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Development and Validation of High Performance Liquid Chromatography Assay Method of Phenytoin in Tablet Dosage Form

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ABSTRACT:
A new, precise and accurate reversed-phase high performance liquid chromatography (RP-HPLC) method was developed for determination of phenytoin. Method was carried out by isocratic technique on a reverse-phase HPLC using a column of C8 inertstil (4.6 x 250) mm, 5µm column with a mobile phase mixture of 500cm^3 water, 400cm^3 absolute methanol and 100cm^3 acetonitrile. The flow rate was adjusted at 2cm^3/min, detection wavelength at 235 nm, temperature at 40°C. The retention time was found to be 4.8min. The results of analysis have been validated statistically and recovery studies confirmed the accuracy of the developed method according to ICH guidelines (ICH Q2 R1, 2005).The assay percentage of phenytoin in tablet dosage form was found to be (100.79± 1.067) % .The % of recovery was found to be (99.6 – 102.5) %. The limit of detection (LOD) and limit of quantization (LOQ) were found to be 0.00040755mg/cm^3 and 0.001235mg/cm^3 respectively.

KEYWORDS: Isocratic, Reverse phase, Anti-epileptic, anticonvulsant, Phenytoin.

INTRODUCTION:

Phenytoin sodium (fig.1) is 5,5-diphenylimidazolidine-2,4-dione sodium salt (USP, 2015). Phenytoin sodium belongs to the category of drugs referred to as anticonvulsant and anti-epileptic. Phenytoin is one of the most commonly used antiepileptic medications in clinical practice for generalized seizures. It is used to prevent and control seizures.

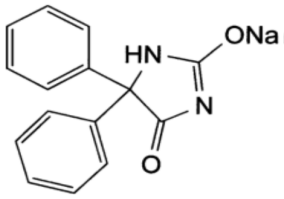

Fig (1): structure of phenytoin.

Several methods have been reported for the determination of phenytoin sodium using LC-MS (Safat, K.,2000), mass spectrometry(Susan S et al., 2011), spectrophotometry [12], HPLC [(Siew YT et al.,2015),(Varaprasad A et al.,2012),(Muralee K et al.,2015),(Ravichandran S et al.,2015),(Jeyaprakash MR et al.,2013),(Hisham H et al.,2013),(Cristina S et al.,2008),(Ouakouak H et al.,2014)], polarography (Serralheiro A et al.,2013), potentiometry (Ashy, A. R et al.,1986).

The objective of this research was to develop and validated an accurate, precise and selective reverse phase HPLC methods of phenytoin. Then validation of this method through elements such as accuracy, linearity, sensitivity, precision, limit of detection and limit of quantification.

MATERIALS AND METHODS:

Material:

Chemicals and Reagent:

Methanol from Duksan pure chemicals Korea, Acetonitrile from Duksan pure chemicals Korea. Phenytoin working standard (purity 98.89%) from Shanghai-Sudan Pharmaceutical CO-LTD, and phenytoin tablet 100mg dosage from the local market.

Instruments:

The instruments used for the study was. High-performance liquid chromatography (HPLC) SHIMADZU Japan, prominence–I LC-2030c.UV-VIS detector software LC solution.

Diluents preparation

Mixture of 500cm^3 Absolute methanol and 500cm^3 acetonitrile.

Mobile phase preparation

Mixture of 500cm^3 water, 400cm^3 absolute methanol and 100cm^3 acetonitrile.

Methods:

Preparation of standard stock solution

27.7mg of phenytoin standard (purity 98.89%) were weighed and transferred to 100cm^3 volumetric flask by 60cm^3 diluents and completed to the mark by diluents. The final concentration of this solution 0.277 mg/cm^3.

Preparation of placebo stock solution

73.8mg of phenytoin placebo were weighed and transferred to 100cm^3 volumetric flask by 50cm^3 of diluents and shaked for 30 minutes (RPM 270) and completed by diluents.

Average weight of tablet 0.17384mg then the theoretical weight of placebo equivalent to 100mg of phenytoin equal (0.17384- 0.1) = 0.0738mg.

