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Research Article

DISSOLUTION METHOD DEVELOPMENT AND VALIDATION BY UPLC FORDETERMINATION OF PHENYTOIN SODIUM IN PHENYTOIN SODIUM CAPSULES

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A B S T R A C T

A rapid, accurate and precise Ultra Performance Liquid Chromatographic (UPLC) method was developed for generating an exhaustive In-Vitro Dissolution profiles of phenytoin sodium capsules in an Immediate Release formulations. The method has been validated. The method employs Waters UPLC system on Acquity BEH C18, 100 x 2.1mm, 1.7µm column with a flow rate of 0.3 mL/min using a mobile phase of 50-50% of Buffer and Acetonitrile. The UPLC was equipped with a uv-visisble Detector and the measurements were taken at 229nm. The immediate release formulations label claim were 300mg, 100mg, 50mg and 25mg for which the injection volume was appropriately selected. The total runtime for each injection was 2mins only with the retention time of the phenytoin peak at about 1.4mins.The method was validated for Linearity, Specificity, precision, Solution Stability and Accuracy. The method validation shows the linearity correlation 0.999.

INTRODUCTION

Phenytoin is an approved antiepileptic drug which is a white to off white crystalline powder with the molecular formula C15H12N2O2 and the chemical name 5,5-diphenylimidazolidine- 2,4-dione and molecular weight of 252.268 gram per mol[1-3]. The primary site of action appears to be the motor cortex where spread of seizure activity is inhibited.

Phenytoin sodium being an anti-epileptic/ anti-convulsant drug, it falls under the category of Narrow Therapeutic Index (NTI) and has a non- linear kinetics. The bioavailability range lies between 90-111.11% as against other drugs with the range of 80-120%. 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]. This NTI molecule needs exhaustive In-vitro dissolution profiles for matching with the reference formulation. In such cases, the analysis by UPLC becomes more significant than using other methods like HPLC [8], UV, liquid chromatography and immunoassays for the estimation of Phenytoin sodium [9]. The UPLC method is developed, equivalency between HPLC and UPLC methodology was established and UPLC method has been validated.

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The ICH validation parameters linearity, repeatability, precision, Accuracy, Solution Stability and robustness were validated [10-11].

MATERIALS AND METHOD

Sr No	Instrument	Make	Software	Detector/Model No
1	UPLC Acquity H class	Waters	Empower Software	TUV Detector
2	UPLC Acquity H class	Waters	Empower Software	PDA Detector
3	Dissolution	Electrolab	NA	TDT-14L
4	Sonicator	Lab India	NA	NA
5	Weight balance	Mettler Toledo	NA	ML204

Development Trial

Table 1 Method development Trials

Chromatography Parameters	Trial 01	Trial 02
Column	Acquity CSH, 100 x 2.1mm, 1.7µm	
Buffer	Water	
Mobile phase	Prepared a mixture of Water and Methanol in the ratio 60:40 v/v	Prepared a mixture of Water and Methanol in the ratio 25:75 v/v
Diluent	Water	Water: Methanol (70:30)
Flow Rate	0.3 mL/min.	0.3 mL/min.
Injection Volume	2.0 µL	2.0 µL
Wavelength	229 nm	229 nm
Column Temp.	25°C	25°C
Runtime	4.0 minutes	4.0 minutes
Standard Concentration	110 ppm	110 ppm
Sample Concentration	Transferred one capsules in to 900mL	Transferred one capsules in to 900mL dissolution

	dissolution vessel	vessel
Conclusion	Main peak was observed at retention time of 2.0mins but peak shape was distorted.	Main peak was observed at retention time of 0.5 mins but peak shape was distorted.
	There is no impact on the dissolution results but peak shape was found distorted	There is no impact on the dissolution results but peak shape was found distorted

