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Research Article

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Stability indicating RP-HPLC method validation for the assay of phenytoin sodium in phenytoin sodium capsules

Muralee Krishna, Meghana Nadre, Aniruddha V. Sherikar and Ranjith Reddy*

Glenmark Pharmaceutical Limited, Plot No.2, Pharma Zone, Pithampur, India

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ABSTRACT

A simple, accurate, rapid and precise High performance liquid chromatographic (HPLC) method was validated for the determination of Assay of phenytoin sodium in phenytoin sodium capsules. The method employs Waters HPLC system on Inertsil ODS 3V, 250 x 4.6mm, 5µmcolumn with an isocratic elution at a flow rate of 1.0 mL/min using a mobile phase of 55-45% of Buffer and methanol. The detection was performed by a photo diode array and UV-visible Detector. In Linearity over concentration range of 50% to 150% correlation observed was 0.999. The intra and inter-day precision are with in limit (overall % RSD not more than 2.0 %). The overall mean recovery over a range 80,100, 120 % of phenytoin sodium was 100.8%. The method is robust even for slight change in chromatographic conditions. The validated method was Specific, Linear, Precise, Accurate, Rugged and Robust for Assay of phenytoin sodium in phenytoin sodium capsules. The method validated as per ICH guideline by High performance liquid chromatography.

Keywords: Phenytoin Sodium, Chromatography, Assay, High performance Liquid Chromatography.

INTRODUCTION

Phenytoin is an antiepileptic drug approved in the USA, Europe and several other countries. Phenytoin is currently used to manage partial onset seizures in humans suffering from epilepsy. Phenytoin has the molecular formula $C_{15}H_{12}N_2O_2$ and the chemical name 5,5-diphenylimidazolidine-2,4-dione with molecular weight of 252.268 gram per mol[1-3]. Phenytoin is an anticonvulsant drug, which is useful in the treatment of epilepsy. The primary site of action appears to be the motor cortex where spread of seizure activity is inhibited. Possibly by promoting sodium efflux from neurons, phenytoin tends to stabilize the threshold against hyper excitability caused by excessive stimulation or environmental changes capable of reducing membrane sodium gradient. This includes the reduction of post tetanic potentiation at synapses. Loss of post tetanic potentiation prevents cortical seizure foci from detonating adjacent cortical areas. Phenytoin reduces the maximal activity of brain stem centers[4-7].The analysis by HPLC is more significant than using other methods like UV, liquid chromatography and immunoassays for the estimation of Phenytoin sodium[8]. The HPLC method is developed, validated and applied. An analytical method validated that allows the determination of assay of phenytoin sodium in Phenytoin sodium capsules. The validation parameters, linearity, repeatability, precision, Accuracy, Solution Stability and robustness were validated.[9-10].

Objective:
The aim describes validation for determination of Assay of phenytoin sodium in phenytoin sodium capsules by using a high performance liquid chromatography.

EXPERIMENTAL SECTION

Instruments used in method validation are tabulated in table no. 1

Table No.1 List of Instrument Used

Sr No	Instrument	Make	Software	Detector/Model No
1	HPLC	Waters	Empower Software	2489 dual wavelength
2	HPLC	Waters	Empower Software	2998 PDA Detector
3	Sonicator	Lab India	NA	NA
4	Weight balance	Mettler Toledo	NA	ML204
5	Oven	Thermo lab	NA	GMP
6	Photolytic Chamber	Thermo lab	NA	GMP

Methodology

Standard: Phenytoin Sodium working standard: Use the standard as such and use % potency on as is basis for calculations. Keep container tightly closed. Determine water content at the time of use. Batch No.: GPWS0151401

Preparation of Buffer solution: Weigh and transfer accurately about 6.8 g of monobasic potassium phosphate in 1 liter of water, adjust the pH to 3.5 ± 0.05 with Orthophosphoric acid. Filter through 0.45µ nylon filter and degas.

Preparation of Mobile phase: Prepare mixture of Buffer solution and Methanol in the ratio of 45:55v/vand degas.