Specificity:

Diluents', mobile phase, placebo, standard solution (100%) and sample solution was injected in HPLC.

System suitability

Standard solution (100%) was injected five times at three different mobile phase mixtures (500cm^3 water, 400cm^3 methanol, 100cm^3 acetonitrile), (500cm^3 water,420cm^3 methanol, 100cm^3 acetonitrile) and (520cm^3 water,400cm^3 methanol, 100cm^3 acetonitrile) then retention time, area under peak, tailing factor, width, resolution (closest peak to phenytoin peak) and number of theoretical plate.

Linearity:

Aliquots 1.25, 2.5, 3.75, 5, 6.25, 7.5and 8.75cm^3 of solution (0.277mg/cm^3) to series 25cm^3 volumetric flask and completed to the mark by diluents to obtain solutions had final concentrations 0.01385, 0.0277, 0.04155, 0.0554, 0.06925, 0.0831and 0.09695mg/cm^3 respectively (25% - 175%).

Accuracy:

The accuracy of the method was done by adding a known amount of standard solution to placebo across the range of linearity (50%, 75%, 100%, 125%and 150%).

Precision:

The precision was done by preparation of a 100 % standard solution (0.0554 mg/cm^3) for both intermediate - precision and repeatability. Using different analysts each one prepared different sample.

Intermediate Precision: The intermediate precision was done by using 100% concentration solution

Repeatability: Six replicates in same concentration were analyzed in same day for repeatability

Limit of detection and the limit of quantification:

Detection limit is the lowest concentration of analyzed sample that can be detected, but not necessary quantized. It can be determined by preparing a solution that expected to produce a response that is approximately 3 to10 time's base line noise

$$LOD = 3.3\sigma/S$$

$$LOQ = 10\ \sigma/S$$

σ = standard deviations of the response

S = mean of the slope of the calibration plot

Results and Discussion:

Specificity:

Showed no interference between the area under peak of phenytoin and diluents, mobile phase, placebo or degradation product. The result showed in table(1) and **Figures No(2)(a, b, c, d, e)**.