Table 2 Method development Trials

Chromatography Parameters	Trial 03	Trial 04
Column	Acquity CSH, 100 x 2.1mm, 1.7µm	Acquity BEH C18, 100 x 2.1mm, 1.7µm
Buffer	Accurately transfer 1 mL of O-Phosphoric acid in 1000 mL of water and filter through 0.2µm filter.	
Mobile phase	Prepare a mixture of Buffer and Acetonitrile in the ratio 50:50 v/v.	Prepare a mixture of Buffer and Acetonitrile in the ratio 60:40 v/v.
Diluent	Water	Water: Methanol (70:30)
Flow Rate	0.3 mL/min.	1.0 mL/min.
Injection Volume	2.0 µL	10 µL
Wavelength	229 nm	220 nm
Column Temp.	25°C	40°C
Runtime	2.0 minutes	2.0 minutes
Standard Concentration	110 ppm	110 ppm
Sample Concentration	Transferred one capsules in to 900mL dissolution vessel	Transferred one capsules in to 900mL dissolution vessel
Conclusion	Main peak was observed at retention time of 1.3 mins but peak shape was distorted	Main peak was observed at retention time of 0.5 mins but peak shape was distorted
	There is no impact on the dissolution results but peak shape was found broad	There is no impact on the dissolution results but peak shape was found distorted

Table 3 Method development Trials

Chromatography Parameters	Trial 05
Column	Acquity BEH C18, 100 x 2.1mm, 1.7µm
Buffer	Accurately transfer 1 mL of O-Phosphoric acid in 1000 mL of water and filter through 0.2µm filter.
Mobile phase	Prepare a mixture of Buffer and Acetonitrile in the ratio 50:50 v/v.
Diluent	Water
Flow Rate	0.3 mL/min.
Injection Volume	0.5 µL
Wavelength	229 nm
Column Temp.	40°C
Runtime	2.0 minutes
Standard Concentration	110 ppm
Sample Concentration	Transferred one capsules in to 900mL dissolution vessel
Conclusion	Main peak was observed at retention time of 1.3 mins
	Peak Shape was found satisfactory and Method can be finalised

Final Methodology

Preparation of Buffer: Accurately transfer 1 mL of O-Phosphoric acid in 1000 mL of water and filter through 0.2µm filter.

Preparation of Mobile Phase: Prepare a mixture of Buffer and Acetonitrile in the ratio 50:50 v/v and degas.

Preparation of Dissolution medium: (Water) Use deaerated and degassed water as dissolution medium.

Dissolution Parameters:

Apparatus	:	Basket
Dissolution Medium	:	Water
Temperature	:	37 ± 0.5°C
RPM	:	50 rpm
Volume	:	900 mL
Time Point	:	45 minutes

Preparation of Standard stock solution: Weigh and transfer accurately about 33 mg of Phenytoin Sodium working standard into a 50 mL volumetric flask, add about 15 mL methanol and sonicate it to dissolve. Cool to room temperature and make up to the mark with methanol and mix.

Standard solution for 25 mg capsules:Transfer 2 mL of Standard stock solution in to 50 mL volumetric flask and dilute up to mark with dissolution medium.

Standard solution for 50 mg capsules: Transfer 2 mL of Standard stock solution in to 25 mL volumetric flask and dilute up to mark with dissolution medium.

Standard solution for 100 mg capsules:Transfer 4 mL of Standard stock solution in to 25 mL volumetric flask and dilute up to mark with dissolution medium.

Standard solution for 300 mg capsules:Transfer 5 mL of Standard stock solution in to 10 mL volumetric flask and dilute up to mark with dissolution medium.

Preparation of Sample solution: Place one capsule in each of the six dissolution vessel containing 900 mL of dissolution media. Carry out the dissolution test. Withdraw 10 mL of aliquots, after each time interval replenish it with fresh dissolution media previously maintained at 37°C. Filter the solution through 0.45µ Nylon filter.