Preparation of Standard solution: Weigh and transfer accurately about 25 mg of Phenytoin Sodium working standard into a 100 mL volumetric flask, add about 30 mL of methanol and sonicate it to dissolve. Cool to room temperature and make up to the mark with mobile phase and mix.

Preparation of Sample solution: Pool the contents of 10 capsules. Weigh and transfer accurately content equivalent to about 100 mg of Phenytoin sodium into 200 mL volumetric flask, add about 60 mL methanol and sonicate for 20 minutes with occasional swirling. Cool to room temperature and dilute to volume with mobile phase and mix. Filter through 0.45 Nylon filter. Transfer 10.0 mL of this solution into 20 mL volumetric flask and make up to volume with mobile phase.

Chromatographic Conditions:

Column	:	Inertsil ODS 3V, 250 x 4.6mm, 5µm
Flow Rate	:	1.0 mL / min.
Detection	:	229 nm.
Column Temp	:	25°C.
Injection Volume	:	10 µL.
Run Time	:	15 min.
Retention time	:	Between 9.5 to 13.0 minutes

Evaluation of System suitability: Inject the five replicate injections of standard solution into the chromatograph and record the chromatograms. Measure the area counts for Phenytoin peak. The RSD of five replicate injections of standard solution should not be more than 2.0%. Tailing factor for Phenytoin peak should not be more than 2.0. Number of theoretical plates should not be less than 3000.

RESULTS AND DISCUSSION

Specificity: Prepared representative Standard solutions and Sample solutions of Phenytoin Sodium Capsules and Injected each of the Diluent, Placebo solutions, Sample solutions and Standard solutions into the HPLC using the Chromatographic system utilizing a photodiode array detector. No interference was observed from Blank and Placebo at the retention time of Phenytoin peak. Also, The Phenytoin peak is pure in Standard solution and Sample solution. Therefore, the HPLC method for the determination of Assay of Phenytoin Sodium in Phenytoin Sodium Capsules is specific. Specificity reported in table no.2.

Table 2: Table for Specificity

Sr. No.	Name	Purity Angle	Purity Threshold
1	Standard solution	0.170	1.059
2	Sample solution	0.164	1.060

