 min. In present study, the method was validated according to ICH guidelines Q2 (R1). The proposed RP-HPLC method has shown adequate separation for Phenytoin Sodium in bulk and the marketed formulation. Force degradation study was performed by applying various conditions and it is observed that 8.66 % degradation of drug in basic condition amongst the other conditions applied to carried out degradation studies. The method was validated in terms of specificity, linearity, accuracy, precision and robustness.

Index Terms- Phenytoin sodium, method development, forced degradation studies, validation, RP-HPLC

I. INTRODUCTION

Phenytoin Sodium is the sodium salt form of phenytoin, a hydantoin derivate and non-sedative antiepileptic agent with anticonvulsant activity. Phenytoin sodium promotes sodium efflux from neurons located in the motor cortex, thereby stabilizing the neuron and inhibiting synaptic transmission. This leads to a reduction in post tetanic potentiation at synapses, an inhibition of repetitive firing of action potentials and ultimately inhibits the spread of seizure activity. The solubility of phenytoin sodium in water increases with temperature. At room temperature, phenytoin sodium is soluble at concentrations up to approximately 20 mg/mL, soluble in methanol, ethanol. Stabilizes neuronal membranes and decreases seizure activity by increasing efflux or decreasing influx of sodium ions across cell membranes in the motor cortex during generation of nerve impulses.

II. RELATED WORK

Stability indicating RP-HPLC method developed and validated for the assay of phenytoin sodium in phenytoin sodium capsules was reported by Muralee Krishna [1]. HPLC method was developed for the determination of Phenytoin Sodium and Phenobarbitone in Bulk and Pharmaceutical Dosage Form by Radhika Shah et al. [2]. High-Performance Liquid Chromatographic Method for Determination of Phenytoin in Rabbits Receiving Sildenafil by Alaa Khedr et al. [3]. RP-HPLC method for the determination of phenytoin sodium residues on the surface of manufacturing equipments and study of its recovery from pharmaceutical formulations by Shashikant B. Bagade et al. [4]. Rapid detection of phenytoin sodium by partial-least squares and linear regression models combined with surface-enhanced Raman spectroscopy by Yinghui Wen et al. [5]. UV Spectrophotometric was developed for the estimation of Phenytoin Sodium in pure and pharmaceutical Formulation by Mangesh A. Mantri et al. [6]. From the literature it is shown that very few stability indicating method has been reported, therefore it was thought worthwhile to develop stability indicating HPLC method for the estimation of phenytoin sodium in bulk and pharmaceutical formulation.

III. METHODOLOGY

Shimadzu LC 2010 AT system equipped with a C18 Chromasil column (4.6 x 250mm, 5µm) was used for the development of stability indication HPLC method for the determination of phenytoin sodium with UV detector. Various conditions like acid, base, oxidative, thermal and UV light for the stability studies.

Name	Phenytoin Sodium, 5,5-Diphenylhydantoin sodium salt
Category	Non-sedative antiepileptic agent with anticonvulsant activity

Table 3: List of Material

Sr.No.	Name of Materials	Supplier
1	Phenytoin Sodium	Yarrow Chem Products
2	HPLC Grade Water	S D Fine-Chem Limited
3	0.1 % Orthophosphoric Acid	Dipa Chemical Industry
4	Acetonitrile	Dipa Chemical Industry
5	Methanol	Dipa Chemical Industry

III. EXPERIMENTAL RESULTS

Preparation of Phenytoin Sodium Standard Solution
Standard solution was prepared by accurately weighed 10 mg of Phenytoin sodium working standard into a 10 ml volumetric flask, added 10 ml of methanol: water (8:2 v/v), shaken and sonicated to dissolve the content, made up the volume with solvent mixture and filtered through 0.45 micron membrane filter. The solution was further diluted with solvent mixture to obtain the required concentration of standard concentrations (2-12 µg/ml) for Phenytoin Sodium.

