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Analytical Method Development for Simultanious Quantification of Ternary Mixture Containing Anticonvulsant Drugs by RP-HPLC Method

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Abstract :- A simple, accurate, rapid and precise isocratic reversed-phase high-performance liquid chromatographic method has been developed and validated for simultaneous determination of Gabapentin, Hesperidine and Fosphenytoin sodium in tablet. The chromatographic separation was carried out on an waters C18 analytical column (150×4.6 mm; 5 µm) with a mixture of water:acetonitrile pH 7.0 adjusted with *o*-phosphoric acid (80:20, v/v) as mobile phase; at a flow rate of 1 ml/min. UV detection was performed at 214 nm. The retention times were 5.2, 7.9 and 16.9 min. for Gabapentin, Hesperidine and Fosphenytoin sodium, respectively. Calibration plots were linear (r2>0.999) over the concentration range 60-180 µg/ml for all three drugs. The method was validated for accuracy, precision, specificity, linearity, and sensitivity. Validation studies revealed that method is specific, rapid, reliable, and reproducible. The high recovery and low relative standard deviation confirm the suitability of the method for routine determination of Gabapentin, Hesperidine and Fosphenytoin sodium in bulk drug and tablet dosage form.

***Key words**: Gabapentin, Hesperidine and Fosphenytoin sodium, RP-HPLC, Anti convulsant, ternary mixture (TM)*

I. INTRODUCTION

Analytical methods development and validation play important roles in the discovery, development, and manufacture of pharmaceuticals. Pharmaceutical products formulated with more than one drug, typically referred to as combination products, are intended to meet previously unmet patients need by combining the therapeutic effects of two or more drugs in one product.[1] These combination products can present daunting challenges to the analytical chemist responsible for the development and validation of analytical methods. There is a scope, therefore to develop newer analytical methods for such drugs. Also quality is important in every product or service but it is vital in medicines as it involves life. [2]

Analytical method development and validation is playing an important role in most of the pharmaceutical industries. The method validation should be very useful for industries when introduction of new methods for their routine work. For reporting of new toxicities and patient resistance method development and validation should be very important criteria in pharmacy field. Analytical methodology provides to an analyst the required data for a given analytical problem, sensitivity, accuracy, range of analysis, precision i.e. the minimum requirements which essentially are the specifications of the method for the intended purpose to be able to analyse the desired analyte in different matrices with surety and certainty. [3] Using multicomponent analysis (MCA), a technique that provides accurate quantitation and identification of unknown components based on known reference spectra, Multicomponent Analysis is a very powerful technique which provides accurate quantitative results from partially resolved peaks. The MCA process is fast and more accurate than conventional single channel integration techniques. In addition the purity assessment feature of MCA provides greater confirmation of peak identity and homogeneity than many other diode array purity evaluation techniques. [4]

The accuracy in spectral measurements, spectral pattern and the nature of the individual components are key factors for obtaining reliable results in the simultaneous spectrophotometric analysis of multi component mixtures. Another important factor contributing towards the precision of analysis of a mixture is relative composition of the individual components. Nevertheless, a higher absorbance in the case of a minor component will improve the precision. The selection of wavelengths in a ternary mixture may pose a little difficulty but it is always preferable to select such wavelengths which may fall at a maximum and where there is least slope of the other two compounds. [5] For each compound in ternary mixture, without searching for the critical point for the separated peaks, the maximum amplitude of the separated peaks can be measured, it can be considered as an advantage of the new method over alternative methods for the resolution of ternary mixtures. [6]

The Fosphenytoin sodium it is chemically known as 5,5-diphenyl-3-[(phosphonooxy) methyl] -2,4-imidazolidinedion disodium salt. This is act as anticonvulsant. Structure is shown in FIG NO.1 [7,8] .The Gabapentin chemically known as 1-(aminomethyl) cyclohexaneacetic acid also act as an anticonvulsant. Structure of Gabapentin is shown in FIG NO.2 [8,9] and the herbal drug Hesperidine chemically know as (23)-5-hydroxy-2-(3-hydroxy-4 methoxy phenyl) 7 [(2S,3R,4S,5S,6R)-3,4,5 trihydroxy-6-{[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy 6 methyloxan -yl]oxymethyl] oxan-2-yl}oxy-2,3-dihydrochromen-4-one. This is act as antioxidant and anticonvulsant also. The structure of Hesperidine shown in **FIG NO.3** [8,10]

