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ORIGINAL ARTICLE **OPEN ACCESS**

Development and Validation of Stability Indicating Assay Method Using RP-HPLC For Determination of Antiepileptic Drugs in Presence of Their Degraded Product

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ABSTRACT

A sensitive, exact, rapid, avaricious and robust RP-HPLC method was developed for the quantification of Sodium Valproate (SV) and Valproic Acid (VA) with DAD detector. In this method, a reversed-phase Fortis C18 (100mm x 4.6ID, Particle size: 2.5 micron) column with a mobile phase of Acetonitrile: (0.1% OPA, PH 2.7) (80:20; v/v) at 0.7ml/min flow rate was used to separate SV and VA with a detection of 210nm.The volume injected was 20 µL. The retention time of VA and SV was obtained as 1.537min and 4.910min respectively. All necessary validation parameters and system suitability tests were carried out in details. The analytical curve was linear (r^2 = 0.999) over a wide concentration range of VA and SV (14.5 -87 µg/ml) and (33.3 -199.8µg/ml). The system shows adequate accuracy with relative standard deviation less than 2.0%. The method showed good duplicability and recovery with % RSD less than 2%. So, the proposed system was found to be simple, specific, precise, accuracy, linear, and rugged. Hence it can be applied for practice analysis of Sodium Valproate (SV) and Valproic Acid (VA) in bulk drug.

Keywords: *RP-HPLC estimation, DAD, Validation, Sodium Valproate, Valproic Acid.*

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INTRODUCTION

Analytical chemistry termed as science of determining the components of materials in terms of the elements or compound contained. The approach of this science is used to recognize the substances which may be present in a material and to determine the exact amounts of the identified substances. Analytical chemistry is important in nearly all aspects of chemistry. Analytical techniques proved in assuring and maintaining the quality of substance and are critical components of QA and QC.

Analytical method should be,

1. Most productive, economical and convenient,
2. As accurate and precise as required,
3. As simple as possible,
4. Most specific

Should be fully optimized before transfer for validation of its characteristics such as precision, accuracy, sensitivity etc.

According to USP, system suitability tests are integral part of chromatographic methods. These tests are used to verify that the reproducibility and resolution of the system are adequate for the analysis to be performed. Framework such as plate count, symmetry factor, resolution and duplicability (%RSD retention time and area for 6 repetitions) are determined and compared against the specifications set for the method.

Reversed phase chromatography has found both logic and preparative appeal in the district of biochemical detachment and cleans. Molecules that possess some level of hydrophobic disposition can be separated by reversed phase chromatography with very good recovery and resolution. The separation device in reversed phase chromatography depends on the hydrophobic binding interaction between the solute molecule in the mobile phase and the immobilized hydrophobic ligand, i.e. the stationary phase. The true nature of the hydrophobic unbreakable interaction itself is an affair of heated discussion but the normal insight assumes the binding interaction to be the result of a favorable entropy effect.

Sodium valproate, chemically sodium-2-propyl pentanoate, is first line drug used for its unique anticonvulsant properties in the treatment of primary generalized seizures, partial seizures and

myoclonic seizures. The mode of action is to stabilize electrical activity in the brain by increasing synthesis and decreasing metabolism of gamma amino butyric acid[18-20]