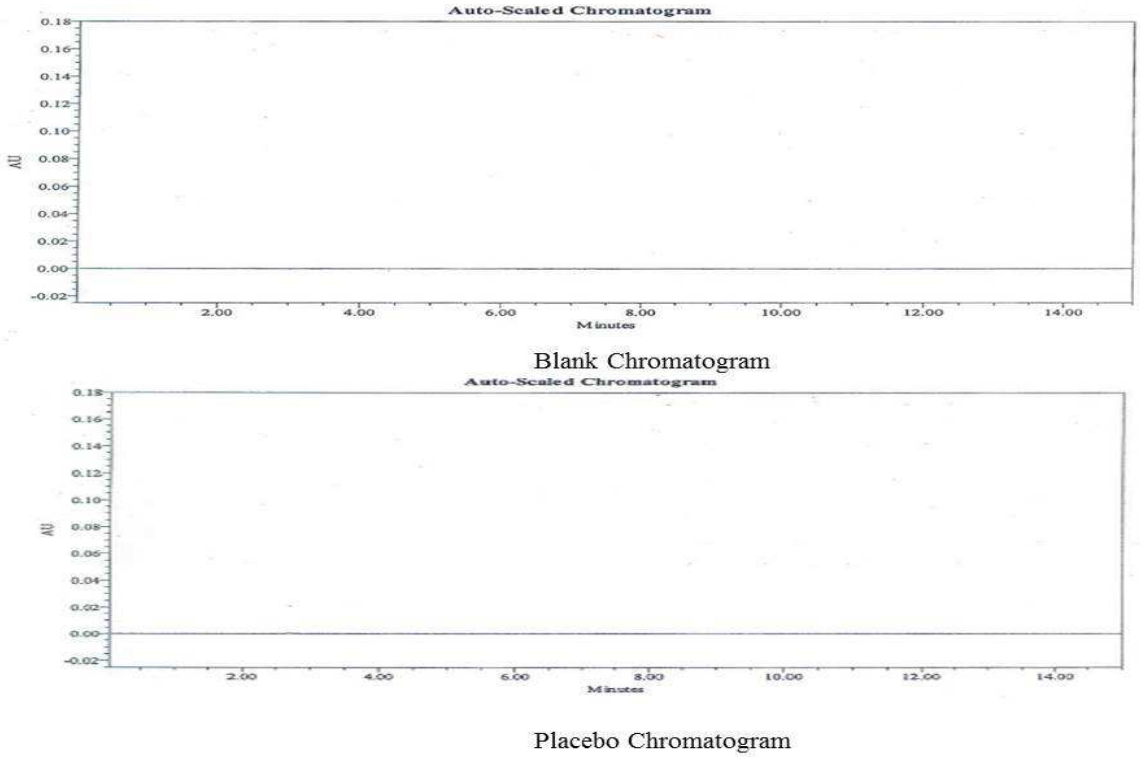

Figure No: 1 Blank and Placebo Chromatogram

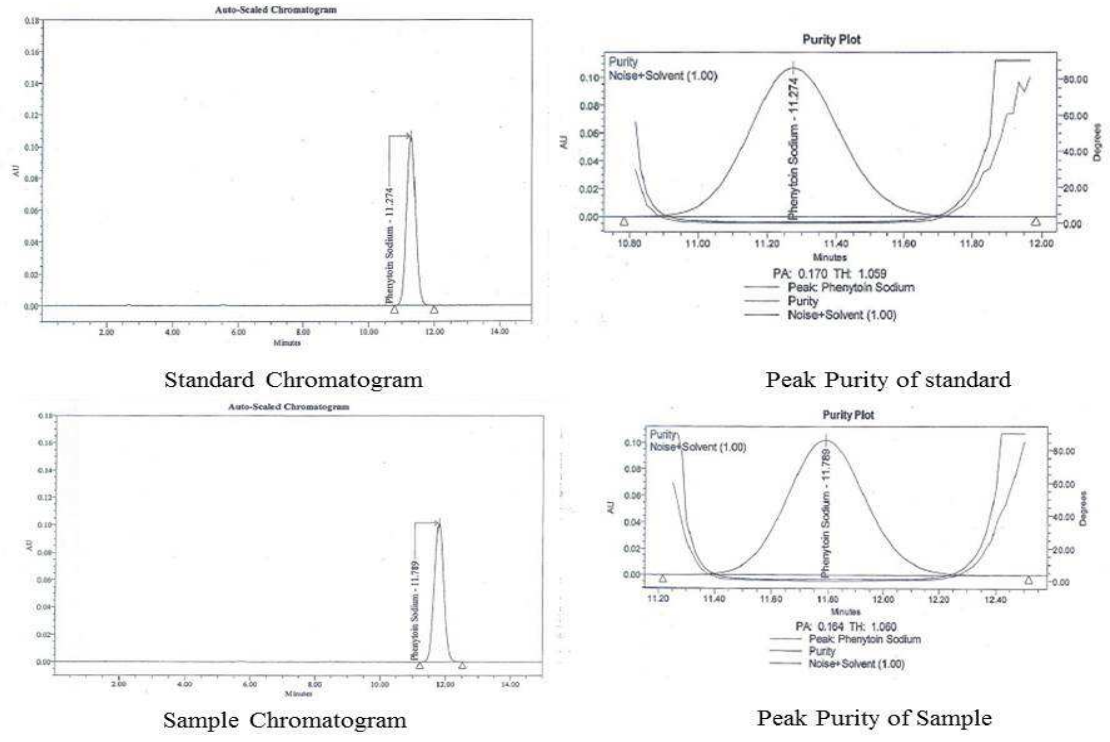

Figure No: 2 Standard and Sample Chromatogram

Linearity and Range: A series of Standard preparations of Phenytoin sodium to be prepared over a range of 50% to 150% of the working concentration of Phenytoin sodium in Phenytoin sodium Capsules. Since the working concentration is 250 µg per ml, of Phenytoin sodium, the range proposed is about 125 µg per ml to 375 µg per ml of Phenytoin sodium. Linearity of phenytoin Sodium reported in table No. 3.