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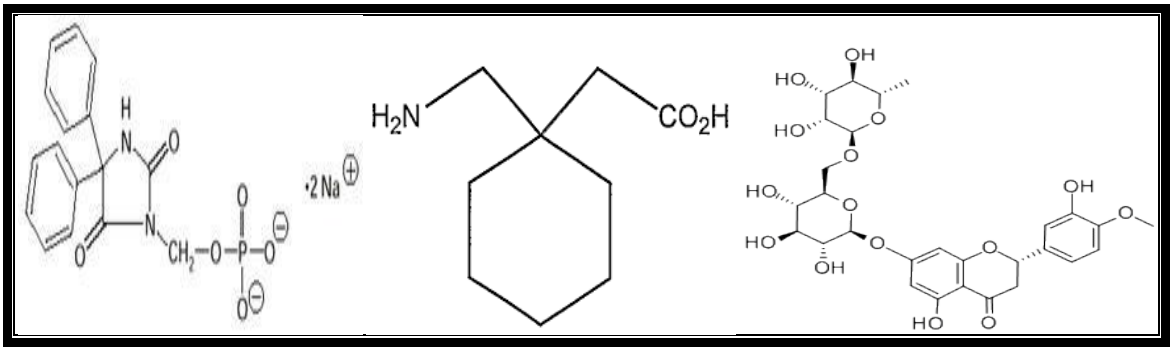
FIG NO.1	FIG NO.2	FIG NO.3



Thus, the aim of the present study is to develop a sensitive analytical method for the simultaneous estimation of three anticonvulsant drugs by Ternary Mixture analysis method along with their validation by RP-HPLC Method.

II. MATERIAL AND METHOD

A. Chemicals And Drugs

The active pharmaceutical ingredients Fosphenytoin sodium, Gabapentin and Hesperidine was obtained from Swapnroop Drugs and Pharmaceutical, Aurngabad. And the chemicals were obtained from Mylochem Mumbai.

B. Experimental Methods

- Selection of detection Wavelength

Solution of Fosphenytoin sodium, Gabapentin and Hesperidine (100µg/ml) was prepared in diluent. The λ max was determined on Agilent UV – visible spectrophotometer (Model Agilent carry 60) the range 200 – 800 nm. The overlay of three drugs are shown in fig no.4

- Selection and optimization of Mobile Phase

The standard solution of Fosphenytoin sodium, Gabapentin and Hesperidine was run and combination of solvent have been tried to get a symmetric and stable peak. Each mobile phase was filtered through 0.47 µ membrane filter. Details for selection of mobile phase are given in Table No. and Table no.1.

- System Suitability Test

System suitability is a Pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from six replicate injection of standard drug solution. See Table No. and Table no.2.

- Preparation of Standard solution

Weigh 12 mg Fosphenytoin sodium, 12 mg Gabapentin and 12 mg Hesperidine Transfer it into 10 ml volumetric flask, add 5 ml of methanol. Sonicate it to dissolve and make up the volume with methanol. Pipette out 1 ml from above solution and transfer in to 10 ml and make the volume with water.

- Preparation of Test solution

Weight an powder of 20 Tablets (lab made) each containing 12 mg Gabapentin,12 mg Hesperidine and 12 mg Fosphenytoin sodium and transfer to a 10 ml volumetric flask containing 5 ml methanol sonicate for 15 min then volume make up with methanol. Pipette out 1 ml from above solution and transfer in to 10 ml volumetric flask and make up the volume with water.

- Preparation of Mobile Phase

The mobile phase was consisting of 0.12% Orthophosphoric Acid and Acetonitrile in the ratio of 80:20%v/v. pH of resulting was 7. The solution was than filtered through 0.47 mm membrane filter and degassed.

- Analysis of pharmaceutical formulation

To determine the content of FOS, GAB and HESP in tablets containing 12 mg each, which were made in lab, Weight an powder of 20 Tablets (lab made) each containing 12 mg Gabapentin, 12 mg Hesperidine and 12 mg Fosphenytoin sodium and transfer to a 10 ml volumetric flask containing 5 ml methanol sonicate for 15 min then volume make up with methanol. Pipette out 1 ml from above solution and transfer in to 10 ml volumetric flask and make up the volume with water. This final solution was filtered through 0.47 mm membrane filter. Aliquots of 10ml of clear filtrate were injected into the RP-HPLC column. Results are shown in Table no.9.