Table (1) Result of specificity for phenytoin

Diluents	No interference
Mobile phase	No interference
Placebo	No interference

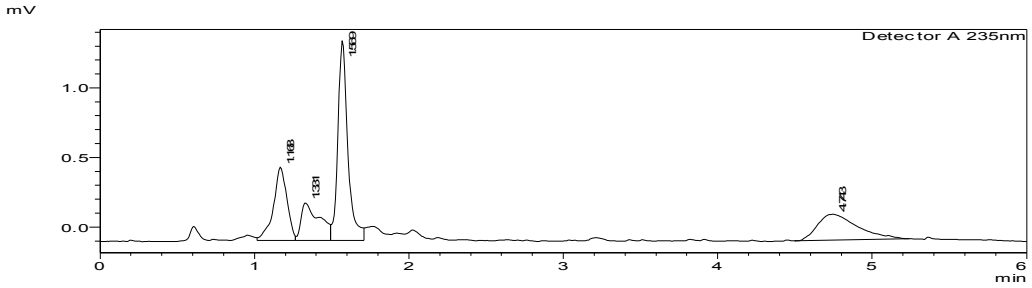

Fig (2-a) Chromatogram of M-ph

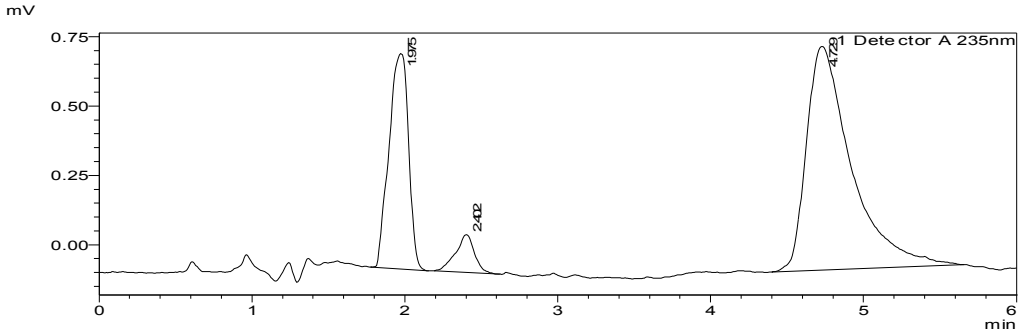

Fig (2-b) Chromatogram of diluents

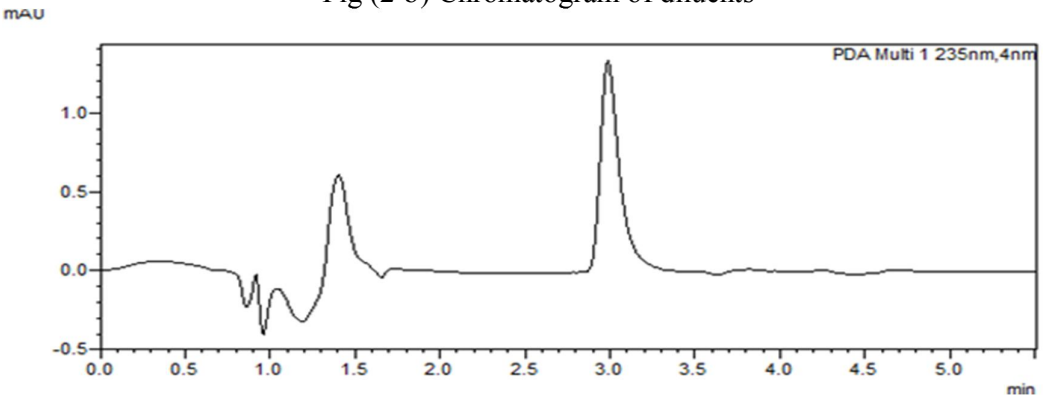

Fig (2-c) Chromatogram of placebo solution for phenytoin.

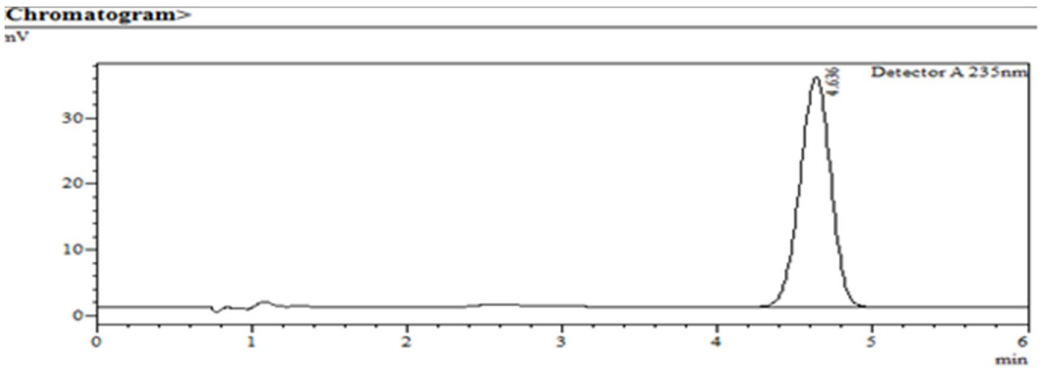

Fig (2-d) Chromatogram of standard.

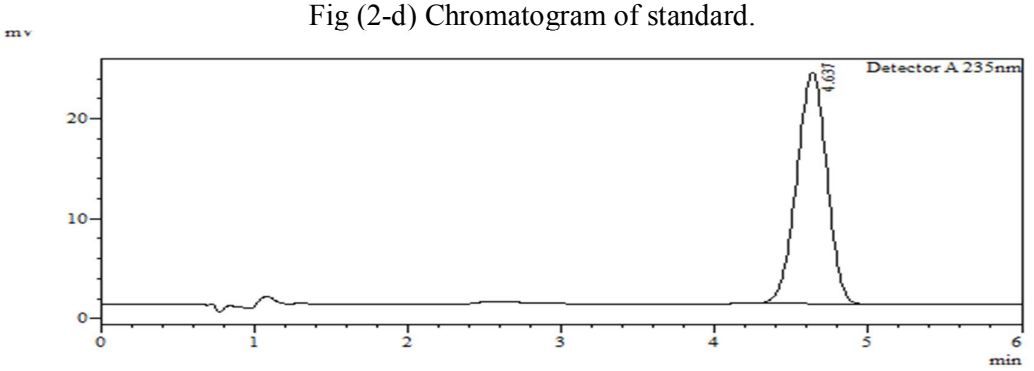

Fig (2-e) Chromatogram of sample.

