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Validation of RP-HPLC method for the determination of phenytoin sodium residues on the surface of manufacturing equipments and study of its recovery from pharmaceutical formulations

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ABSTRACT

Cleaning validation provides assurance to the cleaning procedure that ensures equipment is consistently cleaned from the product, detergent and microbial residues to an acceptable level to avoid contamination and cross contamination. In the pharmaceutical manufacturing it is an important step consists in the removal of possible drug residues from the equipments and areas. The cleaning procedures must be validated and methods to determine trace amounts of drugs. An RP-HPLC method for the determination of phenytoin sodium residues on equipment surfaces was developed and validated in order to control a cleaning procedure. Cotton swabs, moistened with methanol were used to remove any residues of drugs from surfaces. And recovery study conducted for the tablet and injection at three concentration levels of 80, 100 and 120 %. The precision of the results, reported as the relative standard deviation (RSD), were below 3.2 %. The method was validated over a concentration range of 2-10µg mL^{-1}. Low quantities of drug residues were determined by HPLC using a Kromasil® C18 column (100×4.6mm, 5µm) at 20 °C with phosphate buffer pH 3.5±0.05 as mobile phase at a flow rate of 1.0 mL min^{1}, an injection volume of 20 µL and were detected at 254 nm using UV detector. A simple, selective and sensitive HPLC assay for the determination of phenytoin sodium residues on equipment surface was developed, validated and applied. The analysis of variance (ANOVA) is applied to find change occurred, if any while recovery study using two different formulations (using Graphpad Prism Ver. 5.0).

Keywords: Cleaning validation, Residue, Phenytoin sodium, Recovery, ANOVA

INTRODUCTION

Phenytoin sodium is an antiepileptic drug. Phenytoin sodium is related to the barbiturates in chemical structure, but has a five-membered ring [1]. The chemical name is sodium 5, 5-diphenyl-2, 4-imidazolidinedione, having the following structural formula (Fig.1):

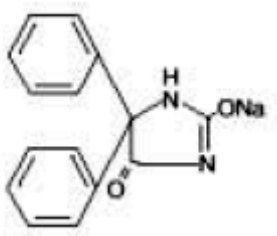

Fig. 1 Phenytoin sodium

The analysis by HPLC is more significant than using other methods like UV, liquid chromatography and immunoassays for the estimation of Phenytoin sodium [2]. The HPLC method is developed, validated and applied. The cleaning of equipment after manufacturing of dosage form is necessary and the cleaning procedures for the equipment must be validated according to goods manufacturing practice (GMP) rules and guidelines [3]. The main objective of cleaning validation is to avoid contamination between different productions or cross contamination. The carryover amount left manufacturing tells how much effective the cleaning is [4-9].

The acceptable limit for residue in equipment is not established in the current regulations. According to the FDA, the limit should be based on logical criteria, involving the risks associated with residues of a determined product. The calculation of an acceptable residual limit, the maximum allowable carryover of active products in production equipment should be based on therapeutic doses, the toxicological index and a general limit (10 ppm). Several mathematical formulae were proposed to set up the acceptable residual limit [10-13]. An analytical method developed and validated that allows the determination of carryover amount of Phenytoin sodium residues in production area and to confirm the efficiency of the cleaning procedure. The validation parameters, linearity, repeatability, precision and limit of detection (LOD) and quantification (LOQ) were validated [14-17].

MATERIALS AND METHODS

2.1 Chemicals and reagents

The certified phenytoin sodium, working standard was received as gift sample from the Abbott India Ltd., Goa. Methanol (HPLC gradient grade) was purchased from Merck. Purified water was obtained from Millipore. The extraction-recovery sampling was realized with Johnson and Johnson swab cotton on a polypropylene handle. The mobile phase was filtered through a 0.45 µm Nylon filter from Pall Life Sciences.

2.2 Instrument

The HPLC system consisted of a degasser Series-200, pump Series-200, a UV- Vis detector Series-200, from Perkin Elmer. Ultrasonicator from Oscar, analytical balance AUX220 from Shimadzu Corporation, Japan and pH meter Pico+ from Lab India Ltd.

2.3 Chromatographic conditions

All chromatographic experiments were performed in the gradient mode. Phosphate buffer pH 3.5±0.05 was used as mobile phase at a flow rate of 1.0mL min^{-1}. The separation was performed at 20 °C on a Kromasil® C18 column ((100×4.6) mm, 5 µm). UV detection was carried out at 254 nm.