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III. METHOD VALIDATION

The method was validated as per ICH guidelines. The method was validated in terms of linearity, specificity, accuracy, precision, Limit of Detection (LOD) and Limit of Quantification (LOQ) (11)

- Accuracy

Accuracy was determined by performing recovery studies by spiking different concentrations of pure drug at three levels i.e.50%, 100% and 150%. Percentage recovery for pure drug was calculated from differences between the peak areas obtained or fortified and unfortified solutions. The result of accuracy study is reported in Table no. 4

- Precision

By injecting six replicates of ternary mixture of three different concentrations (50,100and150µg/ml), on the same day and the values of relative standard deviation (R.S.D.) were calculated to determine intra-day precision. These studies were also repeated on different days to determine inter-day precision. The data obtained from precision experiments are given in Table no.5 and Table no.6 for intra-day and inter-day respectively.

- Linearity

Weigh 12 mg Fosphenytoin sodium, 12 mg Gabapentin and 12 mg Hesperidine Transfer it into 10 ml volumetric flask, add 5 ml of methanol. Sonicate it to dissolve and make up the volume with methanol. Pipette out 1 ml from above solution and transfer in to 10 ml and make the volume with water. 20 µl of each of these standard solutions ranging from 80 to 180 µg/ml were injected into a chromatograph at flow rate of 1 ml/min. Retention time and peak area obtained were recorded and standard calibration curve was plotted and linearity equations were derived. The Correlation coefficient, % curve fitting were also calculated. Result are shown in fig no. 6,8 and 9

- Limit of Detection and Limit of Quantisation

Using linearity data LOD and LOQ was calculated by Steyx Method. Results are shown in Table no.3

- Specificity

The specificity of the RP-HPLC method was checked by comparison of chromatogram obtained from standard and sample. Results are shown in Table no.7

- Robustness

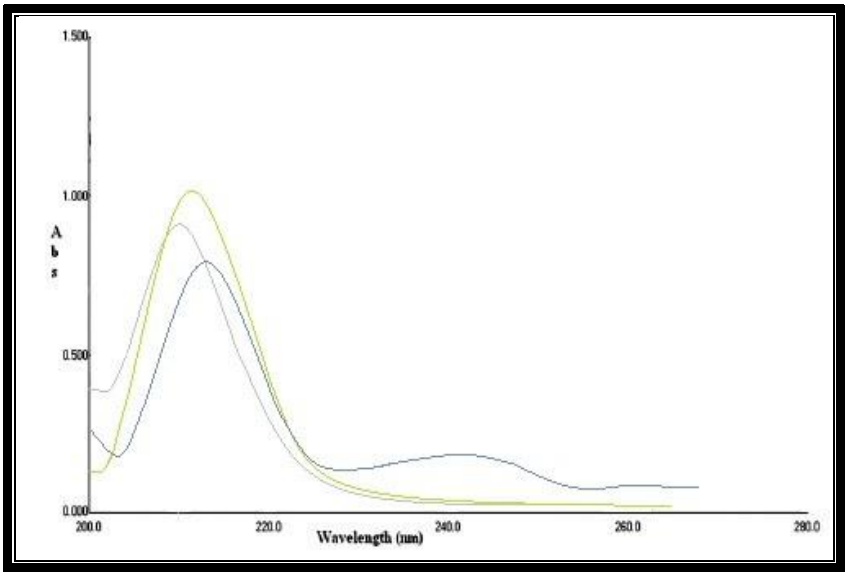
The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The robustness of a method is evaluated by varying method parameters such as mobile phase composition, pH, etc., and determining the effect (if any) on the results of the method. Result are shown in Table no.8

IV. RESULT AND DISSCUSION

A. Selection of Detection Wavelength

The λ max value was found to be for Fosphenytoin sodium 214nm, Gabapentin 215 nm and Hesperidine 216 Using data 214 nm wavelength was selected at which all three components give well resolved peaks.

Fig No: 4. Overlay of UV spectrum of GAB, HES and FOS



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B. Selection of Mobile Phase

From the various mobile phase tried, mobile phase containing Orthophosphoric acid in Water: Acetonitrile (80:20) %v/v was selected, since it gave sharp peaks with symmetry within limits and significant retention time.

