RESEARCH ARTICLE Am. J. PharmTech Res. 2016; 6(6) ISSN: 2249-3387

AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

RP- HPLC Method Development and Validation for the Determination of Valproic Acid In Pharmaceutical Dosage Form and Human Plasma

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ABSTRACT

This research paper describes a procedure for determination of valproic acid (VA) in human serum using salicylic acid (SA) as internal standard (I.S.). The two drugs were separated by using 60% acetonitrile and 40% acidified water with phosphoric acid adjusted pH to 3 ±0.1 as mobile phase. The column employed was C_{18} 150×4.65µm at ambient temperature. Detection wave length, 220nm, flow rate 1ml/min. injection volume,20µL The method was linear over the range of 10-100ug/ml valproic acid. The validated limit of quantification and the limit of detection for valproic acid were11,1 and 1.11ug/ml, respectively. The precision of the method was evaluated and the intra and the interday precision were found to be 0.2 and 2.0, respectively. The method is simple rapid, sensitive, robust and accurate and can be used for therapeutic drug monitoring (TDM).

Keywords: Valproic acid, method development, method validation. Internal standard Salicylic acid.

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Received 21 September 2016, Accepted 27 September 2016

Please cite this article as: Elhag DE *et al.*,- HPLC Method Development and Validation for the Determination of Valproic Acid In Pharmaceutical Dosage Form and Human Plasma . American Journal of PharmTech Research 2016.

INTRODUCTION

Chromatographic methods for the quantitative determination of drugs and their metabolites in biological samples play a significant role in the evaluation and interpretation of bioavailability, bioequivalence and pharmacokinetic data. Throughout the drug development process, method validation is carried out to ensure that an analytical method is accurate, specific, reproducible and rugged over the specific range in which the analyte will be analyzed.

Valprioc acid or 2-propylpenanoic acid is antiepliptic drug used to treat bipolar disorder. Other uses include treatment of certain types of seizure. This drug can be used on its own or as a combination with other anti convulsants drugs to treat epissodes of frenzied mood or seizure. In some cases it can be used for treatment of migraine headache. The chemical structure of valproic acid is shown in Figure 1.

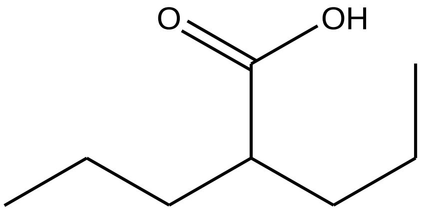

Figure 1: Valproic acid

Several methods had been introduced by employing different analytical techniques such as HPLC, employing different detectors e.g. UV, fluorescence or mass spectrometry detectors[1-8]. The newly introduced method offers simplicity, rapidity, precision and accuracy due to the virtue of the use of internal standard which eliminates the problems of detector drift.

MATERIALS AND METHOD

Chemical and reagent

Valproic acid standard was a donation from Shangahai Pharmaceutical Company, Sudan, Salicylic acid (sd FCL, India),Acetonitrile (Carlo Erba (France), Phosphoric acid,(Lab ware (India) acetonitrile, maize and starch and titanium dioxide (gift from GMC, Sudan) .

Mobile phase preparation:

60% acetonitrile 40% acidified water with phosphoric acid adjusting PH at 3 ±0.1. Isocratic mode was used.

Preparation of valproic acid standard stock solutions

An exact weight of 50 mg of valproic acid standard was weighed and transferred into 50ml volumetric flask to give a stock solution of a concentration of 1000ug/ml. From this stock solution, a series of solution of 10, 20, 30, 40, 50, 60 and 80 and 100 ug/ml were prepared to construct the calibration curve.

Preparation of salicylic acid standard stock solutions (internal standard)

Exactly 50 mg of *salicylic acid* standard was weighed and transferred into 50ml volumetric flask to give a stock solution of a concentration of 1000ug/ml. 1ml of this solution was pipetted into 10 ml volumetric flask and the volume was completed to the mark to give a solution of a concentration of 100ug/ml. A volume of 50ul of this solution were pipette into 10ml volumetric flask and was completed with the mobile phase to the mark to give a solution of a concentration of 0.5ug/ml.