2.4 Standard solutions preparation

The stock solution of standard was prepared by accurately weighing Phenytoin sodium standard (~ 50.0 mg) and transferred into a 100 mL volumetric flask. Accurately measured quantity of methanol about 20 mL was added and the contents of the flask were sonicated for 15 min. The volume of the flask was made up to 100 mL using methanol (i.e. 1000 µg mL^{-1} phenytoin sodium solution). Dilutions were later prepared with water to obtain solutions for calibration (2–10 µg mL^{-1}). These solutions were filtered through a 0.45 µm Nylon filter before analysis and injected in triplicate.

2.5 Recovery studies

The recovery study of the method was ascertained by standard addition method. It was carried out by adding the standard solution of drug in test samples corresponding to three levels viz. 80, 100 and 120%. At each level of the amount three determinations were performed and the results obtained were calculated and compared with expected results.

2.6 Sample preparation

The selected surface(s) (5 cm×5 cm) of equipments, previously cleaned and dried, were sprayed with standard solution, for the positive swab control at all concentration levels, and the solvent was allowed to evaporate (approximate time was 2 hrs). The surfaces were wiped with the first cotton swab soaked with methanol, passing it in various directions, to remove the residues from the various surfaces of equipments. The other dry cotton swab was used to wipe the wet surfaces. The swabs were placed into a 10 mL volumetric flask. The background control sample was prepared from the extraction media. The positive swab sample having concentration of 0.5, 2.5 and 12.5 µg mL^{-1}. The negative swab control was also prepared.