C. Optimized Chromatographic conditions

Table No. : 1. Optimized Chromatographic conditions

Chromatograph	**Agilent 1200HPLC system**
Mobile phase	0.12% Orthophosphoric acid in Water : Acetonitrile (80:20%v/v)
Column	Waters- C_{18} column(150 mm × 4.6 mm, 5 µm particle size)
Detector	VWD
Flow rate	1 ml/min
Wavelength detection	214 nm
Injection volume	20µl
Temperature	Ambient
Run time	20 min
Diluent	Methanol,Water

Table no. 2 result of System Suitability

Sr. No.	Parameters	GAB	HES	FOS
1.	Area(mAU*)	50.90	2149.33	1457.20
2.	Retention Time(min)	5.219	7.94	16.932
3	Theoretical plates	6128.73	7923.51	11738.36

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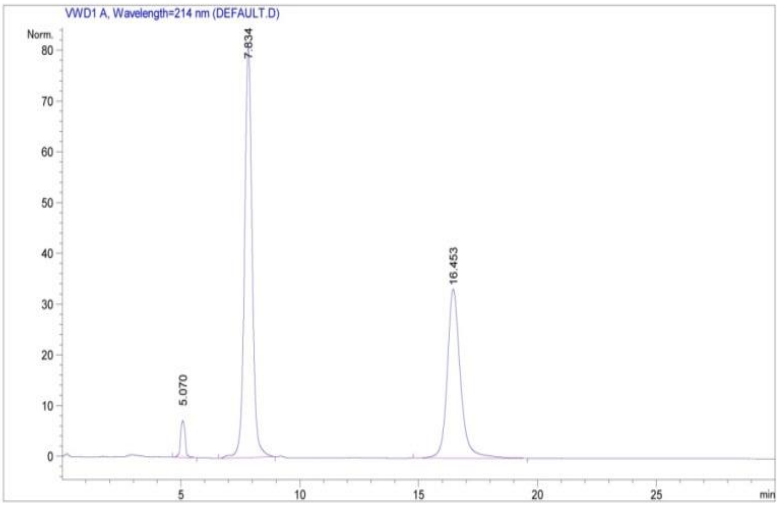
Fig no.5 Chromatogram of standerd Ternary Mixture



D. Linearity

The linearity response for Gabapentin, Fosphenytoin sodium and Hesperidine were observed in the concentration range of 80-180µg/ml for each the drugs respectively, with Correlation coefficient, percentage curve fittings found to be well within the acceptance criteria limit (See Fig. 6,7,8).

