WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 8, Issue 6, 1508-1517. Research Article ISSN 2277– 7105

METHOD DEVELOPMENT AND VALIDATION BY RP-HPLC FOR ESTIMATION OF PHENYTOIN IN BULK AND PHARMACEUTICAL DOSAGE FORM

Sagar S. Yenare*[1] and Gaurav S. Lodha[2]

[1]*Department of Quality Assurance Technique, Dr. Vithalrao Vikhe Patil Foundation College of Pharmacy, Vilad Ghat, Ahmednagar, (MS), India-414111.

[2]Depatment of Pharmaceutics Dr. Vithalrao Vikhe Patil Foundation College of Pharmacy, Vilad Ghat, Ahmednagar (MS), India-414111.

Article Received on
22 March 2019,

Revised on 11 April 2019,
Accepted on 01 May 2019,

DOI: 10.20959/wjpr20196-14958

***Corresponding Author**
Sagar S. Yenare
Department of Quality Assurance Technique, Dr. Vithalrao Vikhe Patil Foundation College of Pharmacy, Vilad Ghat, Ahmednagar, (MS), India-414111.

ABSTRACT

In the Current scenario there is a need for a creation of a useful RPHPLC methods for various types of drug. Thus a easy, reproducible and trustworthy reversed phase high performance liquid chromatographic (RP-HPLC) method is developed and validated for the estimation of Phenytoin. Jasco System was utilized to carry out chromatography with an UV 730 D detector. On C18 (4.6x250 mm) column. The mobile phase used is Mehanol: Distill water (50:50 V/v) at a floe rate of 1 ml / min. The pH of mobile phase 3. Detection was done with a UV detector at 254 nm. As per the ICH guidelines, parameters such as precision, linearity, accuracy, ruggedness, LOD and LOQ were studied. The retention of Pheneytoin was 5.174. the Linearity range was found to be. The correlation coefficients of phenytoin obtained were 0.979. The developed method was found to be reliable, accurate and easy for simultaneous estimation of Phenytoin in pharmaceutical dosage forms. The developed method is also useful for quality control of both bulk manufacturing as well as pharmaceutical dosage forms.

KEYWORDS: Phenytoin, method validation, RP-HPLC, ICH guidelines.

1. INTRODUCTION

Name

Phenytoin

Molecular Formula

C15H12N2O2

Description

1. An anticonvulsant that is used in a wide variety of seizures. It is also an anti-arrhythmic and a muscle relaxant. The mechanism of therapeutic action is not clear, although several cellular actions have been described including effects on ion channels, active transport, and general membrane stabilization. The mechanism of its muscle relaxant effect appears to involve a reduction in the sensitivity of muscle spindles to stretch. Phenytoin has been proposed for several other therapeutic uses, but its use has been limited by its many adverse effects and interactions with other drugs.[1,2]

Structure

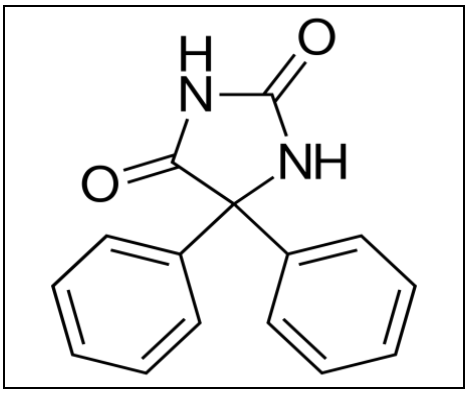


Fig 1: Structure of PHT.

2. MATERIALS AND METHOD

2.1Instrumentation

Jasco HPLC system and C18 column (250 x 4.6 mm) was used. The instrument is equipped with an UV 730 D detector. A 20 microLitre rheodyne injector port was used for injecting the samples.[2,3]

2.2Chemicals and solvents

The working standard of Phenytoin was obtained as gift samples from Glenmark Pharmaceutical Ltd, Goa, India. The tablet market formulation was procured from local market. HPLC grade water was purchased from Lupin, Ltd, Mumbai, India. Methanol (HPLC Grade) was obtained.

