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DEVELOPMENT AND VALIDATION OF A RP-HPLC METHOD FOR ESTIMATION OF PHENOBARBITONE IN A TABLET& BULK DOSAGE FORM

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ABSTRACT

The Present work was to develop a simple, fast, accurate, precise, reproducible, reverse phase high performance liquid chromatographic method for estimation of phenobarbitone in pharmaceutical tablet dosage form marketed as Phenobarbone. Chromatographic separation was done using Inertsil ODS RP C18 column having dimension of 4.6×250mm having particle size of 5µm, with mobile phase consisting of phosphate buffer pH 3 ±0.02 pH adjusted with ortho phosphoric acid and acetonitrile (50:50 %v/v), flow rate was adjusted to 1.0 ml/min and detection wavelength at 263nm. The retention time of Phenobarbitone 2.35 min. The Proposed method has been validated for accuracy, precision, linearity; range and robustness were within the acceptance limit according to ICH guidelines. Linearity for phenobarbitone was found in range of 25µg-150µg and correlation coefficient was found to be 0.999 %RSD for method precision was found to be 0.76 and for system precision was 0.80 respectively, % mean recovery for phenobarbitone was found to be 99.18% The method was found to be robust even by change in the mobile phase ±5% and in less flow condition

INTRODUCTION:

Phenobarbital, also known as phenobarbitone or phenobarb, is a medication recommended by the World Health Organization for the treatment of certain types of epilepsy in developing countries. In the developed world it is commonly used to treat seizures in young children while other medications are generally used in older children and adults. 5-ethyl-5-phenyl-2, 4, 6 (1H, 3H, 5H) - pyrimidinetrione. Mechanism of action through its action on GABA receptors, phenobarbital increases flux of chloride ions into the neuron which decreases excitability. Direct blockade of excitatory glutamate signalling is also believed to contribute to the hypnotic/anticonvulsant effect that is observed with the barbiturates. Phenobarbital is used in the treatment of all types of seizures except absence seizures. It is no less effective at seizure control

than phenytoin, however phenobarbital is not as well tolerated.]Phenobarbital may provide a clinical advantage over carbamazepinefor treating partial onset seizures.

Equipments and Reagents: Shimadzu – LC- 2010 CHT & prominence model with auto sampler equipped with UV-VIS detector with LC solutions software. Waters Alliance HPLC system with 2695 Separation module and 2487 dual wavelength detector with Empower software. Data handling system- LC solutions chromatographic software. Acetonitrile (HPLC Grade)**,** Potassium dihydrogen phosphate (LR Grade)**,** Sodium hydroxide (AR Grade)**,** Purified Water (Milli Q grade) Phenobarbitone Working Standard.

PROCEDURE:

Preparation of Buffer: Dissolved 20.4g of potassium dihydrogen phosphate in 3000ml of water and adjusted p^H to 5.0 ± 0.05 with dilute Sodium hydrogen solution.

Mobile Phase A: Mixed 1980ml of buffer and 20ml of Acetonitrile and mix thoroughly. **Mobile Phase B**: Mixed 800ml of buffer and 200ml of Acetonitrile and mix thoroughly. **Reference solution (a):** Dissolved 30mg of working standard in 50ml of mobile phase A. **Reference solution (b):** Dissolved 4.0mg of Cefadroxil working standard in 50ml of mobile phase A and further diluted 5ml and 5ml of reference solution A and make up to the 100ml with mobile phase A.

Reference solution (c): Transfer 2ml of reference solution (a) to 20ml with mobile phase A and further diluted of this solution 20ml and made up to the mark with mobile phase A**. Preparation of sample:** Weighed and dissolved 30mg of sample in 50ml of volumetric flask and made up to volume with mobile phase A.

Precision:

Preparation of stock solution: Accurately weigh and transfer 10 mg equivalent of Phenobarbitone 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 dilute 1ml stock sol to 10 ml. (stock Solution B)

Preparation of 30 µg/ml solution: Further pipette 3 ml of the above stock solution B into a 10ml volumetric flask and diluteup to the mark with diluent. Mix well and filter through 0.45µm filter

Procedure: The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate Precision: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions are shown in Table no: 1

Preparation of stock solution: Accurately weigh and transfer 10 mg equivalent of Phenobarbitone Working standard into a 10 mL 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 dilute 1ml stock sol to 10 ml. (stock Solution B).