Sample preparation

Samples of 0.2 µl of plasma were taken. To this 250, 500 and 600ul of valproic acid stock solution were added in separate 10ml tubes. 50ul of salicylic acid was added to each volumetric flask and each solution was mixed and vortexed for 1minute. 2ml of acetonitrile were also added. Exactly50ul of the internal standard (salicylic acid) were added to each tube and then the solutions were completed to 10ml with acetonitrile and centrifuged at 3000. Then 20ul of the upper organic layer injected in the instrument.

RESULTS AND DISCUSSION

Method development

Method development process involves many trials for producing ideal chromatograms in terms of peak symmetry, number of theoretical plates, minimal or noise- free and tailing factor.9

The aims of introduction of a new method for drug analysis are numerous. Examples of the basic criteria for introducing a new method may include one of the following: lack of an official method in the pharmacopeia or the absence of a method to analyse the drug in combination with other drugs or the high cost of an existing method.

As per USP (United States Pharmacopeia), validation of an analytical method is the process by which it is established by means of experimental studies, that the performance of the method meets the requirements for the intended analytical application. Performance is expressed in terms of analytical parameters which include for assay validation the determination of precision, accuracy, limit of detection, limit of quantitation, solution stability, selectivity, range, linearity and ruggedness

System Suitability

Before performing the main analysis, the system suitability was evaluated. The parameters investigated at this stage were tailing factor, relative standard deviation of peak area and retention time For this purpose, The results obtained are shown in Table1 and the chromatogram of the two drugs are shown in Table1 and Figure 2.

Table 1: System suitability parameters

Parameter	VA	SA
Tailing factor	1.2	1.4
No. of Theoretical plates	7180	4511
Retention time	4.1 min	2.6min
RSD	0.74	0.99

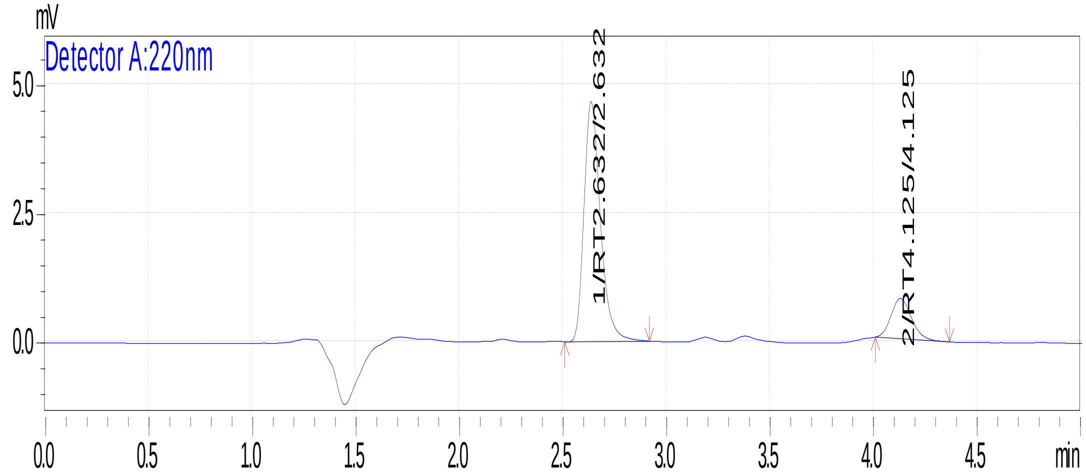

Figure 2: The chromatogram of valproic acid and salicylic acid

The USP chromatography General Chapter States" "System suitability tests are an integral part of gas and liquid chromatographic methods. They are used to verify that the resolution and the reproducibility of the chromatographic system are adequate for the analysis to be done. The tests are based on the concept that the equipment, electronics, analytical operations and the sample to be analyzed constitute an integral system that can be evaluated as such".