2.3 Chromatographic conditions

Column: C18 column (250x 4.6 mm)

Mobile phase: Methanol: Distill water in proportion of 50:50 v/v (PH-3) Detector 254

ƛmax: 254 nm.

Injection Volume:- 20 µl.

Flowrate: 1 ml/min.

Temperature: Ambient.

Run Time: 10 min Diluents: CAN.

2.4 Selection of Mobile Phase

Standard solution of Phenytoin (PHT) was injected into the HPLC system and run in different solvent systems. Mixture of different solvents were tried in order to determine optimum chromatographic conditions for effective separation of PHT. After several permutation and combination, it was found that mixture of methanol: water gives satisfactory results as compared to other mobile phases. Finally the optimal composition of the mobile phase Methanol: water in the ratio of 50:50 (ph-3) was selected, as it gave high resolution of PHT with minimal tailing.[5,6]

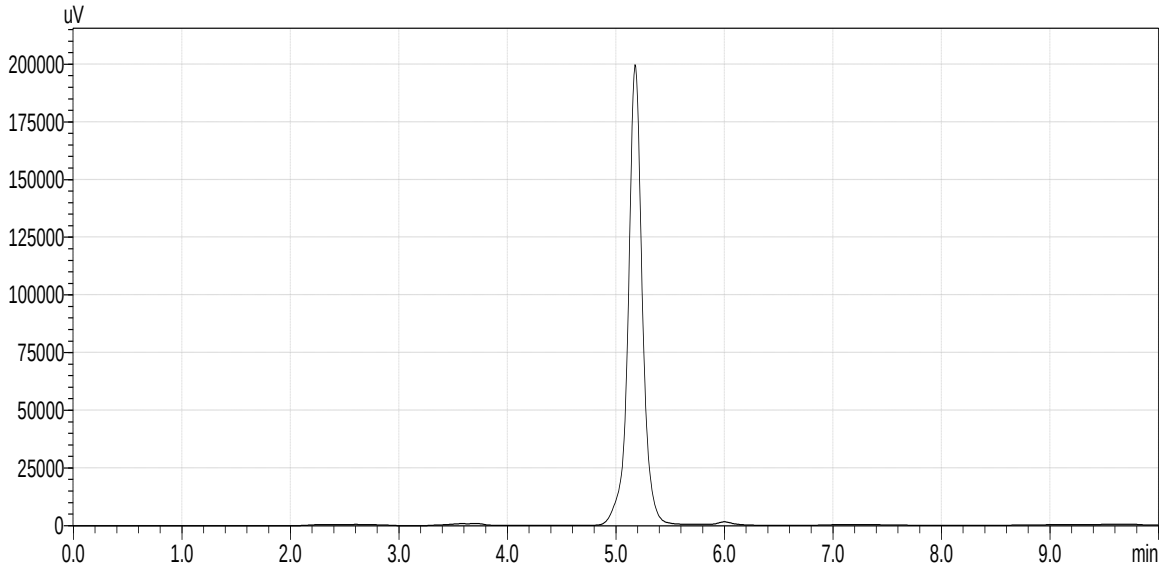
2.5 Preparation of Standard stock solution

Mobile phase was prepared by mixing 50 ml of methanol with 50 ml of Distill water. The pH of mobile phase was adjusted by 0.05% ortho phosphoric acid (PH-3). This mobile phase was filtered through 0.45µ membrane filter and then it was ultrasonicated for 20 minutes.

2.6 Preparation of standard stock solution

About 10 mg PHN are accurately weighed and transferred to 10 ml volumetric flasks. The drug is dissolved in 5 ml of with shaking. The volume was made up to the mark using the mobile phase, thus getting 1000 µg/ml of PHT of the standard stock solution. Ultrasonication was done for 20 minutes and filteration was done with the help of membrane filter of pore size 0.154.

Mobile Phase: Methanol: Water 50:50 (v/v) (pH-3)



ƛ max: 254 nm

Flow rate: 1 ml/min.

Retention Time: 5.174 min

2.7 System suitability parameters

The System suitability parameters were determined as follows.