Chromatographic Condition

Column : Acquity BEH C18, 100 x 2.1mm, 1.7µm
Flow Rate : 0.3 mL / min.
Detection : 229 nm.
Column Temp : 40°C.
Injection Volume : 0.5 µL for 300 mg, 1.0 µL for 100 mg and 2.0 µL for 50 and 25mg
Run Time : 2 min.
Retention time : Between 1.0 to 1.6 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%. Number of theoretical plates should not be less than 5000.

Procedure: Separately inject equal volume of Blank (Dissolution medium) and Sample solution into the chromatograph and record the chromatograms. Measure the area counts for Phenytoin peak.

RESULT AND DISCUSSION

Specificity: Prepared a representative Placebo solution, Sample solution of Phenytoin Sodium Capsules and Standard solutions as per the Methodology. Injected each of the Dissolution media, Placebo solution, Sample solution and Standard solution into the UPLC using the Chromatographic system as per the Methodology utilizing a photodiode array detector. No interference was observed from Blank and Placebo at the retention time of Phenytoin peak. Also, the

peak purity data of Phenytoin peak shows that Phenytoin peak is homogeneous and there are no coeluting peaks. Therefore, the UPLC method for the dissolution of Phenytoin in Phenytoin Sodium Capsules is specific. Specificity reported in table no.4.

Table 4 Table for Specificity

Sr. No.	Name	Purity Angle	Purity Threshold
1	Standard solution	0.541	1.457
2	Sample solution	0.548	1.210

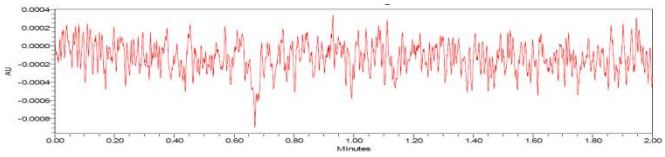


Figure No 1 Blank Chromatogram

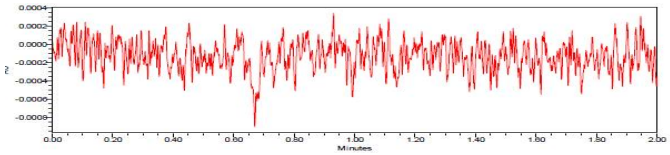


Figure No 2 Placebo Chromatogram

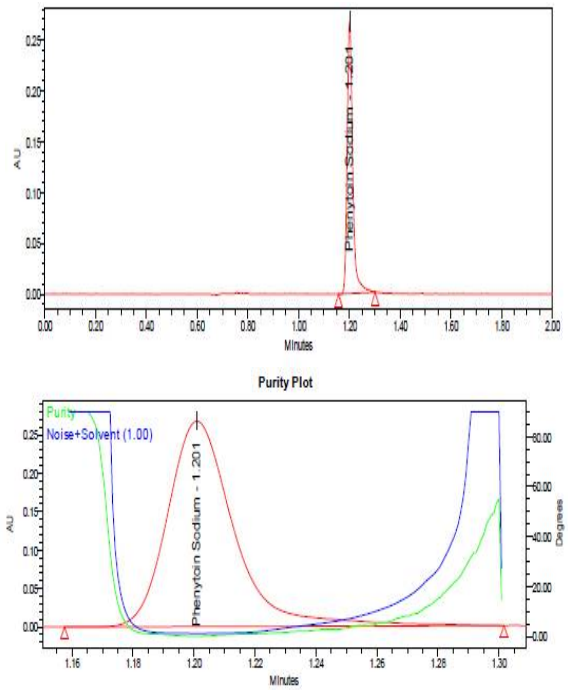
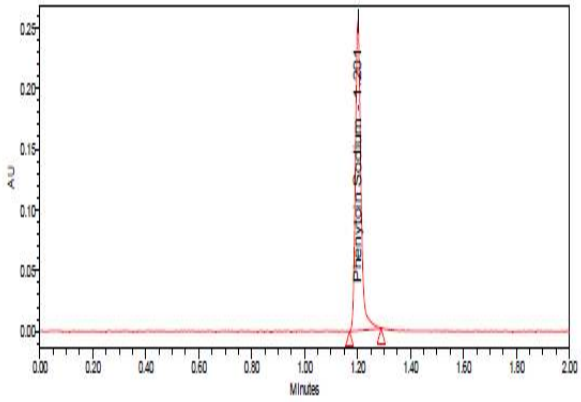