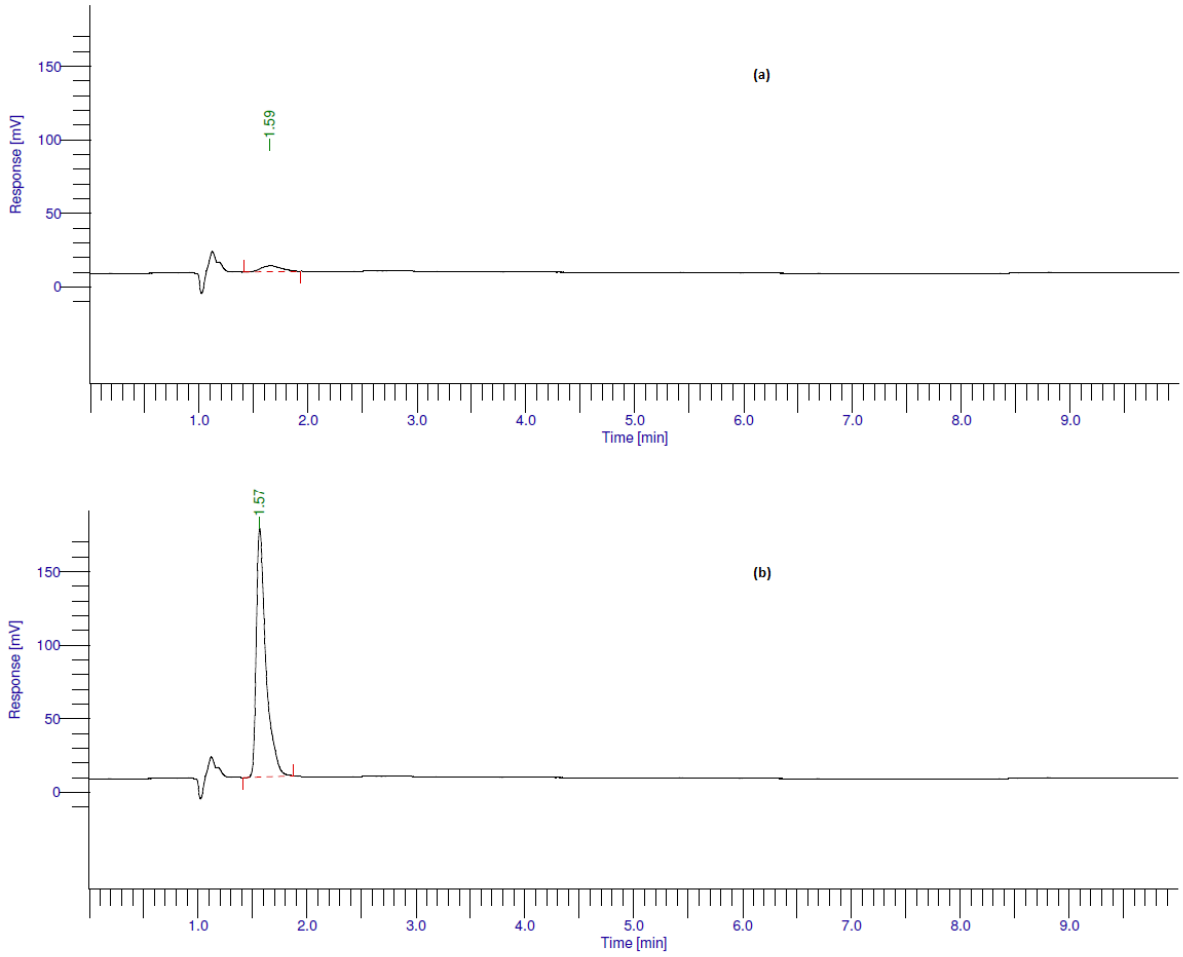

Fig. 2: Chromatograms obtained from: (a) Negative cotton swab, (b) Standard Phenytoin sodium solution (10ppm)

RESULTS AND DISCUSSION

3.1 Calculation of Acceptable Limit

The maximum allowable carryover (MACO) is the acceptable transferred amount from the previous to the following product. The MACO is determined based on the therapeutic dose, toxicity and generally 10 ppm criterion. Once the maximum allowable residue limit in the subsequent product was determined, the next step was the determination of the residue limit in terms of the contamination level of active ingredient per surface area of equipment. The total surface area of the equipment in direct contact with the product was accounted for in the calculations. The limit per surface area was calculated from the equipment surface area and the most stringent maximum allowable carryover (the most stringent criterion being based on the therapeutic dose in this case). The 0.1 % dose limit criterion is justified by the principle that an active pharmaceutical ingredient (API) at a concentration of 1/1000 of its lowest therapeutic dose will not produce any adverse effects on human health. The calculated limit per surface area (LSA) in the case Phenytoin sodium was 1.0 µg $swab^{-1}$ per surface of 25 cm^2. A equipment(s) surface area of 5 cm×5 cm was chosen for practical reasons.

3.2 Optimization of the chromatographic conditions

The gradient mode, applied for the determination of phenytoin sodium residues collected by swabs, without interference of impurities originating from the swabs and extraction media. λ_{max} was found to be 254 nm, so for the analysis it was selected for detection. And low quantities of Phenytoin sodium may be detected correctly. Furthermore, the calibration curve obtained at 254 nm showed good linearity.

Regarding the chromatographic procedure, Kromasil C-18 ((100×4.6) mm, 5 µm) was preferred to improve the peak symmetry and to obtain an appropriate retention time.

A mixture of Methanol–water for the sample preparation in order to get optimum results various proportion were tried amongst which 50:50 v/v proportions found to be desirable. The best separation was achieved with the proposed mobile phase Methanol–water (50:50, v/v) at a flow rate of 1.0 mL min^{-1}. The injection volume was kept 20µL. The retention time found to be 1.57 mins.

3.3 Optimization of the sample treatment

Cotton swabs were spiked with different quantities of Phenytoin sodium and placed into volumetric flasks. The solvent methanol: water (50:50) was used to prepare the sample, the volumetric flasks were sonicated for 15 mins) and the solutions were analyzed using HPLC system.

3.4 Method Validation

Once the chromatographic conditions had been selected, the method was validated, whereby attention was paid to the linearity, limit of detection, limit of quantification, precision and repeatability [18-19].

3.4.1 System Suitability test: System suitability testing is essential for the assurance of the quality performance of a chromatographic system. During performing the system suitability tests, the USP tailing factor observed was 1.48±0.02.

3.4.2 Linearity: Linearity data were obtained by plotting the area of the Phenytoin sodium peak, expressed in area units, against the concentration of Phenytoin sodium expressed as µg mL^{-1}. A linear regression least square analysis was performed in order to determine the slope, intercept and coefficient of determination. The standard curve was linear from 2-10 µg mL^{-1}. The values of the slope, intercept and coefficient of determination of the calibration curve for Phenytoin sodium are given in Table 1. The high value of the coefficient of determination indicated good linearity.