Table 1: System suitability parameters.

Sr No.	Parameters	PHT
1	Retention time (min) 5.174	4.55
2	No. of theoretical plate (N)	6055.60
3	Tailing Factor	0.70

Time(min).

Table 2: Standard Calibration curves.

Sr No.	Conc. (µg/ml)	Mean peak area
1	10	566.42
2	20	1234.50
3	30	1463.25
4	40	2110.00
5	50	2510.00

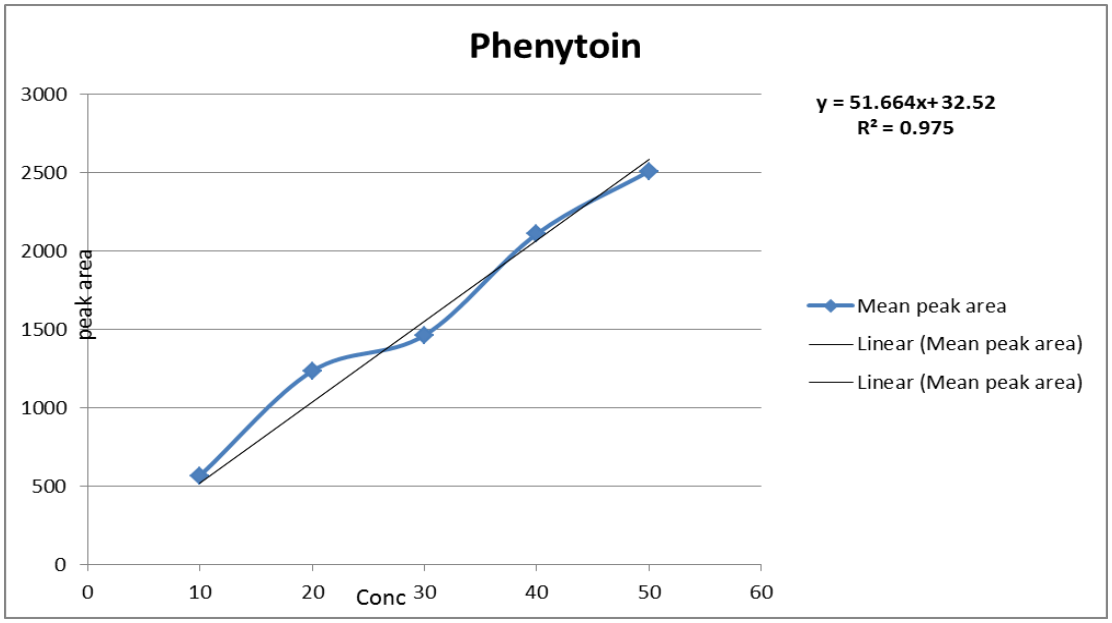


Fig 3: Caliberation curve of Phenytoin by HPLC.

2.8 Study of beers-Lambert's law

Preparation of Standard calibration curves and selection of analytical concentration ranges: Appopriate & necessary aliquots were tranfered to a series of 10 ml volumetric flasks. Concentration ranges were 10-30 which were made upto mark by the mobile phase. The prepared diluted solutions were filterd through 0.2 µ membrane filter. Optimized chromatographic conditions were used for recording of chromatogram.The Filtrate (20µl) was injected into the column. Area for each peaks for PHT were measured at 254 nm. The mean peak area was calculated by each sample solution being chromatographed five times. Standard calibration graph for PHT are given in Fig. no.3.

3. Analysis of marketed formulation

Drug estimation in marketed formulations was done by taking twenty tablets containing 10 mg of PHN are weighed and average weight was calculated. The taken 20 tablets were crushed to powder in a morter. For the analysis drug quantity of drug equivalengt to 10 mg was transffered to 10 ml volumetric flask containing the mobile phase of 5 ml. It is then ultrasonicated for 10 mins. Then mobile phase was utilized to make up the volume upto the mark. The solution was then filtered, This solution was diluted further to obtain mixed sample solution containing 10 Ug/ml of PHT. The solution was filtered through 0.30µ membrane filter. A 20 µl of sample solution was injected into sample injector for seven times under chromatographic conditions as described above. Areas of each peak was measured at

254 nm. Peak area of PHT found in the pure mixture was used to determine the amount of each drug present in the sample. The results obtained are provided in table nos. 3 & 4.

