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New RP - HPLC Method for the Determination of Valproic acid in Human Plasma

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Abstract:
A simple reverse phase HPLC method was developed for the determination of Valproic acid present in human plasma. A Denali, Grace, C18, 4.6 X 150mm, 5µ in an isocratic mode with mobile phase containing 40mM Ammonium dihydrogen phosphate buffer (pH 4.3 ± 0.4): Acetonitrile (30:70 v/v) was used. The flow rate was 1.2 ml/ min and va;proic acid was monitored at 254 nm. Nanonic acid was used as the internal standard. The retention times of Valproic acid and Nonanoic acid are 9.5 ± 1.5 minutes and 14.0 ± 2.0 minutes. The method was established for the calibration range of 0.500 to 80.000 µg/mL and the method was validated for Accuracy, Precision, specificity.
Key words: *Valproic Acid, Nanoic Acid, HPLC and Plasma*

Introduction:

Valproic acid is mainly indicated for the treatment of epilepsy and other neurological disorders.After oral administration, the drug is rapidly absorbed from the gastrointestinal tract and metabolized in the liver. Fatal hepatic failure has been reported in patients on valproic acid therapy, especially those on chronic use. Central nervous system depression and convulsions may occur. The drug crosses the placental barrier and has been found in breastmilk. Pancreatitis has also been reported, usually seen in patients receiving normal therapeutic dosage.Reports showed that acute toxicity is rare, and usually follows a benign course (Ellenhorn, 1988). Fatal hepatic failure is usually seen following chronic use of valproic acid. The most commonly reported adverse effects are anorexia, nausea and vomiting.

Valproic acid may be synthesized from 4-heptanol by successive conversions to 4-bromoheptane with HBr, to 4-cyanoheptane with HCN and to 2-propyl pentanoic (valproic) acid by alkaline hydrolysis of the 4-cyanoheptane (Gennaro,1985).

The routine therapeutic monitoring of anticonvulsant drug concentrations in serum or plasma is necessary for the management of epilepsy treatment. In epileptic patients, a minimal, effective level of drug is maintained to assess compliance and to optimize the therapy.

Materials and Methods:

Chemicals and Reagents

Valproic acid and Nonanoic acid are qualified working standards and are obtained commercially. All other reagents like 2,4 Dibromo acetophenone(Synthesis), 18-Crown ether (Synthesis), Acetonitrile (HPLC grade), Ammonium dihydrogen phosphate (AR grade), Disodium hydrogen phosphate (GR grade), Potassium dihydrogen phosphate (GR grade) are of reagent grade. Human plasma was obtained from a commercial supplier.

Apparatus and Chromatographic Conditions

HPLC analysis was performed on Shimadzu HPLC system equipped with a Photo Diode Array Detector. Separations were carried on a Denali, Grace, C18, 4.6 X 150mm, 5µm column using isocratic elution. The flow rate was 1.2 mL/min. UV detection was performed at 254 nm. Peak identity was confirmed by retention time comparison and the HPLC was operated at room temperature.

Preparation of Solutions

40mM Ammonium dihydrogen phosphate buffer (pH 4.3 ± 0.5)

Weigh about 4.6 gm of Ammonium dihydrogen phosphate and transfer into a 1000mL reagent bottle and dissolve in 1000mL of Milli-Q water. Sonicate for 5 minutes, check the pH of the solution, it shall be 4.3 ± 0.5. Filter the solution through

0.45µm membrane filter. Store the solution at room temperature and use within 3 days from the date of preparation.

Phosphate buffer (pH 7.0 ± 0.5)

Weigh about 1.94gm of Potassium dihydrogen phosphate, 3.94gm of Disodium hydrogen phosphate and transfer into a 100mL reagent bottle and dissolve in 100mL of Milli-Q water. Sonicate for 5 minutes, check the pH of the solution, it shall be 7.0 ± 0.5. Store the solution at room temperature and use within 3 days from the date of preparation.