Linearity and range

The Linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. For the establishment of Linearity, a minimum of 5 concentrations is recommended. The linearity of the method was investigated by measuring nine solutions in the range of 10-100µg/ml for valproic acid. The obtained calibration curve is shown in Figure 3. Also another calibration curve for valproic acid and salicylic acid was plotted and shown in Figure 4.The y xis represents (VA peak area/SApeak) area and the x-axis represents the conc of (VA/conc of SA).

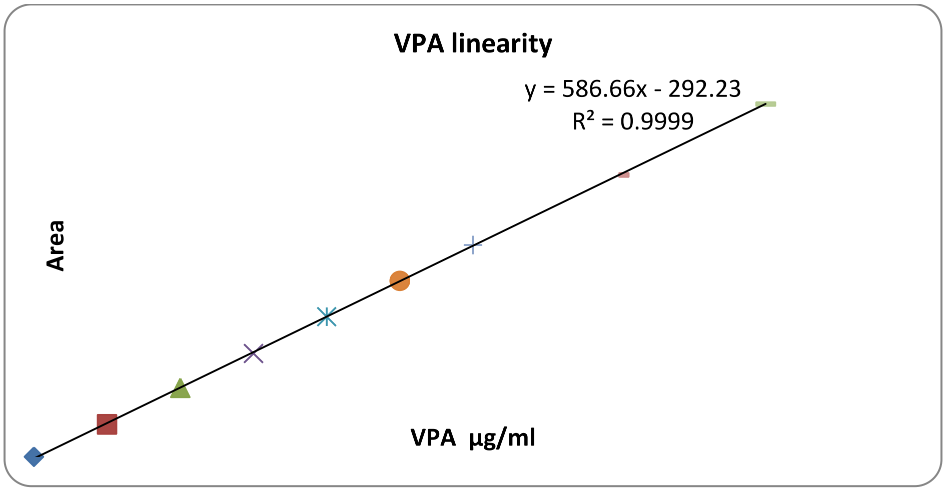

Figure 3:Calibration curve of valproic acid

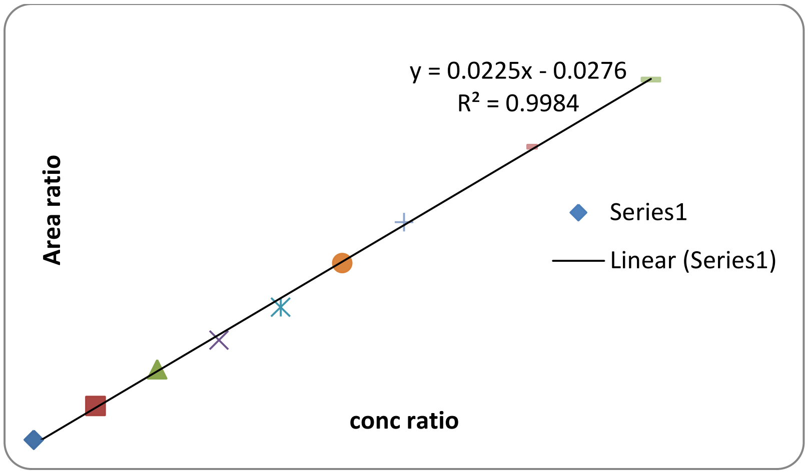

Figure 4: Calibration curve obtained by plotting thea peak area ratio of valproic acid to salicylic acid Vs their respective conc ratios

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

The intra-day and inter-day precisions of the proposed methods were determined by estimating the corresponding responses 9 times on the same day and on 3 different days, for one concentrations of 50 µg/ml valproic acid and 0.5ug/ml salicylic acid. Method repeatability (intra-day precision) was evaluated and the results are shown in Table 2.