System suitability:

Average of five injection, Concluded that change in the mobile phase mixture ratios (± 20 ml) will generate (average ± % RSD) in retention time (4.9109±6.42%) minute , area under peak (270116±1.6%), number of theoretical plate (25020.5±1.849%), tailing factor (0.971±0.6%), width (0.32±5.80%) and resolution (7 ±11.2%). Results are shown in Table (2).

Table; (2) System suitability of phenytoin

Mobile phase	Retention time	Area under peak	NO. of theoretical plate	Tailing factor	Width (USP	Resolution (USP)
500cm^3 water,400cm^3 methanol, 100cm^3 acetonitrile	4.818	274239	24862	0.965	0.316	6.856
	4.823	272711	24990	0.965	0.315	6.895
	4.834	274906	25051	0.965	0.315	7.023
	4.843	272333	25073	0.967	0.316	6.978
	4.853	272952	25130	0.97	0.316	6.98
average	4.8342	273428	25021	0.9664	0.3156	6.9464
500cm^3 water,420cm^3 methanol, 100cm^3 acetonitrile	4.635	275800	24300	0.975	0.307	6.379
	4.635	274840	24413	0.975	0.306	6.343
	4.642	271838	24693	0.979	0.305	6.368
	4.647	268733	24733	0.976	0.305	6.39
	4.646	267596	24649	0.981	0.306	6.398
average	4.641	271761	24558	0.9772	0.3058	6.3756
520cm^3	5.061	266413	21443	0.968	0.357	7.195

water,400cm^3 methanol, 100cm^3 acetonitrile	5.061	267345	21415	0.967	0.357	7.193
	5.391	264164	28191	0.969	0.332	8.439
	5.391	264415	28179	0.969	0.332	8.438
	5.384	263460	28186	0.968	0.331	8.435
average	5.26	265159	25482.8	0.9682	0.3418	7.9
Average	4.9109	270116	25020.5	0.971	0.32	7
SD	0.3154	4373	462.600	0.0058	0.019	0.79
%RSD	6.42	1.6	1.849	0.60	5.80	11.2

Linearity:

The linearity was determined by terms of correlation coefficient and results are given in Figure No 3 and Table No 3. The correlation coefficient ($R^2 \geq 0.99$) demonstrates linearity. In addition, the y-intercept must not be significantly different from zero according to ICH guide lines.

The method has linear responding in the range of concentration (25% to 175%).

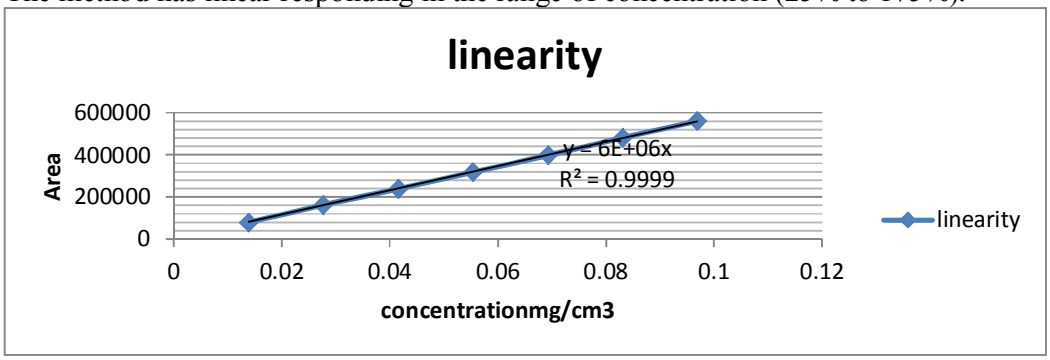

Fig **(3); c**alibration curve of phenytoin**.**

$R^2 = 0.999$ and intercept =6E+06

(Acceptance criteria for Linearity ≥ 0.99)

Conclusion that method has linear responding in the range of concentration (25% to 175%)

Table; (3) linearity result of phenytoin.