Derivatizing reagent

Weigh about 0.5gm of Crown ether, 1.0gm of 2,4 Dibromo acetophenone and transfer into a 50mL reagent bottle and dissolve in 50mL of Acetonitrile. Sonicate for 5 minutes. Store the solution at room temperature and use within 15 days from the date of preparation.

Rinsing solution

Transfer 500mL of Acetonitrile into a reagent bottle and add 500mL of Milli-Q water mix and sonicate for 5 minutes. Provide a batch number. Store the solution at room temperature and use within 7 days from the date of preparation.

Preparation of Drug stock solution (w/v)

Weigh about 100mg of Valproic acid working standard and transfer it into a 10mL volumetric flask, mix with about 5mL of Acetonitrile and make up the volume with the same to get 10.0mg/mL concentration solution. Correct the final concentration of Valproic acid accounting for its potency and actual amount weighed. Provide a batch number. Store the stock solution in the refrigerator at below 10°C.

Preparation of Internal standard stock solution (w/v)

Weigh about 100mg of Nonanoic acid working standard and transfer it into a 50mL volumetric flask, mix with about 10mL of Acetonitrile and make up the volume with the same to get 2.0mg/mL concentration solution. Correct the final concentration of Nonanoic acid accounting for its potency and actual amount weighed. Store the stock solution in the refrigerator at below 10°C.

Drug stock dilution

Transfer 0.039mL of Valproic acid (10mg/mL) stock solution into a 5mL volumetric flask and make up the volume with Acetonitrile. Mix and provide a batch number. Store the solution at room temperature and use a fresh dilution daily.

Internal standard dilution

Transfer 0.375mL of Nonanoic acid (2mg/mL) stock solution into a 25mL volumetric flask and make up the volume with Acetonitrile. Store the solution at room temperature and use fresh dilution daily.

Preparation of Spiking Solutions

Prepare CC spiking solutions from Valproic acid CC stock solution and these spiking solutions are spiked in to the plasma to get the calibration curve range of 80.000µg/mL to 0.501µg/mL.

Results and Discussion:

Accuracy:

Typical chromatogram of Valproic acid and nanoic acid was represented in figure 1. To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (HQCSS, MQCSS, LQCSS, and LOQQCSS) of samples of along with internal standard within the linearity ranges were taken and the results were shown in Table 1.

Linearity:

The linear fit of the system was illustrated graphically in calibration curve. Least square regression analysis was carried out for the slope, intercept and correlation coefficient found acceptable as per regulatory requirements. The calibration curve was represented in figure 2.

Precision:

The precision of the method was ascertained separately from the peak area ratios obtained by actual determination of eight replicates of