Figure No 3 Standard Chromatogram and peak purity plot



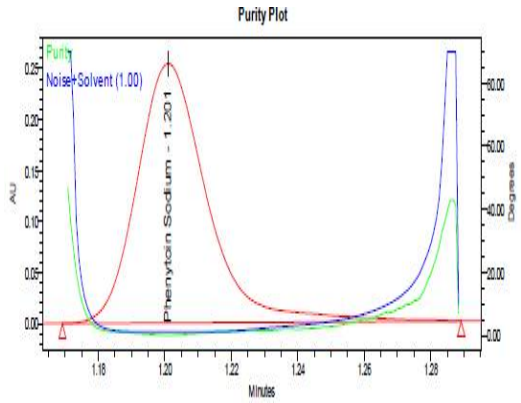


Figure No 4 Sample Chromatogram and peak purity plot

Linearity and Range: A series of Standard preparations of Phenytoin were prepared over a range of 20% to 150% of the working concentration of Phenytoin in Phenytoin Sodium Capsule.The Correlation coefficient is 0.999. Therefore, the UPLC method for the dissolution of Phenytoin in Phenytoin Sodium Tablets is linear. Linearity reported in table no.5.

Table 5 Linearity Table

% Linearity Range	Concentration (ppm)	Response (Area)	Statistical analysis	
20	5.20	6626		
50	32.50	42243	Slope	1252
80	65.01	77997		
90	162.52	188975		
100	260.04	319463	Intercept	-5050
110	325.04	383917		
120	390.05	483945	Correlation Coefficient	0.999
150	487.57	619956		

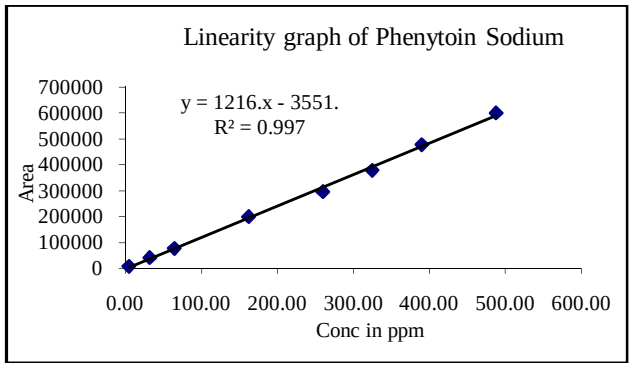


Figure No 5 Linearity plot

Table 6 Accuracy Table

Sample No.	Amount added (mg)	Amount recovered (mg)	% Recovery
60%-25mg Sample-1	15.09	15.47	102.5
60%-25mg Sample-2	15.40	15.46	100.4
60%-25mg Sample-3	15.01	15.21	101.3
60 %-300mg Sample-1	179.11	162.81	90.9
60 %-300mgSample-2	178.98	165.21	92.3
60 %-300mg Sample-3	179.11	164.61	91.9
80 %-300mg Sample-1	238.81	226.32	94.8
80 %-300mg Sample-2	238.89	223.19	93.4
80 %-300mg Sample-3	238.92	219.49	91.9
100%-300mg Sample-1	298.57	273.50	91.6
100%-300mg Sample-2	298.33	278.80	93.5
100%-300mg Sample-3	298.58	276.46	92.6
120%-300mg Sample-1	358.02	343.96	96.1
120%-300mg Sample-2	357.92	342.12	95.6
120%-300mg Sample-3	358.16	344.12	96.1
	Mean		96.1
	SD		3.216
	% RSD		3.35

Accuracy (Recovery): Weighed placebo of Phenytoin Sodium Tablets equivalent to 1 tablet of 20 mg in separate 1000 ml volumetric flasks & spiked Phenytoin API at 60% of 25 mg, 60%, 80%, 100% and 120% in triplicate of 300 mg, added dissolution medium and sonicated for 30 mins. The mean recovery is 93.4% Therefore the UPLC method for the dissolution of Phenytoin in Phenytoin Sodium Capsule is accurate. Accuracy reported in table no.6.

