Varaprasad A. *et al.* / *International Journal of Biological & Pharmaceutical Research.* 2012; 3(1): 126-129.

ISSN 0976 - 3651
2229 - 7480

International Journal of Biological
&
Pharmaceutical Research
Journal homepage: www.ijbpr.com

IJBPR

# METHOD DEVELOPMENT AND VALIDATION OF PHENYTOIN SODIUM IN BULK AND ITS PHARMACEUTICAL DOSAGE FORM BY RP-HPLC METHOD

***Varaprasad A., [2] Sriram N.,[3] Godwin Isaac Blessing A.,[4] Jawahar M., [5]Thangamuthu S.**

* Sri balaji College of Pharmacy, Choppadandi, Karimnagar, A.P. India – 505 415.
[2,4,5.]Smt.Sarojini Rammulamma College of Pharmacy, Mahabubnagar, A.P., India - 509001.
[3]Research scholar, Prist University, Thanjavur, Tamilnadu.

**ABSTRACT**

To establish a method and validation developed for the determination of Phenytoin sodium in its pure form as well as in tablet dosage form by reverse phase high-performance liquid chromatographic method. Chromatography was carried out on a Symmetry C18 (4.6 x 150mm, 5 µm) column using a mixture of m and phosphate buffer (60:40 v/v) as the mobile phase at a flow rate of 0.7 mL/min, the detection was carried out at 225nm. The retention time of the drug was 2.49±0.04 minutes. The method produced linear responses in the concentration range of 10 and 20mg/ml of Phenytoin sodium. The method precision for the determination of the assay was below 1.0%RSD. The method is useful in the quality control of Bulk and pharmaceutical formulations.

**Key words:** Phenytoin sodium, RP-HPLC, Development, Validation.

## INTRODUCTION

Phenytoin sodium is widely used in the clinic for the treatment of epilepsy, which is a common disease. Patients with epilepsy need to take drugs for a long time to control the disease. Some patients even need to take drugs for their whole lives. Phenytoin sodium has some disadvantages, such as low treatment indexes, low security range and big differences among individuals. If the dosage and usage of these drugs are incorrect, they can result in serious side and bad effects. Different patients of epilepsy have no close relationship with the dosage of these drugs but have close connection with the concentrations of drugs in plasma. The concentrations of these drugs often directly affect their effects. So, monitoring the concentrations of these drugs in plasma can provide scientific evidence for adjusting the dosage of drugs, improving the condition of patients and reducing the side effects.

Previous studies have shown that many methods, such as ultraviolet spectrophotometer, analysis lamina chromatogram, enzyme immunoassay, Fluorescent polarization immunity and reversed-phase high-performance liquid chromatography, can be used to test the plasma concentrations of anti-epilepsy drugs. Moreover, reversed-phase high-performance liquid chromatography is the best for testing the plasma concentrations of anti-epilepsy drugs among them. This method is simple, sensitive, convenient and reliable. It is not necessary to revise the construction of sample drugs, which can be tested directly and even several drugs can be tested at the same time (Li Hong-jian *et al*., 2001). So, more and more people favor it to monitor the plasma concentrations of anti-epilepsy drugs during the treatment period of epilepsy.

Corresponding Author

**Varaprasad A.**
Email: varaprasadadepu@gmail.com

Our present study established a reversed-phase HPLC that can determine the concentrations of Phenytoin sodium. Phenytoin sodium is related to the barbiturates in chemical structure, but has a five-membered ring (Anonymous 1). The chemical name is sodium 5,5-diphenyl-2, 4-imidazolidinedione, having the following structural formula:

## MATERIALS AND METHODS

### *Chromatographic conditions*

A prominence isocratic HPLC system (Waters high-performance liquid chromatography with Auto Sampler and UV detector) column Symmetry C18 (4.6 x 150mm, 5 µm). A 20µL Rheodyne injection syringe was used for sample injection. HPLC grade, Methnol and Phosphate buffer were used for the preparing the mobile phase.

A freshly prepared, Methanol: 0. 05M potassium dihydrogen phosphate buffer ($P^H$ -2.8) (60 : 40 v / v) was used as the mobile phase. The solvent was filtered through a 0.45µ membrane filter and sonicated before use. The flow rate of the mobile phase was maintained at 0.7 mL/min., column temperature was maintained at room temperature and the detection of the drug was carried out at 220nm (Kazakevich Y and Lobrutto R, 2007).

### *Preparation of Phosphate buffer*

Weigh 7.0 grams of Potassium di hydrogen phosphate into a 1000ml beaker, dissolve and diluted to 1000ml with HPLC water. Adjusted the pH to 2.8 with Orthophosphoric acid

### *Preparation of mobile phase*

Mix a mixture of above buffer 400mL (40%) and 600 mL of Methanol HPLC (60%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

### *Diluent Preparation*

Mobile phase as diluents.