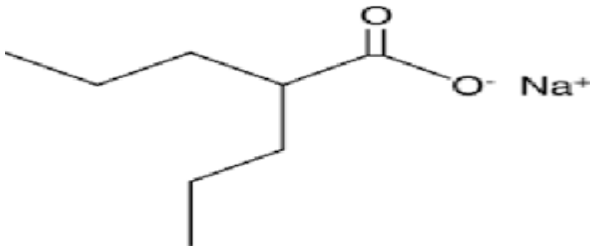

Figure 1: Chemical structure of Sodium Valproate

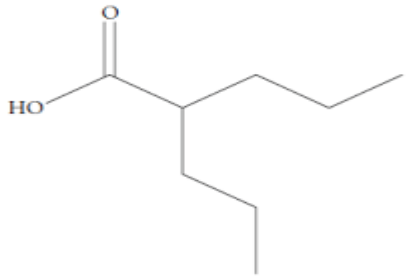

Figure 2: Chemical structure of Valproic acid

Valproic acid (2-propylpentanoic acid) is an anticonvulsant drug, widely used in the treatment of epilepsy, affecting both children and adults. It is also used to treat bi- polar disorder and to prevent migraine headaches. Antiepileptic drugs (AEDs) are used in clinical practice to aid the treatment of patients with epilepsy. In order to obtain information for Therapeutic drug monitoring (TDM), the development of a reliable analytical method for the determination of AEDs in biological fluids is of great importance [4]. Serum and plasma represent the matrices of choice for TDM of AEDs [21-23].

MATERIAL AND METHODS

Reagents and Chemicals

Water, Acetonitrile, Ortho-phosphoric acid, Acetonitrile, Sodium hydroxide, Hydrogen peroxide (H_2O_2), and Hydrochloric acid were used in the study.

Instrumentation:

Agilent (1100series) with Auto sampler and DAD detector with Chemstation software were used.

Chromatographic condition:

A High performance liquid chromatogram equipped with DAD detector, the purity determination performed on a Fortis C18 (100mm x 4.6ID, Particle size: 2.5 micron) at ambient temperature using mobile phase consisting of Acetonitrile: 0.1% ortho phosphoric acid (80:20).

Preparation of standard solution VA and SV:

Weighed accurately about 14.5mg of VA and 33.3mg of SV standard and transffered into 10mL of volumetric flask, added about 10 mL of diluent, shaked to dissolved and volume was made up to the mark with diluent. (concentration of VA and SV is 1450 µg/ml and 3330 µg/ml) A-grade bulb pipette into 10 ml volumetric flasks and the solutions were made up to volume with mobile phase to give final concentrations of 14.5,29,43.5,58,72.5and 87 µg/ml for VA, 33.3,66.6,99.9,133.2,166.5 and 199.8µg/ml for SV.

Preparation of Sample solution VA and SV:

Twenty tablets were weighed and finely powdered. An accurately weighed amount of powder equivalent to 14.5 mg of VA and 33.3 mg of SV was transferred into a 10.0 ml volumetric flask. Then 5.0 ml of diluent was added in it. The flask contents were sonicated for 10 min to make the contents homogeneous. This solution was then diluted up to the mark with diluent. The resultant solution was filtered through Whatman Grade I filter paper. One milliliter of the filtrate was transferred to a 10 ml volumetric flask and then the volume was made up to the mark with diluent to furnish a sample solution containing 58 µg/ml of VA and 133.2µg/ml of SV.

Method validation :

The developed method was validated following ICH guidelines (ICH Q2R1) for accuracy, precision, specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), robustness.

Linearity:
Linearity is the ability of the analytical procedure to obtain a response that is directly proportional to the concentration (amount) of analyte in the sample. If the method is linear, the test results are directly or by well-defined mathematical VA and SV transformation proportional to the concentration VA and SV of an analyte in samples within a given span at which the involved response is proportional to the analyte concentration.

Accuracy:
Accuracy is the nearness of a measured value to the true or accepted value. Accuracy designate the digression between the convey merit found and the true merit. It is determined by applying the method to samples to which known amounts of analyte have been added. These should be analyzed against the standard and blank solutions to ensure that no interference exists.

Precision:
The exactness of an logic method is the level of accord among single test results get when the method is pragmatic to many sampling of a similar sample. Precision is a measure of the dependability of the whole analytical process.

Limit of Detection (LOD):
The detection limit of an individual analyte procedure is the lowest amount of analyte which can be detected not necessarily quantified as an exact value. LOD was calculated using the following formula.

LOD = 3.3σ/S
Where σ is the standard deviation calculated from accuracy of the response and S is the slope from linearity

Limit of Quantification (LOQ)
The quantification limit of an individual analytical procedure is the lowest amount of analyte which can be quantitatively determined. LOQ was calculated using the following formula.

LOQ = 10.σ/S
Where σ is the standard deviation calculated from accuracy of the response and S is the slope from linearity.