3.4.3 Repeatability: Repeatability data were obtained by injecting 6 ppm solution 6 times and areas were calculated which found to have RSD 1.09.

3.4.4 Limit of detection (LOD) and Limit of quantification (LOQ): LOD and LOQ were determined based on the standard deviation of the response (Y-intercept) and the slope of the calibration curve at low concentration levels according to ICH guidelines. The LOD and LOQ for Phenytoin sodium were found to be 0.176 and 0.535 µg mL^{-1}, respectively.

TABLE 1 Linear regression data in the analysis of Phenytoin sodium

Statistical parameters*	Values
Concentration range, µg mL^{-1}	2.0–10.0
Regression equation	y = 4148x + 1809
Coefficient of determination	r^2 = 0.993

**n=6(Number of observations)*

3.4.5 Precision: Precision was determined by six replicate applications and measurement of a sample solution at the analytical concentration. The repeatability of sample application and measurement of peak area for active compound were expressed in terms of relative standard deviation (%R.S.D. Method repeatability was obtained from R.S.D. value by repeating the assay six times in same day for intra-day precision. Intermediate precision was assessed by the assay of two, six sample sets on different days (inter-day precision). The intra- day and inter-day variation for determination of Phenytoin sodium was carried out at three different concentration levels 6, 8 and 10 µg mL^{-1}.

TABLE 2 Inter-day and Intra-day Precision

Conc. (µg mL^{-1})	Inter-day precision (% RSD)	Intra-day precision (% RSD)
6	0.852	2.568
8	0.460	3.267
10	0.806	1.788

3.5 Recovery studies

The proposed method when used for extraction and subsequent estimation of Phenytoin sodium from tablet and injection after spiking with additional drug afforded recovery of 99–102% and mean recovery for Phenytoin sodium from the marketed formulation are listed in Table 3.

TABLE 3 Recovery data of tablet and injection solutions

Formulation	0%	80%	100%	120%
		Mean % recovery*		
Tablet	99.97±0.98	99.58±0.899	99.61±0.19	99.27±1.02
Injection	99.54±1.10	99.32±0.90	99.77±1.18	99.82±1.08

**n=3(Number of observations); ±SD*

3.6 Assay of Swab samples

Assay of swab samples collected from different locations from the equipment. Swab samples from different locations within the manufacturing equipment train were submitted to the laboratory for analysis of residual Phenytoin sodium. These samples were prepared and analyzed by the proposed method and the results obtained for these samples are presented below in Table 4.

TABLE 4 Cotton Swab Analysis (Cotton swab solutions recovered from various surfaces of equipment)

Sampling area	Concentration (µg mL^{-1})*
Granulating bowl	0.371(< LOQ)
Sieves	0.365(< LOQ)
Turret	0.329(< LOQ)
Chute	0.364(< LOQ)
Collecting vessel	0.370(< LOQ)

**n=3(Number of observations)*

3.7 Application of ANOVA to the recovery studies [20-22]:

The analysis was carried using the data obtained from two different formulations, i.e., Phenytoin sodium 100 mg tablet and Phenytoin sodium injection (40mg/2ml).

The paired t- test was implicated to check whether the significant difference is present between the areas obtained through the spiked samples of same concentrations prepared from the two different formulations.

Two tailed, paired t-test was implicated using Graphpad Prism Ver. 5.0 software.

The P value (>0.05) indicates that there is no significant difference between the two different formulation samples.

TABLE 5 ANOVA (t-test applied to the results obtained from recovery samples of tablet and injection solution)

Factors	Concentration*			
	0%	80%	100%	120%
P value	0.4288	0.6330	0.8521	0.6771
R^2	0.3263	0.1347	0.0218	0.1042
Df	2	2	2	2

**n=3(Number of observations)*

CONCLUSION

From the above results we can conclude that RP-HPLC method is simple and effective method developed for the estimation of Phenytoin sodium which is efficient and validated. The recovery samples of the both formulation (tablet and injection) were observed in the range 99-102 % which shows method developed is significant. The cotton swab samples were effectively quantified and residual amount was found below LOQ and hence we can apply the method to ensure the cleaning validation is upto the mark. Also, the data obtained from ANOVA test concludes that there were no significant difference found between the results of recovery study of tablet and injection.

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