### *Standard Solution Preparation*

Accurately weigh and transfer 10mg of Phenytoin sodium Working standard into a 10mL volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

### *Sample Solution Preparation*

Weigh 5 phenytoin sodium Tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10 mg of Phenytoin sodium into a 10mL volumetric flask. Add about 7mL of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45µm filter. Further pipette 0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter (Yang Wen-jing and Zou Chun-mei, 2000).

## METHOD VALIDATION

### *Linearity*

The linearity of the method was demonstrated over the concentration range of 10 and 20mcg /ml of the target concentration. Aliquots of 10 and 20mcg/ml were prepared from above prepared stock solution. Different concentrations of the pure drug were injected into the chromatographic system. Calibration curve of Phenytoin sodium was constructed by plotting peak area vs. applied concentration of Phenytoin sodium. A typical chromatogram is shown in Fig 1. The obtained results showed an excellent correlation between peak area and concentration of pure drug within the concentration range. The correlation coefficient for the average area at each level versus concentration of analyte was calculated (Meyer VR, 1993) and is presented in Table 1 and their calibration parameters were shown in Table 2.

### *Precision Method*

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intra-day studies, six repeated injections of standard solution was made and the response factor of drug peak and % RSD were calculated and present in Table 3. The chromatogram was shown in Fig 1. In the inter-day variation studies, six repeated injections of standard solution were made for six consecutive days and response factor of drugs peak and % RSD were calculated shown in Table 3. From the data obtained, the developed method was found to be precise.

### *Accuracy*

A Study of recovery of Phenytoin Sodium from spiked placebo was conducted at three different spike levels, i.e.50, 100 and 150 samples were prepared with Phenytoin Sodium raw material equivalent to about the target initial concentration of Phenytoin Sodium. Sample solutions were prepared in triplicate for each spike level and assayed as per proposed method (Riley CM and Thomas WR, 1996). The % recovery was given in Table-4. The mean recoveries of Phenytoin Sodium from spiked were found to be in the range of 98.67- 101.5%.

### *LOD and LOQ*

The LOD and LOQ were separately determined based on the standard deviation of response of the calibration curve. The residual standard deviation of the regression lines and slope of the calibration curves were used to calculate the LOD and LOQ (Table 2).

***System suitability***

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time ($R_t$), number of theoretical plates (N) and tailing factor (T) were evaluated (Wang Li *et al.*, 1994) for six replicate injections of the drug at a concentration of 100 mcg / ml. The results given in Table 5. were within acceptable limits.

**Table 1. Linearity results for Phenytoin Sodium**

| Conc. (mcg / ml) | 10 | 20 |
|---|---|---|
| **Avg. Area** | 495789* | 876587* |
| **Correlation** | 0.998 | |

* Average of six determinations.

**Table 2. Characteristic parameters of Phenytoin Sodium for the proposed RP-HPLC method**

| **Parameters** | **RP-HPLC** |
|---|---|
| Calibration range (mcg / ml) | 10-50 |
| Detection wavelength | 220 nm |
| Mobile phase (Methanol: Buffer) | 60:40 |
| Retention time | 2.49±0.04 |
| Regression equation (Y*) | y = 42950x + 19250 |
| Slope (b) | 42950 |
| Intercept (a) | 19250 |
| Correlation coefficient($r^2$) | 0.998 |
| Intraday Precision (% RSD*)<br>Interday Precision (% RSD*) | 0.52<br>1.64 |
| Limit of detection (mcg / ml) | 0.04 |
| Limit of quantitation (mcg / ml) | 1.90 |

**Table 3. Precision results for Phenytoin Sodium**

| **Sr. No.** | **Concentration (mcg / ml)** | **Intraday precision** (Area) | **Interday precision** (Area) |
|---|---|---|---|
| 1 | 40 | 1838427 | 1846162 |
| 2 | 40 | 1816348 | 1803849 |
| 3 | 40 | 1805536 | 1796339 |
| 4 | 40 | 1796847 | 1794528 |
| 5 | 40 | 1828856 | 1820824 |
| 6 | 40 | 1790992 | 1788097 |
| Mean | | 1812835 | 1808300 |
| Std.Dev | | 1849.25 | 21685.2 |
| %RSD. | | 1.04 | 1.22 |

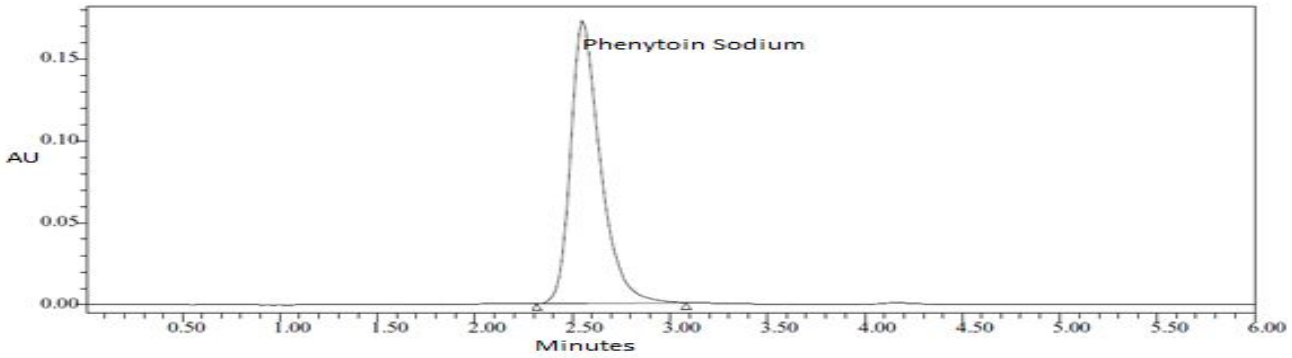
**Table 4. Accuracy results for Phenytoin Sodium**