Preparation of 30 µg/ml solution: Further pipette 3 ml of the above stock solution B into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter

Procedure: The standard solution was injected for six times and measured the area

for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy: Preparation of stock solution: Accurately weigh and transfer 10 mg equivalent of Phenobarbitone Working standard into a 10 mL 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)

Preparation Sample solutions: For preparation of 50% solution (With respect to target Assay concentration): Accurately weigh and transfer 5 mg of Phenobarbitone API sample into a 10 ml 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).

For preparation of 100% solution (With respect to target Assay concentration): Accurately weigh and transfer 10 mg of Phenobarbitone API sample into a 10 mL volumetric flask add about 7 ml of diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

For preparation of 150% solution (With respect to target Assay concentration): Accurately weigh and transfer 15 mg of Phenobarbitone API sample into a 10 mL 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.

Procedure: Take three volumetric flasks of 10ml and it can be labelled as Accuracy 50%, 100% and 150% ,to all the above flasks add 0.3ml of stock solution B and 0.3ml of Accuracy 50%,100% and 150% solutions to the respective volumetric flasks and make up the volume to 10ml with diluent. Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Phenobarbitone and calculate the individual recovery and mean recovery values.

LINEARITY

Preparation of stock solution: Accurately weigh and transfer 10mg of Phenobarbitone sample into a 10 mL volumetric flask and 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 dilute 1ml stock sol to 10 ml. (stock Solution B).

Preparation of Level – I (10µg/ml): 1.0 ml of stock solution B has taken in 10 ml of volumetric flask dilute up to the mark with diluent

Preparation of Level – II (20µg/ml): 2.0 ml of stock solution B has taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (30µg/ml):

3.0ml of stock solution taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (40µg/ml): 4.0 ml of stock solution B taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (50µg/ml): 5.0 ml of stock solution taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Procedure: Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient

Injection	Area
Injection-1	650042
Injection-2	646642
Injection-3	648021
Injection-4	643798
Injection-5	643467
Injection-6	646394
Average	2798.351
Standard Deviation	0.433
%RSD	**650042**

Table No: 1 Precision results showing of Phenobarbitone

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	338951	5.48	5.4	101.50%	99.33%
100%	609543	10.0	9.86	98.56%	
150%	908528	15.0	14.69	98%	

Table No: 2 Accuracy results showing of Phenobarbitone

S. No	Linearity Level	Concentration µg/ml	Area
1	I	10	229992
2	II	20	416046
3	III	30	653373
4	IV	40	800954
5	V	50	1034032
Correlation Coefficient			**0.998**

Table No: 3 Linearity results showing of Phenobarbitone

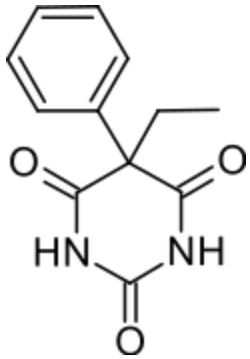

Fig No: 1 Structure of Phenobarbitone

CHROMATOGRAPHIC PARAMETERS:

Column	:	Hypersil ODS 250×4.6nm×5µ or equivalent
Flow rate	:	1.0ml/min
Detection	:	UV at 254nm
Injection volume	:	50µL
Run time	:	20min
Elution	:	Gradient
Column Temperature	:	Ambient
Run time	:	20 minutes

LIMIT OF DETECTION:

Preparation of 100µg/ml solution: Accurately weigh and transfer 10mg of Phenobarbitone Working standard into a 10 ml. Volumetric flasks 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 1.0 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.

Preparation of 0.1% solution At Specification level (0.052µg/ml solution): Further pipette 0.5 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**.** Pipette 0.1mL of abovesolution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

LIMIT OF QUANTIFICATION:

Preparation of 30µg/ml solution: Accurately weigh and transfer 10mg of Phenobarbitone Working standard into a 10 mL volumetric flasks 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 dilute 1ml stock solution to 10 ml. (stock Solution B). Further pipette 3 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

Preparation of 0.4% solution At Specification level (0.174µg/ml solution): Further pipette 0.45 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent .Pipette 0.4 mL of solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

ROBUSTNESS: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The Organic composition in the Mobile phase was varied from 60% to 80%. Standard solution 100 µg/ml was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

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