System suitability parameter:
System suitability parameter is the evaluation of a composition of an analytical system to show that the performance of the system meets the standard required by the method. This parameter can be calculated experimentally to provide a quantities system suitability test report number of theoretical plates (efficacy) capacity factor, separation (relative retention), resolution, telling factor relative standard deviation (precision).

RESULTS AND DISCUSSION

Determination of λ max
UV absorption of 10 µg/mL solution of VA and SV in METHANOL was generated and absorbance was taken in the range of 200-400 nm.λmax of VA and SV in Methanol was found to be 220 nm and 254 nm respectively. This is essential since HPLC detection is basically UV based, thus a 10µg/mL solution of VA and SV in Methanol was used to get the following spectra.

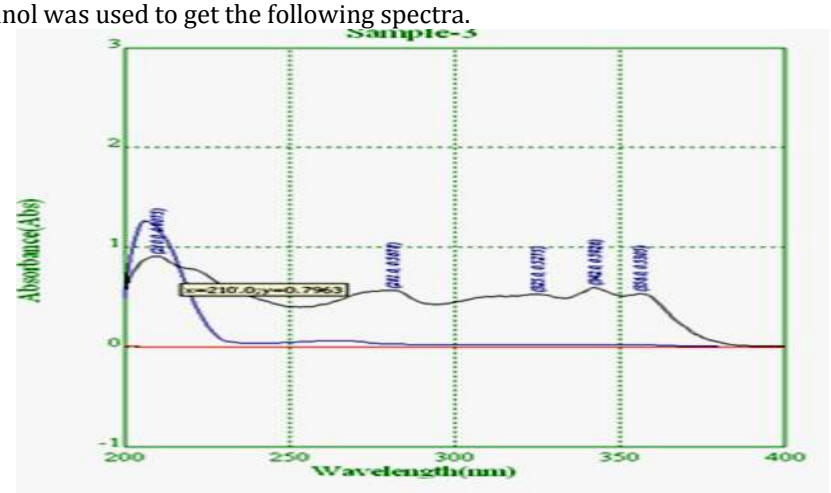

Figure 3: Iso-absorptive point of VA and SV

HPLC method development and optimization
Initially, pure drugs solution was chromate graphed using a mobile phase consisting of a mixture of 0.1% ortho-phosphoric acid in water (pH2.7) and acetonitrile in the ratio of (80:20) v/v at a flow rate of 0.7 ml/min. gives well- resolved peaks of drugs. Detection was carried out at 210 nm. The retention

time under the optimized condition of valporic acid and Sodium Valproate was found to be 1.537 min. & 4.910 min. respectively. The total run time of the chromatogram was about 15 minutes. A typical chromatograph of a mixture of standard and sample VA and SV is summarized by Fig. 6 and Fig. 7 respectively.