Table 2: Intra-day precision test results

Day 1	No	VA area	SA area	area ratio	VA µg/mL	SAµg/mL
	1	29336	17867	1.6419	50	0.5
	2	29623	18028	1.6432	50	0.5
	3	29630	18058	1.6408	50	0.5
	4	29730	17754	1.6746	50	0.5
	5	29639	17940	1.6521	50	0.5
	6	29741	18018	1.6506	50	0.5
Average		29616.5				
SD		146.8				
RSD		0.4				
Day 2	1	30408	26768	1.1360	50	0.5
	2	29945	26217	1.1422	50	0.5
	3	30057	26443	1.1368	50	0.5
	4	29858	26581	1.1233	50	0.5
	5	29810	27262	1.0935	50	0.5
	6	30131	27031	1.1149	50	0.5
Average		30034.8				
SD		218.58				
RSD		0.70				
Day 3	1	31084	26916	1.1549	50	0.5
	2	31129	26886	1.1578	50	0.5
	3	30912	26993	1.1452	50	0.5
	4	30928	27572	1.1217	50	0.5
	5	31003	27427	1.1304	50	0.5
	6	31026	27258	1.1382	50	0.5
Average		310131				
SD		85.1				
RSD		0.3				

The interday precision was estimated by using the average peak area for measurements conducted over three days showed that the RSD was 2.0 which within the range according to the ICH guidelines.

Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution. The ICH guidelines state that repeatability must be assessed using a minimum of nine determination covering the specified range for the procedure under the same operating conditions .e.g. analyst, instrument over a short period of time or a minimum of 6 determinations at 100% of the test concentration.

Accuracy and recovery

Accuracy is the measure of exactness of an analytical method, or the closeness of agreement between the measured value and the value that is accepted as a conventional true value or an accepted reference value. The accuracy was evaluated by adding known amount of valproic acid at three different levels (80%, 100%, 120% level) to the tablet solution (50ug/ml) and then assayed. The results are shown in Table 3and Table 4

Table 3: The results of the accuracy test

Con cug/ml	practical W	theoretical W	recovery %	recovery AVG
80%	37.7241	40	94.31	
80%	36.0912	40	90.23	
80%	35.5744	40	88.94	
100%	48.6464	50	97.29	
100%	47.6905	50	95.38	
100%	48.9545	50	97.91	96.9
120%	60.2857	60	100.48	
120%	61.3858	60	102.31	
120%	60.7886	60	101.31	101.4

Blank human plasma was spiked with 25, 50 and 60ug/ml valproic acid and the solution was treated as described in the experimental part. 20ul of the final solution was injected into the instrument to estimate the recovery and the results obtained are shown in Table.

Table 4: The results of the accuracy test using human plasma

Con cug/ml		practical W	recovery %	Average recovery %
25	plasma 50% 1	23.51548	94.06	
	plasma 50% 2	23.5424	94.17	93.9
	plasma 50% 3	23.4210	93.69	
50	plasma 100% 1	47.3276	94.66	
	plasma 100% 2	47.6033	95.26	94.9
	plasma 100% 3	47.2405	94.48	
60	plasma 120% 1	57.1824	95.30	
	plasma 120% 2	56.6389	94.40	95.5
	plasma 120% 3	58.0572	96.76	

From the Table 4, it could be observed that the recovery of the drug from the expients is higher than recovery % from the plasma and this is due to the complex nature of the plasma matrix. However, accuracy may be inferred once precision, linearity and specificity have been established.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of sample matrix components. Such components include expients or degradation products or impurities, etc. Lack of specificity of an individual analytical procedure may render the method invalid.

Placebo sample solutions were prepared in triplicate by taking a weight of placebo approximately equivalent to its weight in the tablet. Chromatograms of placebo solutions have shown no peaks at the retention time of valproic and salicylic acids. This indicates that the excipients used in the formulation do not interfere in estimation of valproic acid tablets. Figure 5 shows the chromatogram of valproic acid excipients.