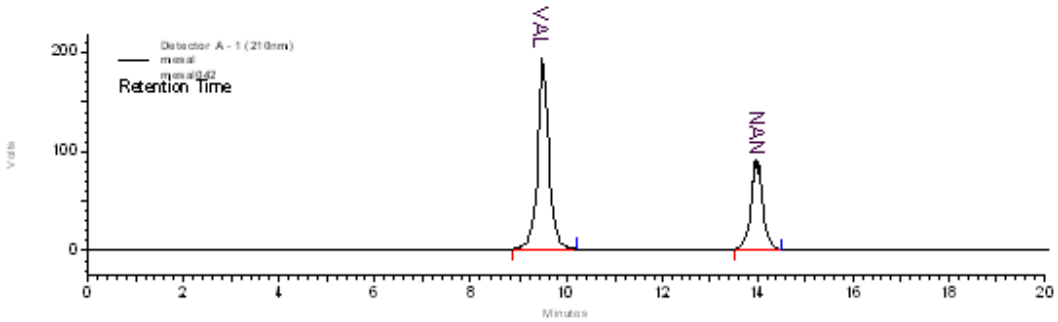

Figure 1: Typical Chromatogram Of Valproic Acid

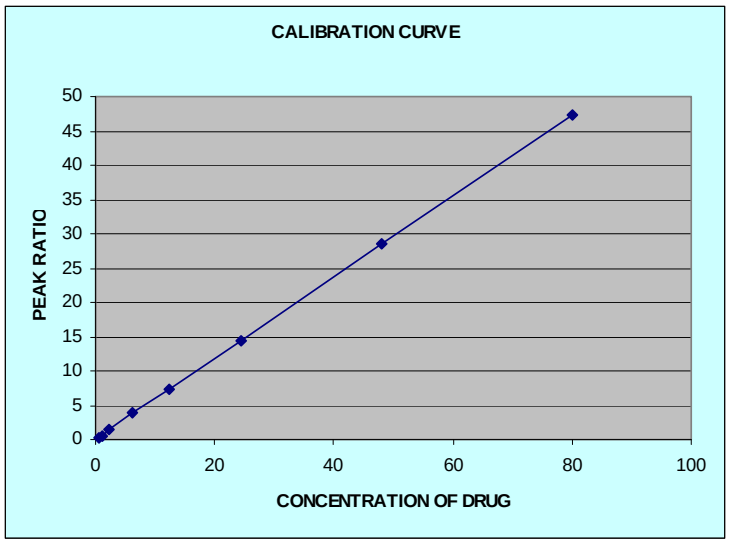

Figure2: Calibration Curve

Table 1: Accuracy

Sample ID	Peak area ratio (drug/I.S.)	Statistical Analysis	
HQCSS	34.94288	Mean	38.04517
HQCSS	38.24538	SD	3.01
HQCSS	40.94725	% RSD	**7.90**
MQCSS	20.43227	Mean	21.6206
MQCSS	24.16855	SD	2.21
MQCSS	20.26098	% RSD	**10.21**
LQCSS	0.97301	Mean	0.931537
LQCSS	1.01356	SD	0.11
LQCSS	0.80804	% RSD	**11.69**
LOQQCSS	0.27199	Mean	0.32051
LOQQCSS	0.33718	SD	0.04
LOQQCSS	0.35236	% RSD	**13.32**

Table 2: Precision

S.No.	Concentration (µg/ml)	Retention time	Statistical analysis
1	12.485	9.52	
2.	12.485	9.57	**Mean:** 9.55
3.	12.485	9.54	
4.	12.485	9.56	**SD:** 0.017548
5.	12.485	9.55	
6.	12.485	9.57	**%RSD:** 0.18
7.	12.485	9.56	
8.	12.485	9.53	

Table 3: System suitability parameters

S.No	Parameters	Obtained Values
1.	Theoretical plates (N)	2959
2.	Resolution (R) between drug and I.S.	3.75
3.	Tailing factor (T)	0.924

a fixed amount of drug and internal standard. The percent relative standard deviations were calculated for Valproic acid and presented in the table 1.4.

Specificity:

Specificity is the ability to measure accurately and specifically the analyte of interest in the presence of other components that may be expected to present in the sample matrix. It was found that the proposed method was specific as there is no interference from any of the expected endogenous substances.

System suitability parameters

System suitability parameters are defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The requirements for system suitability are usually developed after method development and validation has been completed. (or) The USP (2000) defines parameters that can be used to determine system suitability prior to analysis. The system suitability parameters like Theoretical plates (N), Resolution (R), Tailing factor (T) were calculated and were compared with the standard values to to confirm the suitability of the developed method. Theoretical plates, resolution and tailing factor values were represented in table 3.

Conclusion:

The calibration curve was drawn between 80.000 (µg/mL) and 0.501(µg/mL) and for this curve correlation coefficient was found to be 0.99998. The proposed method was also validated for Accuracy, Linearity, Precision, Specificity, and System suitability parameters. For accuracy the percentage RSD was found to be 7.90%, 10.21%, 11.69% and 13.32% and the limit is %RSD should not be more than 15%. As the calibration curve correlation is 0.99998 this method is linear. %RSD for retention time of Valproic acid is 0.18% so the method is Precision. It was found that the proposed method was specific as there is no interference. System suitability parameters

like theoretical plates, resolution and tailing factor are within the limits.

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