Determination of λ max (Selection of Wavelength)
The standard solution of Phenytoin sodium was scanned in the wavelength range of 200-400 nm on a UV-Visible Spectrophotometer from this, wavelength corresponding to maximum absorbance (λ max) was found to be 254 nm for Phenytoin sodium.

Development of standard curve for the Phenytoin Sodium
Various dilutions of Phenytoin sodium from the standard solutions were prepared for the Phenytoin sodium 2 ppm, 4 ppm, 6 ppm,8 ppm,10 ppm and 12 ppm were prepared whereas, by using optimized solvent mixture at the fixed wavelength 254 nm.

Method Development by Reverse Phase High Performance Liquid Chromatography
Optimization of Chromatographic Conditions

In order to achieve the optimized chromatographic conditions to separate and quantify Phenytoin sodium one or two parameters were modified at each trial and chromatograms were recorded with all specified chromatographic conditions. Various trials were carried out to finalize the optimized chromatographic conditions. Poor resolution, bad peak shapes, disturbances in base line were the few reasons of the rejections of the trials. The blank and HPLC chromatogram of phenytoin is shown in figure 1 and 2.

HPLC (Shimadzu LC 2010 with UV detector)	Mobile Phase-Methanol: Water (0.1% OPA) with 0.1 % formic acid 70:30 Column - Inertsil C18 (4.6 x 250mm, 5µm) Flow rate- 1 ml/min Injection Volume- 20µl Pump mode- Isocratic Column temperature- Ambient Wavelength- 254 nm	Peaks shape were good, with good resolution and intensity	Accepted

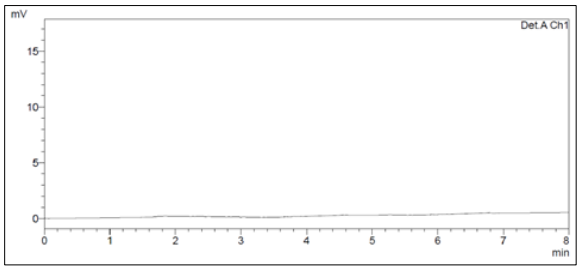


Figure 1: Blank Chromatogram

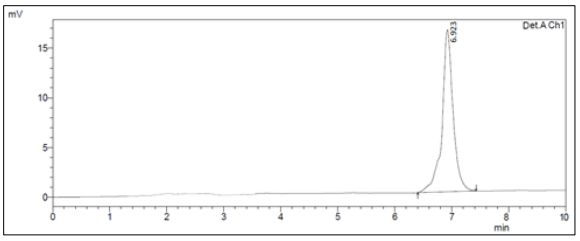


Figure 2: HPLC Fingerprinting of Phenytoin Sodium

Table 1: Evaluation parameter of HPLC method

Sr. No.	Name	Retention Time (min)	Area (µV*sec)	Height (µV)
1	Phenytoin Sodium	6.923	219146	16345

Linearity and Range

Linearity for Phenytoin sodium was found to be in the range of 10 - 60µg /ml with correlation coefficient value (r2) 0.9989. The results were tabulated in table 24 and graphically represented in figure 3.

Table 2: Linearity and Range for Phenytoin Sodium

oncentration (µg/ml)	age PeakArea
10	219826
20	516860
30	749808
40	989339
50	1235854
60	1495637
Slope	25073
CC	9669.1
R^2	9989

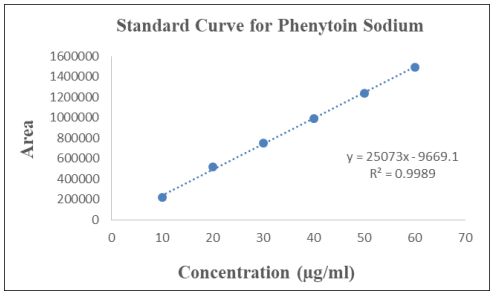


Figure 3: Standard Curve for Phenytoin Sodium

Accuracy (Recovery Study)

The accuracy of the assay method was evaluated by standard addition method in triplicate at 80%, 100 % and 120% level of the labelled claim and the percentage recovery was calculated. The mean % recovery was found to be 99.96%, 99.82% and 100.17 % respectively for Phenytoin Sodium. The results of the recovery study are shown in the table 3.