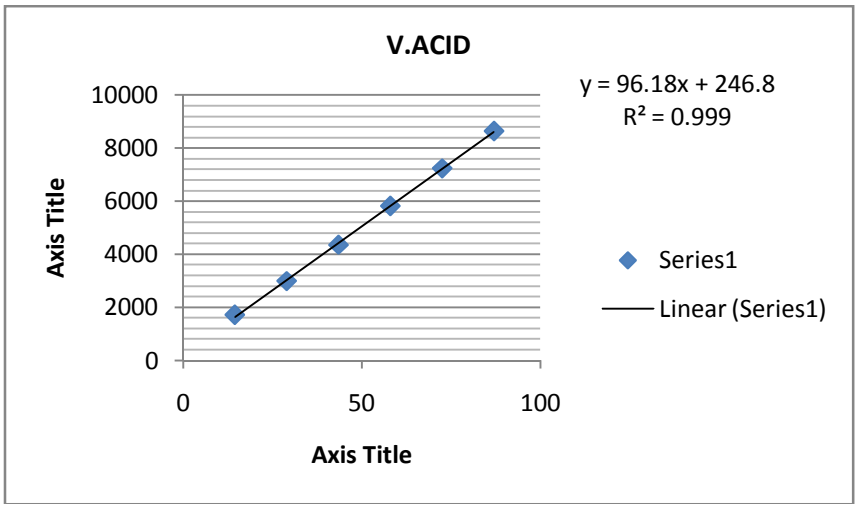

Figure 4: Linearity curve of VA

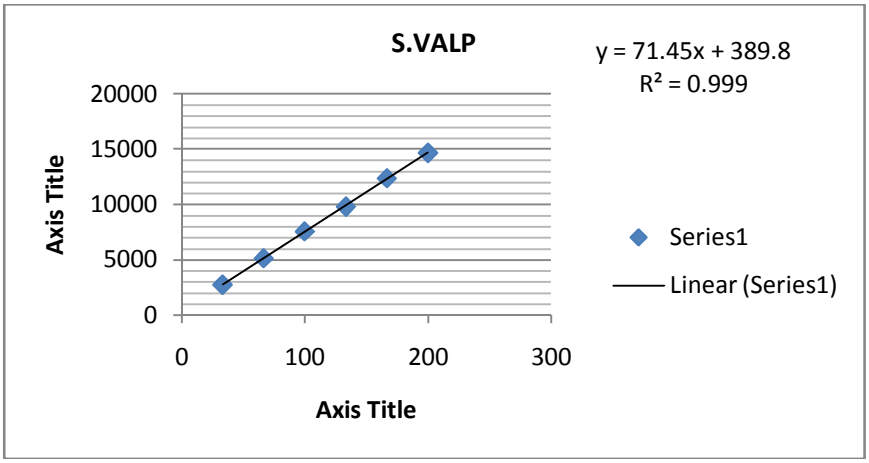

Figure 5: Linearity curve of SV

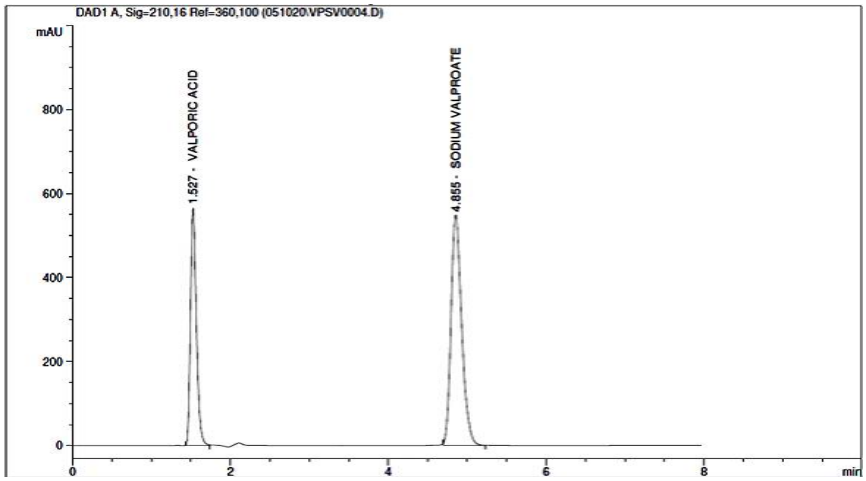

Figure 6: Chromatograph of mixture of standard VA and SV

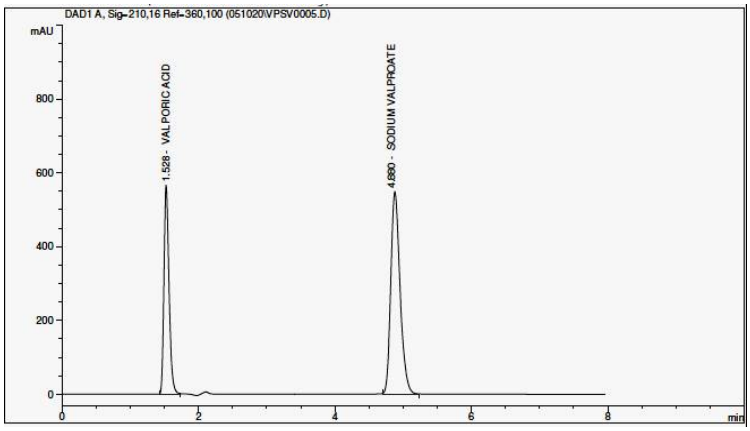

Figure 7: Chromatograph of mixture of sample VA and SV

Validation of the method

System Suitability:

The suitability of the system was demonstrated by assessing various parameters. It was established by injecting two replicate injections of the standard solution. Theoretical plates were found to be 4009 and 6935, tailing factor of 0.73 and 0.75, and %RSD of peak area was 0.61 and 0.66 for both VA and SV respectively (Table 1). All the system suitability parameters were well within the limits, indicating that the system was well suitable for performing the analysis.