Table 3: Table for Linearity

% Concentration	Concentration (PPM) (µg per mL)	Response (Area)	Statistical analysis	
50%	123.202	1101313	Slope	8864
80%	197.122	1764719	Intercept	12354
90%	221.763	1980510		
100%	246.403	2199437	Correlation Coefficient	0.9999
110%	271.043	2428506		
120%	295.684	2655227		
150%	369.605	3282838		

Accuracy (Recovery):Placebo of Phenytoin sodium was spiked with Phenytoin sodium drug substance at three different levels: 80%, 100% and 120% in triplicate (total nine determinations). Each of the sample preparations was injected in duplicate and the average area count to be taken for calculation. Accuracy reported in table no.4

Table 4: Table for Accuracy

Sample No.	Amount added (mg)	Amount recovered (mg)	% Recovery
Acc. 80% -1	80.37	79.130	100.4
Acc. 80% -2	80.09	79.190	100.9
Acc. 80% -3	80.35	79.320	100.7
Acc. 100% -1	101.07	98.730	99.6
Acc. 100% -2	100.72	99.140	100.4
Acc. 100% -3	100.16	99.040	100.9
Acc. 120% -1	120.19	119.710	101.6
Acc. 120% -2	120.08	119.170	101.2
Acc. 120% -3	120.09	119.260	101.3
Mean			100.8
SD			0.557
% RSD			0.553

Precision: System Precision Five replicate injections of the Standard preparation were made into the HPLC. The RSD of system precision is reported in Table no. 5

Table 5: Table for System Precision

Injection	Area
1	2148082
2	2132656
3	2143821
4	2150024
5	2145287
Mean	2143974
SD	6769.835
%RSD	0.316

Method Precision**:** Six sample preparations of Phenytoin Sodium Capsules were prepared and injected into the HPLC. The HPLC method for the determination of Assay of Phenytoin Sodium in Phenytoin Sodium Capsules is reproducible. Result of method precision reported in Table no.6

Table 6: Table for Method Precision

Sample	% Assay
1	102.2
2	102.4
3	102.7
4	102.9
5	102.6
6	103.0
Mean	102.6
SD	0.301
%RSD	0.293

Ruggedness (Intermediate Precision):Six sample preparations of the same lot (as used in Precision) of Phenytoin Sodium Capsules, was made by a different analyst, using different column on a different day and injected into a different HPLC system. Ruggedness reported in table no. 7

Table 7: Table for Ruggedness

Sample	Analyst -1 % Assay	Analyst -2 % Assay
1	102.2	102.3
2	102.4	101.8
3	102.7	102.2
4	102.9	101.5
5	102.6	102.4
6	103.0	102.5
Mean	102.6	102.1
SD	0.301	0.387
%RSD	0.293	0.379
Overall Mean	102.4	
Overall SD	0.427	
Overall %RSD	0.417	

Stability of Analytical solution: The sample and standard preparations were stored at room temperature and tested against freshly prepared standard preparations for 72 hours. Solution Stability of phenytoin Sodium Reported in Table no. 8

Table 8: Stability of Analytical solution at Room Temperature

Sr. No.	Name	% Content	% Correlation
1	Standard Solution - 0 hours	100	--
2	Standard Solution -24 hours	99.3	99.3
3	Sample Solution - 0 hours	102.2	--
4	Sample Solution -48 hrs	100.2	98.0
5	Sample Solution - 96 hours	100.2	98.0

Standard is stable for 24 hours and sample solutions is stable for 96 hours at room temperature

Robustness: Three Sample preparations of the same lot of Phenytoin Sodium Capsules was prepared and The samples along with standard was injected in duplicate under different chromatographic conditions as shown below. Result of robustness reported in table no. 9 to 13

Table 9: Table for Change in organic phase composition. (± 2% absolute)