%sample concentration	Concentration	Area	SD of area under peak	RSD
25%	0.01385	79346		
		79532		
		79473		
Average		79450.3	95.05	0.11963
50%	0.0277	161323		
		161719		
		161544		
Average		161529	198	0.12285
75%	0.04155	238680		
		238684		
		238672		
Average		238678.7	6.1	0.00256

100%	0.0554	317876		
		318155		
		317742		
Average		317924	211	0.06627
125%	0.06925	397977		
		398719		
		398024		
Average		398240	415.5	0.10433
150%	0.0831	478817		
		484949		
		481626		
Average		481797.3	3070	0.63711
175%	0.09695	559893		
		562154		
		561692		
Average		561246.3	1195	0.21284
SD Average			741	

Accuracy:

Accuracy is closeness of test results with the true value which is express as % of recovery. Results are given in Table(4)

%	Actual Conc. mg/cm^{33}	Area of std with placebo	Estimated conc. mg/cm^3	Area of std only	Recovery %
50	0.0277	161498	0.02760	162082	99.6
		161290	0.02751	162419	99.6
		161498	0.02763	161934	99.7
Average		161428.7	0.02758	162145	99.6
75	0.04155	240200	0.0415	240320	100.0
		240400	0.04176	239165	100.5
		240786	0.04187	238954	100.8
Average		240462	0.0417	239480	100.4
100	0.0554	325303	0.0568	317520	102.5
		326280	0.0569	317515	102.8
		325218	0.0567	318011	102.3
Average		325600.3	0.0568	317682	102.5
125	0.06925	403508	0.06979	400398	100.8
		403509	0.06990	399901	100.9
		403916	0.06998	399727	101.0
Average		403644.3	0.06988	400009	100.9
150	0.0831	482293	0.0834	480637	100.3
		483799	0.0837	480456	100.7

		484052	0.0836	481032	100.6
Average		483381.3	0.0836	480708	100.6
Mean					100.79
SD					1.076
RSD%					1.067

Table; (4) Accuracy result of phenytoin.

Precision:

Intermediate Precision:

Result of intermediate precession of two analysts were found to be in the range (273952, 272869.7, 278209.3) %. Results are shown in Table (5).

Table; (5) Intermediate precision of phenytoin.

Run	First analyst	Second analyst	Third analyst
1	274239	272333	277384
2	272711	272952	278935
3	274906	273324	278309
Mean	273952	272869.7	278209.3
Mean	275010.3		
SD	2822.8		
RSD%	1.03		

Repeatability:

Results were found to be within acceptable limits (RSD <2) as shown in table 6**.**

Table; (6) Repeatability precision of the system of phenytoin.

NO	Area	Tailing factor	NO. Theoretical plate(USP)	Resolution
1	318736	0.954	16864	11.077
2	318630	0.955	16944	11.29
3	318560	0.954	16911	11.19
4	318953	0.953	16898	11.302
5	319387	0.953	16899	11.307
6	319548	0.96	17212	11.589
Mean	318969	0.95	16954.67	11.293
SD	411.5123	0.002639	128.6634	0.17039
RSD%	0.129013	0.27643	0.758867	1.508887

Limit of detection and the limit of quantification:

The limit of detection (LOD) may be expressed as

$$LOD = 3.3\sigma/S$$

$\sigma = 741$

S = 6E+06

LOD = 3.3* 741 (6E+06) = 0.00040755mg/cm^3

Limit of Quantization can be determined in the same manner but using the formula

$LOQ = 10\ \sigma/S$

LOQ = 10 *741/ (6E+06) = 0.001235mg/cm^3.