Table 1: System suitability results

Parameter	VA	SV
Theoretical Plate	4009	6935
Retention Time (Rt)	1.542	5.012
Tailing factor	0.73	0.75
% RSD	0.61	0.66

Rt: Retention time, %RSD: Percentage relative standard deviation

Linearity

Linearity was established by the least-squares linear regression analysis of the calibration data. Calibration plots were linear over the concentration range of 14.5-87 µg/ml for VA and 33.3-199.8 µg/ml for SV. Peak areas were plotted against the respective concentrations and linear regression analysis performed on the resulting curves. The linear curve of VA and SV was shown in Fig. 4 and Fig.5 respectively. The linear regression equation obtained was Y=96.18x+246.8 for VA and Y=71.45x+389.8 for SV with correlation coefficient 0.9999 and 0.999 respectively. The results of linearity are shown in Table 2.

Table 2: Linearity results of VA and SV

Parameter	VA	SV
Theoretical Plate	4029	6798
Retention Time (Rt)	1.537	4.910
Tailing factor	0.72	0.74
%RSD	0.66	1.03

Accuracy:

Accuracy was computed by recoveries studies. The mean percentage recoveries values for three levels were found to be between 99.01-101.58% and 98.28-101.58% for VA and SV respectively. The percentage of recoveries values within the limits, indicating the method developed was accurate. The results of recovery are shown in Table 3.

Table 3: Recovery Result

Drug	Level	Amount taken	Amount Found	% Recovery
VA	80	11.6	26.00	99.22
	100	14.5	28.92	99.49
	120	17.4	32.11	101.58
SV	80	26.64	60.16	100.83
	100	33.3	66.11	98.52
	120	39.96	73.75	101.58

***Average of three determinations**

Precision

The %RSD of intraday precision and interday precision were 0.72 and 0.73 for VA. The %RSD of intraday precision and interday precision were 0.74 and 0.71 for SV. The percentage RSD of system, method, and intermediate precision study was well within the limits (<2%), indicate that the method was precise.

LOD and LOQ:

The LOD was found to be 0.57µg/ml For VA and 0.7310 µg/ml for SV. The LOQ was found to be 1.73µg/ml for VA and 2.215 µg/ml for SV. The values of LOD and LOQ indicate that the method was greatly sensitive (Table 6).

Robustness

The robustness of the method was designed by changing the optimized condition adequately. To evaluate the robustness of the developed method, the chromatographic conditions were deliberately altered and the resolution between VA and SV was evaluated. On the assessment of the result it can be deduced that the variation in the changing wavelength, the flow rate does not affect the method significantly. %RSD <2% specifies that the developed method was robust. The results of robustness are shown in Table 4.

Table 4: Robustness results

Condition		Sodium Valproate		Valproic Acid	
		SD	%RSD	SD	%RSD
Change in wavelength (210±1 nm)	209nm	41.42	0.09	12.00	0.24
	211nm	25.12	0.46	37.07	1.00
Change in flow rate (1.0±0.1 ml/min)	0.6	4.20	0.05	2.25	0.05
	0.8	6.28	0.10	0.04	0.0
Change in mobile phase (1.0±0.1 ml/min)	19+81	0.17	0.10	38.47	0.88
	21+79	0.707	0.01	35.37	0.85

*Average of three determinations, %RSD: Percentage relative standard deviation

Analysis of VA and SV from marketed tablets

The percentage assay of tablet formulation was found to be 98.71 and 101.80% for VA and SV respectively. The stability of the drug solutions was observed for 2 h. In degradation studies, the drug was exposed to various stress conditions. From the chromatograms of stressed samples, it was found that no interference from degradants was observed at the retention time of VA and SV. Optimum degradation was observed in the presence of acid and alkali. Substantial degradation was observed in the presence of water, light, and peroxide. minor degradation was observed in the presence of peroxide and thermal for VA and thermal for SV. The results of the percentage of degradation are presented in Table 5 and Fig. 8-12. Hence, the method was found to be specific.