Table 3: HPLC Assay of PHN (n=3).

Amt. taken (µg/ml)	Peak Area	Amt. found (µg/ml)	% of drug found
10	650.55	10:5	100:40
10	665.80	10:15	100:10
10	655.80	10:12	100:50

Table 4: Statistical evaluation of marketed formulation.

Drug	% Mean*	S.D.	% RSD
PHN	102.52	0.32	0.31

*Average of six determination.

4. Validation

The developed method was validated as per the given ICH guidelines.

4.1 Linearity and Range

The linearity of measurement was evaluated by analysisng different concentrations of the standard solutions of PHT. At the conc. range of 10-50µg/ml for PHT respectively the Beer's law was well obeyed.[11,12]

Table 5: Linear regression data for calibration curves.

Parameters	Phenytoin
Linearity range	10-30 µg/ml
r^2	0.975
Slope	51.664
Intercept	32.52

r^2 Correlation coefficient; S.E.: Standard error.

4.2 Precision

4.2.1 Repeatability

To check the degree of repeatability of the method, six samples of the marketed formulation were analyzed. The results of the Repeatability are given below in Table no. 6.[10]

Table 6: Result of repeatability.

Drug	Conc (µg/ml)	Area mean*	Amount found	% Amount found	S.D.*	%RSD*
PHN	10µg/ml	657.38	10:12	100:33	1.32	1.37

*Average of six determination.

Table 7: Results of Intra-day and Inter-day precision studies.

Parameter	Conc. (µg/ml)	Area mean*	Amount found*	% Amount found*	S.D.*	%RSD*
Intraday	10	657.38	10.13	100:10	1.06	0.15
	20	668.75	20.15	100:10	0.65	0.12
	30	1080.00	30.40	100:00	1.30	0.10
Interday	10	665.40	10:14	100:20	0.8	0.10
	20	700.00	20:18	100:10	1.68	0.08
	30	1060.01	29:48	100:30	0.60	0.06

**Average of three determinations*

Low %RSD values for intra and inter day confirmed that the method is precise

4.2.2 Intermediate precision

The Intra and Inter-day precision was determined by analysis of the marketed formulation of PHN on the same day at different time intervals and on different days as well respectively. The results are provided in a tabulated form in table no. 7.

4.3 Accuracy

According to ICH guidelines, recovery studies were done to check the accuracy of the proposed method by applying the standard solution method to known amount of TA and EMT corresponding to 80,100 and 120%. Analysis was performed as per the procedure of analysis of PHN tablets. The recovery were done three times at every level. The results of recovery studies are provided below in Table no.8 and 9.

Table 8: Recovery studies results.

Recovery Level (%)	Conc of Drug (µg/ml)		Total conc.of drug (µg/ml)	Toatal amt. Recovered (µg/ml)	% Recovery*	%RSD
70	5	7	17	17.20	100	0.50
90	5	9	19	19.05	100.20	0.36
110	5	11	20	20.10	100.30	1.20

*Average of three determinations.

Table 9: Robustness evaluation of PHN.

Sr. No.	Factor	Level	Retention time
1	Flow rate (ml/min) 0.9 1 1.1	 -1 0 +1	 5.5 5.1 5.05
	Mean +/- S.D. (n=6)		5.17+/- 0.22
2	Mobile phase volume (v/v) 48:50 52:45 50:55	 -1 0 +1	 5.5 5.10 5.05
	Mean +/- S.D. (n=6)		5.17+/-0.12
3	Wavelength 245 247 254	 -1 0 +1	 5.17 5.35 5.17
	Mean +/- S.D. (n=6)		5.11+/- 0.092

Table 10: LOD and LOQ of PHN.