Table 3: Recovery study for Phenytoin Sodium

Phenytoin Sodium							
Level	Set	Amount added (µg/ml)	Amount found (µg/ml)	%Recovery	Mean	SD	%RSD
80 %	1	32	32.06	100.19	99.96	0.234	0.234
	2	32	31.99	99.97			
	3	32	31.91	99.72			
100 %	1	40	40.00	100.00	99.82	0.275	0.275
	2	40	39.80	99.50			
	3	40	39.98	99.95			
120 %	1	48	48.02	100.04	100.17	0.150	0.150
	2	48	48.06	100.13			
	3	48	48.16	100.33			

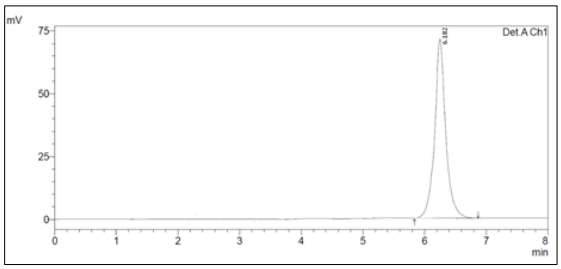


Figure 4: Chromatogram of Phenytoin Sodium accuracy at 80% level 1

Precision

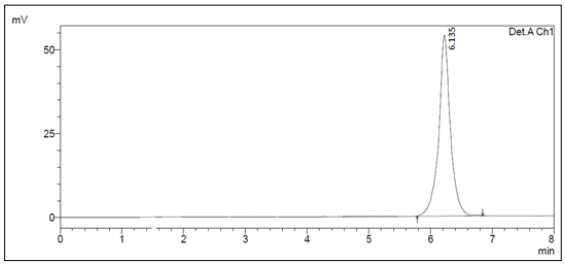
a) System Precision

The system precision was performed by measuring the peak response for standard drugs solutions (30 µg/ml) in six replicates. Peak responses, mean, and % relative standard deviation (%RSD) for Phenytoin Sodium was

found to be 0.0810 %. The results are shown in table 4 and were found well within the acceptable criteria.

Table 4: System Precision Data of Phenytoin Sodium

Sr. No.	Peak areas of Phenytoin Sodium
1	745308
2	745876
3	745987
4	746542
5	746987
Mean	746140
SD (±)	645.047
RSD (%)	0.0810



System Suitability

The HPLC method has been developed for the determination of the percentage assay of Phenytoin sodium. The Mobile phase was used, Methanol: Water with 0.1 % Orthophosphoric acid 70:30 with Column Inertsil C18 (4.6 x 250mm, 5µm) at Flow rate of 1 ml/min, Injection Volume was 20µl at 254nm. The chromatograms of standard and blank are shown in figure 6. The Retention time for Phenytoin sodium was found to be 6.178 min respectively and other parameters like, resolution, tailing factor, and theoretical plates were found to be within acceptable limit.

Table 6: System Suitability Parameters for Phenytoin Sodium

Sr. No.	Name	etention Time*	Area*	USP Tailing *	SP Plate Count*
1	Phenytoin Sodium	6.178	219078	1.63	2146

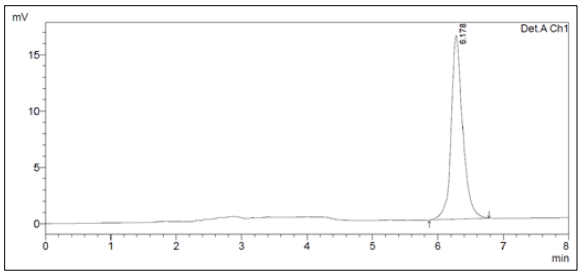


Figure 6: Standard Chromatogram of Phenytoin Sodium

Ensuring Drug Quality and Safety

Drug analysis plays a pivotal role in ensuring the quality and safety of pharmaceutical products. It involves the comprehensive examination of drug formulations to confirm the presence of the active pharmaceutical ingredient (API) in the correct concentration and to identify and quantify any impurities or contaminants.