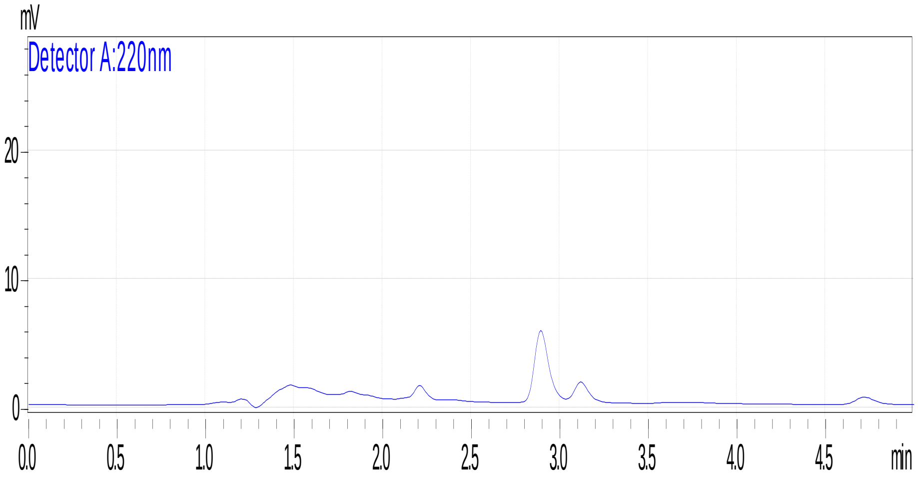

Figure 5: chromatogram of placepo

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 evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. Temperature, wavelength, pH and the flow rate were the parameters used to investigate the robustness of the method and the results obtained are shown in Table of valproic acid.

Parameter	RSD	No. of Theoretical plates	Tailing factor	Retention time
Temp 32^{0}C	0.57	7124	1.3	4.1
pH	0.8	7143	1.29	4.1
Wavelength(nm)	1.4	07139	1.29	4.1
Flow rate	0.7	6625	1.3	3.7

If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure. Establishment of the robustness of the method ensures that -in addition to the other parameters- the proposed method is does fit the purpose of analysis. From the results shown in Table it could be concluded that the method is unaffected by minor variation in the operating conditions.

Limit of detection and limit of quantitation

The limit of detection and the limit of quantification of the drug were calculated using the following equations.

Limit of detection = 3.3SD/S.

SD = the standard deviation of the response,

(S) = the slope of the calibration curve

Limit of quantitation = 10SD/S.

Based on the above equations, the limit of detection and limit of quantitation for valproic acid were found to be 1.11 and 11.10ug/ml.

Solution Stability

The stability of the standard solution of the drug and the internal standard was investigated by storing the stock of the two solutions for six hours. No significant changes were observed in the results.

Application of the Method to Tablets

The method was used for determination valproic acid in tablet formulation The proposed method was also applied to the pharmaceutical dosage valproic acid tablet. For this purpose 3 batches were selected and 6 replicates of each batch were analyzed by the HPLC, from the results, it was observed that the obtained results are in good agreement with the claimed amount of valproic acid.

DISCUSSION

Method validation is the process of demonstrating that analytical procedures are suitable for their intended use and that they support the identity, strength, quality, purity and potency of the drug substances and drug products. Method validation is primarily concerned with identification of the sources of potential errors quantification of the potential errors in the method

Internal standard calibration involves the comparison of the instrument responses from the target compounds in the sample to the responses of reference standards added to the sample or sample extract before injection. The peak area of the drug or the substance to be determined is normalized to the peak area of the reference standard. One direct consequence or benefit is the elimination of the detector drift between measurements and thus leading to more accurate data. in other words, it accounts for routine variation in the response of the chromatographic system

The use of internal standard in method development is a further guarantee of the accuracy and the validity of the method as it provides the following advantages; different methods are used to obtain quantitative chromatographic data. The choice of the internal standard is not trivial and among the considerations of the choice is the absence of any chemical reaction between the analyte and the internal standard. Secondly well resolved peaks must be obtained by the proposed method. Equally important, internal standard does compensate for the irreproducibility in the injected volume and which implies that minimization of the uncertainty associated with the measurements.

CONCLUSION

In the entire experimental work, a new method for determination of valproic acid in the tablet dosage form and plasma was introduced and validated. All the testing results were significant and in good agreement with the requirements of the ICH guidelines.

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