| **Sample No.** | **Spike Level** | **Amount (mcg / ml) added** | **Amount (mcg / ml) found** | **% Recovery** | **Mean % Recovery** |
|---|---|---|---|---|---|
| 1 | 50 % | 20 | 19.70 | 98.51 | 98.67 |
| | 50 % | 20 | 19.75 | 98.75 | |
| | 50 % | 20 | 19.81 | 98.81 | |
| 2 | 100 % | 40 | 40.59 | 101.48 | 101.5 |
| | 100 % | 40 | 40.49 | 101.24 | |
| | 100 % | 40 | 40.47 | 101.18 | |
| 3 | 150 % | 60 | 59.70 | 99.50 | 99.57 |
| | 150 % | 60 | 59.75 | 99.58 | |
| | 150 % | 60 | 59.76 | 99.60 | |

**Table 5. System suitability studies of Phenytoin Sodium by RP-HPLC method**

| Property | Values | Required limits |
|---|---|---|
| Retention time ($R_t$) | 2.556 ± 0.02 | RSD ≤ 1% |
| Theoretical plates (N) | 2158.5 | $N > 2000$ |
| Tailing factor (T) | 1.4 | $T \leq 2$ |

**Fig: 1. Chromatogram of Phenytoin Sodium at 220 nm**




## DISCUSSION AND CONCLUSION

In HPLC method, HPLC conditions were optimized to obtain, an adequate separation of eluted compounds. The objective of this study was to develop a rapid and sensitive RP-HPLC method for the analysis of Phenytoin Sodium in bulk drug and pharmaceutical dosage form by using the most commonly employed RP C-18 column with UV-detection.

The run time was set at 6 min and the retention time for Phenytoin Sodium was 2.49±0.04 min. Each sample was injected 5 times, and the retention times were same. When the concentrations of Phenytoin Sodium and its respective peak areas were subjected to regression analysis by least squares method, a good linear relationship ($r^2$= 0.998) was observed between the concentration of Phenytoin Sodium and the respective peak areas in the range 10 and 20mcg / ml. The regression equation was used to estimate the amount of Phenytoin Sodium, either in tablet formulations or in validation study (precision and accuracy). For the proposed RP-HPLC method, characteristic parameters were shown in Table 2.

To analyse tablet formulations, RP-HPLC method has been developed. Phenytoin Sodium tablets were analyzed as per the procedure described above. The low % RSD values (≤ 2) indicated that the method was precise and accurate. The mean recoveries were found in the range of 98.67 – 101.5 %. No interfering peaks were found in the chromatogram indicating that excipients used in the tablet formulation did not interfere with the estimation of the drug by the proposed RP-HPLC method.

The proposed RP-HPLC method was also validated for intra and inter-day variation. When the solution containing 40 mcg/ml of Phenytoin Sodium was repeatedly injected on the same day, the %RSD in the peak area for six replicate injections was found to be 1.02% Also the inter day variation (6 days and six injections) was found to be 1.20%. The results are presented in Tables 3. The % RSD values were within 2 and the method was found to be precise. It can concluded that the proposed HPLC method is rapid, sensitive, precise and accurate for the determination of Phenytoin Sodium and can be reliably adopted for routine quality control analysis of Phenytoin Sodium in Bulk and its pharmaceutical formulations.

## REFERENCES

Kazakevich Y, Lobrutto R. HPLC for pharmaceutical scientists. London: John Wiley and Sons; 2007:10-22.

Li Hong-jian, Ran Guo-xia, Cao Li-meng, et al. Determination of the serum concentrations of three kinds of antiepileptic drugs and the experience of individualized drug administration. *China Pharmacy*, 2001; 12(3): 160-161.

Meyer VR. Practical High Performance Liquid Chromatography. 2nd ed. London: John Wiley and Sons; 1993: 26-258.

Riley CM, Thomas WR. Development and Validation of Analytical methods. Vol.III. United States of America: Elsevier Sciences Inc; 1996: 305-309

Wang Li, Xu Zhong-xi, Cheng Yan. Clonazepam determination in human serum by high performance liquid chromatography. *Chinese Journal of Clinical Pharmacology*, 1994: 10(3): 181-183.

Yang Wen-jing, Zou Chun-mei. Simultaneous determination of plasma concentration of four medicines by HPLC. Journal of Shanxi Medical University, 2000: 31(5): 500-501.