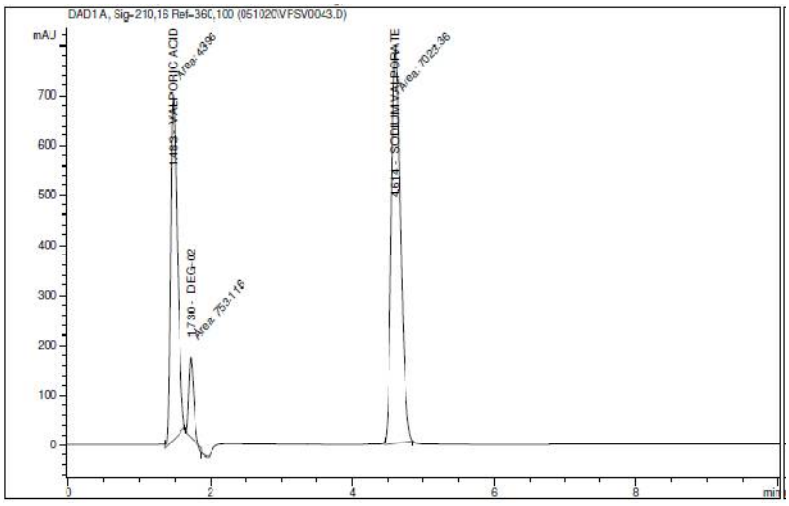

Figure. 8: Chromatogram of VA and SV degraded with acid hydrolysis

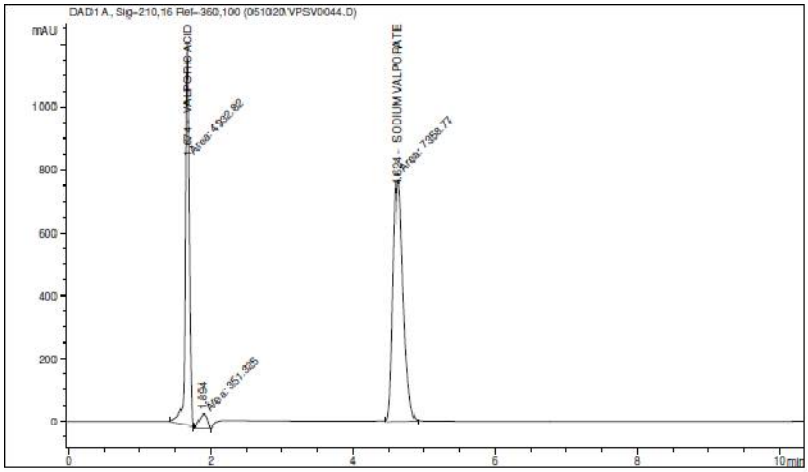

Figure. 9: Chromatogram of VA and SV degraded with alkali hydrolysis

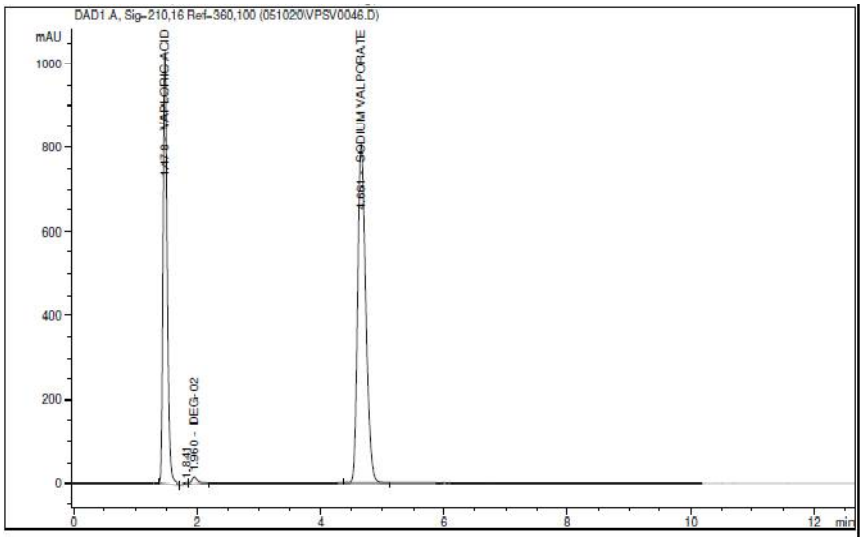

Figure. 10: Chromatogram of mixture of VA and SV degraded with neutral hydrolysis