Parameter	TPM
LOD (µg/ml)	0.045 µg/ml
LOQ (µg/ml)	0.142 µg/ml

4.4 Robustness

The robustness of the method is very necessary to check and determine in analytical works. During development robustness was thus studied by small but purposeful and deliberate ariations in flow rate, percentage of methanol in the mobile phase and wavelength. Each factor selected to examine and check were changed at three particular levels which are -1,0,1. One factor at a time was changed to estimate the effect and to the effect that it maked on the retention time of the drugs. The results hus obtained are provided in table no. 10.[3,8,9]

Flow rate variation, % of methanol in the mobile phase and wavelength did not affect the results obtained. Rt and tailing factors of both the drugs at different levels of variations were found to be similar. Hence, the method used in the present analytical work was found to be robust.

4.5 Limit of Detection (LOD)

The limit of detection (LOD) is the smallest concentration that can be detected but it is not necessarily quantified as an exact value obatained. LOD can be calculated by the formula;

$LOD = 2.4\sigma / S$

Where, σ = standard deviation of theresponse, S = slope of calibration curve.

4.6 Limit of Quantitation (LOQ)

The limit of quantitation is defined as the lowest amount of analyte in the sample that can be quantitavely determined with proper precision and accuracy. LOQ can be calculated by the formula.

LOQ = 10σ /S

Where, σ = standard deviation of the response, S = slope of calibration curve LOD,

LOQ are shown in the Table no. 11.

CONCLUSION

From all the observations and its obatained results it was concluded that the developed RP – HPLC method for the estimation of Phenytoin in bulk and pharmaceutical dosage form was accurate, precise, linear, robust, simple, easy, systematic and rapid. Percentage recovery obtained in the study shows that the method was free from interference of excipients used in the formulation.

CONFLICT OF INTERESTS

Declared None.

ACKNOWLEDGEMENT

Authors are grateful to Dr.M.S.Tare, HOD of Pharmaceutics Sitabai Thite College of Pharmacy, shirur for his rightful guidance.

REFERENCES

1. P.D. Sethi, Volume-1, High Performance Liquid Chromatography, CBS publishers and distributers pvt; ltd.
2. Guanyun. A. Xu, Lawrence A Trissel, Stability indicating HPLC Methods for Drug Analysis, Alpha Pharmaceutical press, 827-828. 669-670.
3. P.D. Sethi, Rajat Sethi, Santosh V. Gandhi, Nitin Dubey, High Performance Liquid Chromatography, Sethu's Quantitaive analysis of pharmaceutical formulation, Volume-5, CBS publisher & distributers pvt. Ltd.
4. Dr. P.D. sethi, Quantitative analysis if pharmaceutical formulations, method with reqction mechaniary, 4th edition, volume 1, cbs. Publishers and distributors, pvt. Ltd, 1997.
5. Ledhicer mitscher, The organic chemistry of drug synthesis, volume 2, a wiley interscience publication.

6. John A.Admovics.Chromatographic analysis of pharmaceuticals, 2nd edition, revised and expanded, volume 74.
7. R.O, C norvan and J.M. Coxon Principle of organic synthesis, 3rd edition, CRC press.
8. Ra Ma Roo nandella Medicinal chemistry, Revised edition by Pharmamed press.
9. S.M. Khapkar Basic concepts of analytical chemistry, new age, 3rd edition by New age international publishers.
10. Mahadev B. Kshirsagar P. Mahajan, Sanjay D. sawant, Department of quality. Method development and validation by RP-HPLC for estimation of drug in bulk and pharmaceutical dosage form.
11. Radhika Shah, Ragini Shah, Develoment and validation of RP-HPLC Method for Phenytoin sodium and phenobarbitone in bulk and pharmaceutical dosage forms.
12. Siew, yong teo, michaelid rathbane Development and validation of a stability indicating isocratic reverse phase liquid chromatography, assay for determination of phenytoin in bulk and pharmaceutical dosage form, IJPRS, 2015.
13. Beckett A. H. and Stenlake J.B., practical pharmaceutical chemistry, 14th edition, new delhi, CBS publisher and distributor, 2002, page no.: 275-288.