Control	(+2% absolute)	(-2% absolute)
102.2	100.7	100.5
102.4	101.0	100.7
102.7	100.8	106.2
Cumulative Mean	101.6	102.5
Cumulative SD	0.896	1.100
Cumulative %RSD	0.882	1.084

Table 10: Table for Change in pH of Buffer (± 0.2 units)

Control	(+0.2 units)	(-0.2 units)
102.7	104.2	105.2
101.7	101.6	101.9
102.1	101.9	102.I
Cumulative Mean	102.4	102.6
Cumulative SD	0.979	1.309
Cumulative %RSD	0.956	1.276

Table 11 Table for Change in Flow rate (±0.1 mL/min.)

Control	(+0.1 mL/min.)	(-0.1 mL/min.)
102.2	101.8	101.3
102.4	101.8	101.5
102.7	101.9	101.9
Cumulative Mean	102.1	102.0
Cumulative SD	0.367	0.537
Cumulative %RSD	0.359	0.526

Table 12: Table for Change in column temperature (+5°C)

Control	(+ 5°C)
102.2	99.6
102.4	100.1
102.7	100.1
Cumulative Mean	101.2
Cumulative SD	1.391
Cumulative %RSD	1.375

Table 13: Table for Change in wavelength (± 5 nm)

Control	(+5nm)	(-5nm)
104.2	103.5	103.5
103.3	103.3	103.3
103.6	103.8	103.7
Cumulative Mean	103.6	103.6
Cumulative SD	0.343	0.335
Cumulative %RSD	0.331	0.323

Filter equivalency: Pooled the contents of 10 capsules. Weighed and transferred accurately content equivalent to about 100 mg of Phenytoin sodium into 200 mL volumetric flask, added about 60 mL methanol and sonicated for 20 minutes with occasional swirling. Cooled to room temperature and diluted to volume with mobile phase and mix. Centrifuged in and filter in triplicate through different membrane filters such as Teflon 0.45µ, Nylon 0.45µ filters discarding first few mL of the filtrate .Transferred 10.0 mL of this solution into 20 mL volumetric flask and made up to volume with mobile phase. The Mean Filtration Recovery is within limits for Nylon 0.45µ and Teflon 0.45µ filter.. Result reported in table no 14.

Table 14: Table for Filter Equivalency

	% Assay		
No.	Centrifuged	Nylon 0.45µ	Teflon 0.45µ
1	102.8	102.8	102.9
2	102.3	102.6	102.9
3	102.7	102.7	103.0
Mean	102.6	102.7	102.9
RSD	0.258	0.097	0.056
% Correlation with centrifuged	--	100.1	100.3

System Suitability: The RSD of five replicate injections of standard solution should not be more than 2.0%. Tailing factor for Phenytoin peak should not be more than 2.0. Number of theoretical plates should not be less than 3000. Result of system suitability reported in table no 15.

Table 15: Table for System Suitability

Parameter	%RSD	USP Tailing	USP tangent
Specificity/Forced degradation	0.144	1.1	7695
Linearity, Accuracy	0.279	1.1	7593
Filter Equivalency	0.103	1.1	7291
Precision, Solution Stability	0.316	1.1	7412
Ruggedness	0.220	1.1	7441
Robustness			
Mobile phase - Organic +2%	0.267	1.1	7437
Mobile phase - Organic - 2%	0.091	1.1	7801
pH +0.2 units	0.113	1.1	7122
pH -0.2 units	0.121	1.1	7209
Flow -0.1 mL/min.	0.063	1.1	8101
Flow +0.1 mL/min.	0.232	1.1	7141
Wavelength +5nm	0.163	1.1	8198
Wavelength -5nm	0.191	1.1	8187
Temp. + 5°C	0.313	1.1	8629

CONCLUSION

The test method is validated for Specificity, Linearity and Range, Precision, Accuracy (Recovery), Ruggedness, Stability of Analytical solution, Filter equivalency and Robustness and found to be meeting the predetermined acceptance criteria. The validated method is Specific, Linear, Precise, Accurate, Rugged and Robust for Phenytoin Sodium in Phenytoin Sodium Capsules.

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