Precision

System Precision: Five replicate injections of the Standard Preparation for Phenytoin Sodium Capsule were chromatographed into the UPLC using the method as described under Methodology. The RSD of system precision is reported in Table no. 7.

Table 7 System precision

Sr. No	Response
1	403949
2	400143
3	397293
4	401708
5	391381
Mean	398895
SD	4848.711
%RSD	1.216

Method Precision: Experiment: Six Sample Preparations of Phenytoin Sodium Capsule 300 mg was analyzed using the method as described under Methodology. The RSD of method precision is 2.409% refer Table 7. Therefore; the UPLC method for the dissolution of Phenytoin in Phenytoin Sodium Capsule is precise

Ruggedness: Six Sample preparations of the same lot as used in method precision of Phenytoin Sodium Capsule were analyzed by a different analyst, using different column, on a different day, on a different UPLC. The Over all %RSD of intermediate precision is 2.186%. Therefore, the UPLC method for the dissolution of Phenytoin in Phenytoin Sodium Capsule is reproducible. Comparison of Precision and Ruggedness reported in table no.8.

Table 8 Table for Precision and Ruggedness

Sample	Analyst -1 (Precision) % Drug release	Analyst -2 (Ruggedness) % Drug release
1	92	100
2	97	97
3	98	97
4	97	100
5	95	98
6	98	97
Mean	96.2	98.2
SD	2.317	1.472
% RSD	2.409	1.499
Overall Mean	97.2	

Stability of Analytical solution: The sample and standard preparations for Phenytoin Sodium Capsule were analyzed initially and at different time intervals stored at room temperature. The cumulative area RSD of Standard solution and sample Solution Reported in Table No.9.

Table 9a Stability of Analytical Solution-(Standard)

No.	Time (hr.)	Area
1	INITIAL	415989
2	3 HRS	417151
5	10 HRS	429773
9	20 HRS	420355
13	30 HRS	421795
17	40 HRS	416753
% RSD		1.03

Table 9b Stability of Analytical Solution-(Sample)

No.	Time (hr.)	Area
1	INITIAL	421380
2	3 HRS	418407
3	4 HRS	426435
4	7 HRS	426509
5	9 HRS	414699
6	12 HRS	418329
7	14 HRS	420916
8	17 HRS	418690
9	20 HRS	403463
%RSD		1.65

System Suitability: Standard solution

Recordedthe RSD of five replicate injections of standard solution and the number of theoretical plates refer table no: 10

Table 10 System suitability data

Experiment	%RSD of Standard	Theoretical plates
Specificity, Precision/Filter Equivalency300 mg	1.216	16419
Accuracy	1.32	6889
Ruggedness, Linearity, Solution Stability	0.254	7013
Precision/Filter Equivalency25 mg	1.890	22781
Precision/Filter Equivalency50 mg	0.637	22398
Precision/Filter Equivalency100 mg	1.526	19306

CONCLUSION

The Developed and Validated UPLC method for Dissolutionof Phenytoin Sodiumis linear, precise, accurate and specific. The results of the method are well within the acceptance limits and as per the International Conference on Harmonization requirements.

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List of abbreviations

No.	Number
Hrs	Hours
mL	MilliLiter
UPLC	Ultra performance Liquid Chromatography
SD	Standard Deviation
RSD	Relative Standard Deviation

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