Fig. No. : 6.Linearity graph of Gabapentin

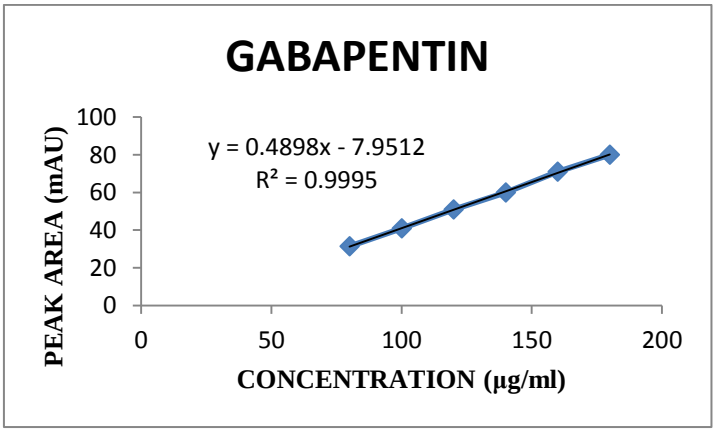


Fig no: 7. Linearity graph of Hesperidine

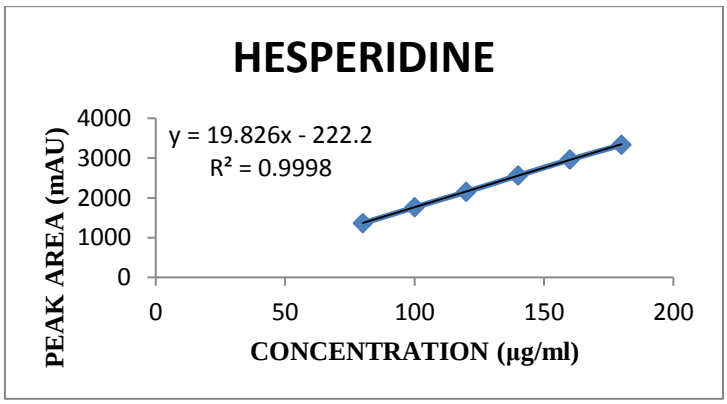


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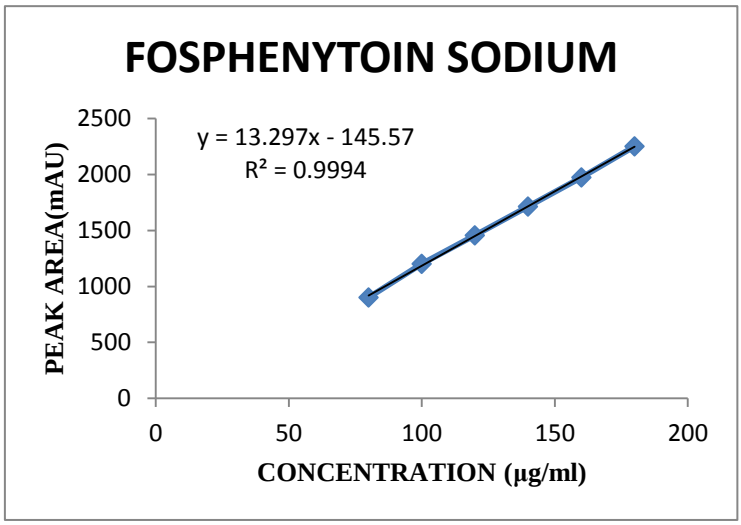
Fig. No. : 8. Linearity graph of Fosphenytoin sodium



E. LOD and LOQ

Table No. : 3 result of LOD & LOQ

Parameter	Gabapentin (µg/ml)	Hesperidine (µg/ml)	Fosphenytoin sodium(µg/ml)
LOD	3.17	2.01	3.28
LOQ	9.79	6.11	9.96

F. Accuracy

Table No.: 4. Result of accuracy study

Drug	% Level	Amount added (µg/ml)	Amount found (µg/ml)	% recovery	% mean recovery	Stdev	% RSD
GAB	50	59.99	59.97	99.96	99.93	0.030	0.03
	100	120.02	119.9	99.90			
	150	180.00	179.9	99.94			
HES	50	59.97	59.99	100.3	100.3	0.248	0.24
	100	120.02	119.8	99.81			
	150	179.97	179.95	99.98			
FOS	50	60.01	60.0	99.98	99.98	0.005	0.005
	100	120.02	120.01	99.99			
	150	179.98	179.97	99.99			

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G. Precision

Table No.: 5. Summary of Intraday Precision

TM level %	Intraday %RSD					
	at 9.00 AM			at 2.00 PM		
	Gab	Hes	Fos	Gab	Hes	Fos
50	0.003	0.009	0.004	0.001	0.001	0.004
100	0.0005	0.0006	0.02	0.0007	0.0004	0.01
150	0.023	0.0009	0.001	0.024	0.0007	0.0006

Table No.: 6. Summary of Inter day Precision

TM level %	Inter day %RSD					
	Day 1			Day 2		
	Gab	Hes	Fos	Gab	Hes	Fos
50	0.009	0.0009	0.004	0.004	0.001	0.001
100	0.0005	0.002	0.02	0.0005	0.002	0.02
150	0.024	0.0009	0.001	0.023	0.0009	0.0008

H. Specificity

Table No: 7. Result of Specificity studies

TM Conc.(µg/ml)	Drug	Area (mAU*)	
		Std	Test
100	Gab	40.9036	41.0231
	Hes	1762.17	1794.8
	Fos	1201.70	1276.36

I. Robustness

Table No. : 8. Summary of Robustness

TM Level %	sample	%RSD (Mobile phase ratio)		%RSD (pH)	
		(75:25)%v/v	(85:15)%v/v	6.8	7.2
100	GAB	0.04	0.06	0.05	0.001
	HES	0.0004	0.0004	0.0004	0.002
	FOS	0.017	0.01	0.01	0.02

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J. Analysis of Pharmaceutical Formulation: (lab made tablet)

Table No. : 9 Result of Analysis of Pharmaceutical Formulation

Drug	**Label containing (mg/tablet)**	**Amount found (mg/tablet)**	**% Assay**
Gab	12	11.89	99.08
Hes	12	11.9	99.16
Fos	12	11.96	99.66

The proposed method was applied successfully for the analysis of GAB, HES and FOS in tablet dosage forms, satisfactory results were obtained.

V. CONCLUSION

The developed RP-HPLC method for the simultaneous estimation ternary mixture was accurate, precise, linear and reproducible. This makes the methods suitable for routine analysis of the combination product in quality control laboratories.

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