Stability in Analytical Solution

No significant difference was found in the % Assay of drug before and after storing for 24 hrs in refrigerator and room temperature. This confirms the stability of the drugs in solutions. The percentage assay is tabulated in table 7.

Table 7: Solution Stability Data of Phenytoin Sodium

Time level	Refrigerator (25°C)	Room Condition (37°C)
Time in (hrs)	% Assay of Phenytoin Sodium	% Assay of Phenytoin Sodium
Initial	100.25 (±0.34)	100.02 (±0.032)
After 24 hrs	100.14 (±0.58)	99.46 (±0.046)

Force Degradation study

In forced degradation studies, purity angle and purity threshold are parameters used to assess the degradation products formed during the degradation of a Phenytoin sodium. These parameters help in evaluating the stability of the Phenytoin sodium under stress conditions. The acid, base, thermal and UV conditions are applied to understand the effect of these condition on the stability of phenytoin sodium. The HPLC chromatogram are shown in figure 8 to 11 and results are shown in table 10.

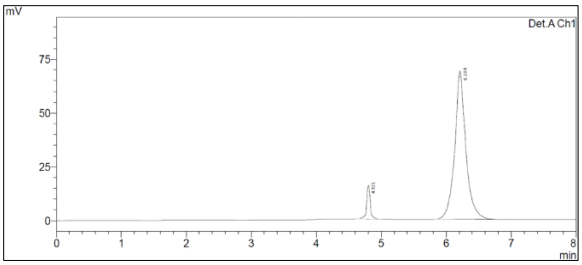


Figure 8: Chromatogram of Phenytoin Sodium in acidic stress condition

It is very important to know when to perform forced degradation studies for the development of new drug substance and new drug product. FDA guidance states that stress testing should be performed in phase III of regulatory submission process. Stress studies should be done in different pH solutions, in the presence of oxygen and light, and at elevated temperatures and humidity levels to determine the stability of the drug substance. These stress studies are conducted on a single batch.

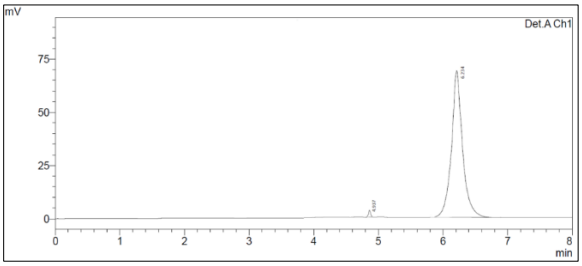


Figure 9: Chromatogram of Phenytoin Sodium in Oxidative stress condition

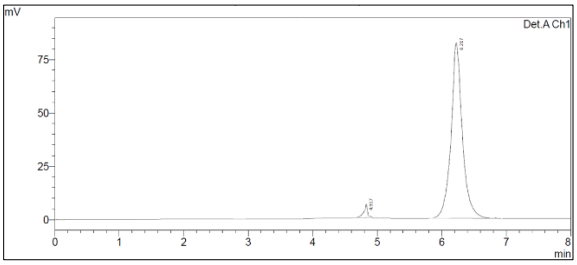


Figure 10: Chromatogram of Phenytoin Sodium in UV light stress condition

Table 9: Force degradation study parameters

Sr. No.	Degradation Condition	% Degradation	Purity Angle	Purity Threshold
1	Acid Degradation	5.59	4.325	6.834
2	Base Degradation	8.66	4.746	9.385
3	Oxidative Degradation	1.78	4.638	5.186
4	Thermal Degradation	3.45	3.863	5.976
5	UV Degradation	2.17	3.358	5.493