Results showed that detection limit and quantization limit for phenytoin by using this method is 0.00040755mg/cm^3 to 0.001235mg/cm^3 respectively.

Conclusion:

In the present work, a simple and sensitive HPLC method has been developed for the determination of phenytoin. The method was completely validated by using sensitivity, stability, specificity, linearity, accuracy and precision parameters for determination of phenytoin in the pharmaceutical tablet formulations. This method can easily, conveniently and accurately adopted for routine quality control analysis of phenytoin in pharmaceutical dosage forms. These data show that the proposed method is sensitive for the determination of phenytoin.

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References:

ICHQ2 (R1) Harmonized triptite guideline. (2005) Validation of analytical procedure: text and methodology International Conference on Harmonization ICH, Genava. .

Ashy, A. R., Sayed, Y. M & Islam, S. I . (1986) Comparision of fluorescence polarization immunoassay and high performance liquid chromatography for the quantitative determination of phenytoin, phenobarbital and carbamazepine in serum. J.Pharm. Pharmacol. **38,** 572

Cristina S, Karin VL, Celia EO, Maria AB, Maria DP & Silvia RC.(2008) Micromethod for quantification of carbamazepine, phenobartital and phenytoin in human plasma by HPLC-UV detection for therapeutic drug monitoring application. Latt Am J Pharm;27:485-91.

Hisham H, Ayman AG & Hanna S.(2013) Development and validation of rapid stability indicating HPLC-determinations of antiepileptic drugs phenobarbital in suppositories and phenytoin in capsules as well as in urine sample. J Liq Chromatogr Relat Technol;36:2292-306.

Jeyaprakash MR, Sireesha V & Meyyanathan SN.(2013) Development and validation for the estimation of benzil impurity in phenytoin formulations by reverse phase HPLC. Asian J Pharm Anal Med Chem;1:79-87.

Muralee K, Meghana N, Aniruddha VS & Ranjith R. (2015) Stability indicating RP-HPLC method validation for the assay of phenytoin sodium in phenytoin sodium capsules. J Chem Pharm Res;7:230-6.

Ouakouak H, Ben MM, Ben CM & Abdelhamid Z.(2014) Phenobarbital analysis in biological matrix (Blood) by high performance liquid chromatography (HPLC). Int Lett Chem Phys Astron;20:31-40.

Ravichandran S, Valliappan K & Ramanathan M.(2015) Validated RP-HPLC method for concurrent determination of phenytoin sodium and clopidogrel bisulphate in tablet dosage form. J Pharm Sci Res;7:934-7.

Safat, K. (2000) Comparative LC-MS and HPLC analyses of selected antiepileptics and beta-blocking drugs. Farmaco; **55,**136

Serralheiro A, Alves G, Fortuna A, Rocha M & Falcao A. (2013) First HPLC-UV method for rapid and simultaneous quantification of phenobarbital, primidone, phenytoin, carbamazepine, carbamazepine-10,11-epoxide, 10,11-Trans-dihydroxy-10,11-Dihydrocarbamazepine, lamotrigine, oxcarbazepine and licarbazepine in human plasma. J Chrom B;925:1-9.

Siew YT, Michael JR, Allan GAC, Siang Y& Seng NG. (2015) Development and validation of a stability-indicating isocratic reverse phase-liquid chromatography assay for determination of phenytoin in bulk and pharmaceutical formulations. Int J Pharm Pharm Sci;7:258-63.

Susan S.-C. Tai, Chia-Yi Yeh & Karen W. Phinney. (2011) Development and validation of a reference measurement procedure for certification of phenytoin, phenobarbital, lamotrigine, and topiramate in human serum using isotopedilutionliquid chromatography/tandem mass spectrometry Anal Bioanal Chem 401:1915–1922.

United states pharmacopeia. (2015) USP 38, The national formulary NF 33, United states pharmacopeia convention,pp 3073.

Varaprasad A, Sriram N, Godwin Isaac Blessing A, Jawahar M & Thangamuthu S. (2012) Method development and validation of phenytoin sodium in bulk and its pharmaceutical dosage form by RP-HPLC method. Int J Bio Pharm Res;3:126-9.