Forced degradation studies indicated that all the degrading peaks obtained during degradation were well resolved from the main drugs i.e. Phenytoin sodium and the peak purity was passed i.e. purity angle was less than purity threshold states that, the method is stable in different stress conditions. By systematically studying the degradation of phenytoin sodium under different conditions, a comprehensive degradation profile can be established. This information is crucial for understanding the stability of the drug and ensuring its efficacy and safety throughout its shelf life. The observed percentage degradation under various conditions provides insight into the robustness of phenytoin sodium and helps in developing appropriate storage and handling guidelines.

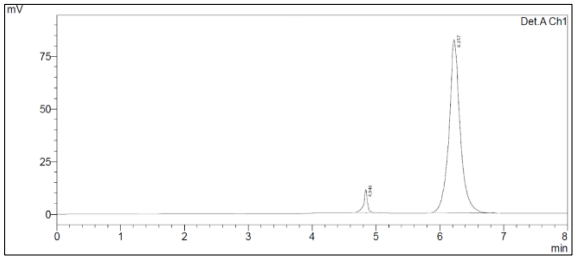


Figure 11: Chromatogram of Phenytoin Sodium in temperature stress condition

RP-HPLC method has shown adequate separation for Phenytoin sodium. For the method development, several preliminary trial runs were taken on the pre-validated RP-HPLC system by varying the concentration of mobile phase, different solvent

containing various ratios of methanol, water and acidic were tried for separation and resolution of peaks of Phenytoin sodium to get the optimized parameters.

The proposed method involves the utilization of a Shimadzu LC 2010 AT system equipped with a C18 Chromasil column (4.6 x 250mm, 5µm). A systematic optimization of chromatographic conditions, including mobile phase composition [Methanol: Water (0.1% Ortho-phosphoric acid) (70:30], before use, the mobile phase was filtered through 0.45 µm membrane filter and degassed by ultra-sonication. The flow rate was 1.0 mL/min, column temperature 30°C, the injection volume was 20 µl, and detection was performed at 254 nm using a UV detector. The retention time was obtained to be 6.15 min.

In present study, the method was validated according to ICH guidelines Q2 (R1). The method was validated in terms of specificity, linearity, accuracy, precision and robustness [8,9].

The specificity of the method was monitored by analyzing the blank, standard sample solution.

Linearity for Phenytoin Sodium was found to be in the range of 10 – 60 µg/ml with correlation coefficient value (r2) 0.9989 which was near to 1.

System and method precision showed that the method is precise within the acceptable limits. i.e. RSD, tailing factor, and number of theoretical plats were calculated for both solutions, all the results are within limits. The % RSD was found to be less than 2.0%, tailing factor are less than 2.0 and number of theoretical plates is greater than 2000. Intraday and Interday precision result also found in acceptable limit, hence method is precise.

Robustness of the method checked after deliberate calibrations of the analytical parameters showed that areas of peaks of interest remain unaffected by small changes of operational parameters.

The mean % recovery was found to be 99.98 % for Phenytoin Sodium.

CONCLUSION

The results clearly indicate that the stability indicating conditioned was applied to selected phenytoin drug by RP-HPLC technique and it is developed for the estimation of Phenytoin sodium in bulk and pharmaceutical formulation. The method was validated in accordance with ICH guidelines. The mobile phase is simple to prepare and economical and as the process is precise and accurate, drug is also stable for 24 hours. In addition, the main features of the developed method are short run time and retention time around 6.15 min. In the current research, the method shows good reproducibility, moreover the RP-HPLC method is accurate, precise, specific, reproducible, sensitive and cost effective for the analysis of Phenytoin Sodium. Hence this method can be easily and conveniently adopted for routine quality control analysis of Phenytoin sodium.

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