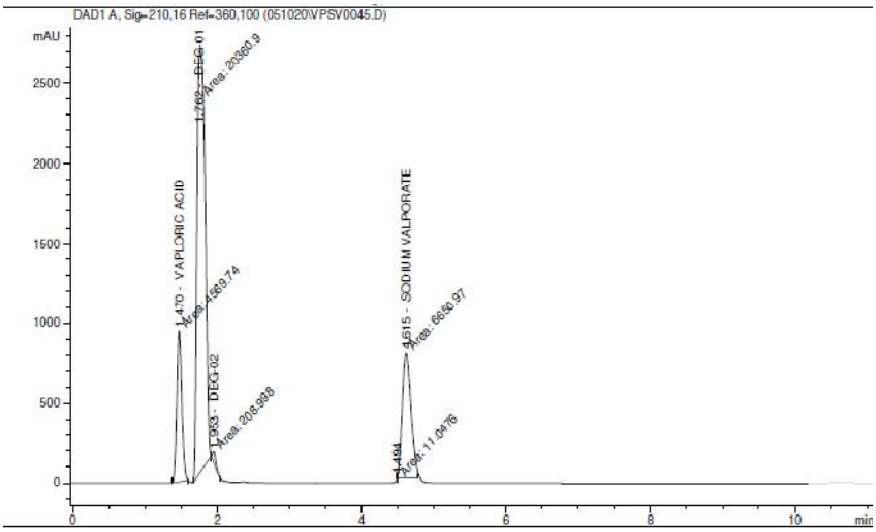

Figure. 11: Chromatogram of VA and SV degraded with oxidative hydrolysis

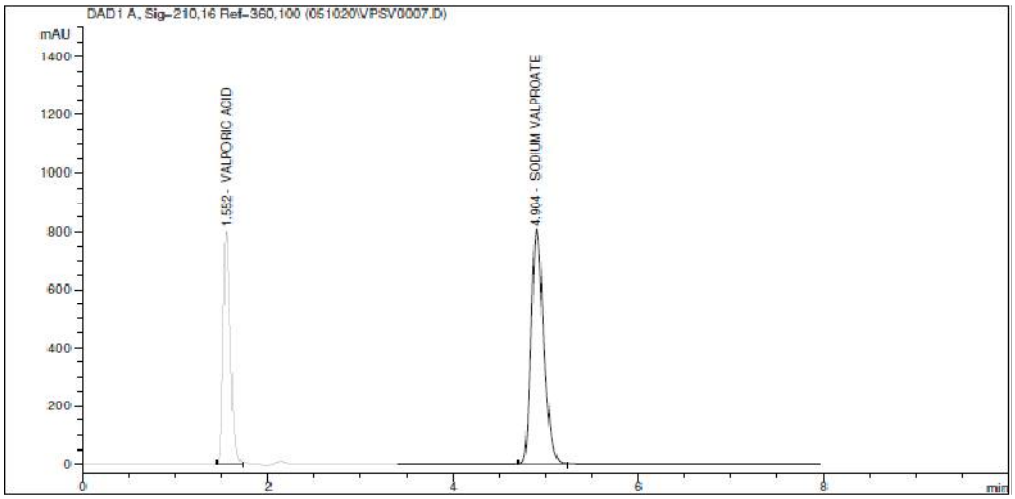

Figure. 12: Chromatogram of VA and SV degraded with exposed to direct sunlight

Table 5: Stability-indicating method data for VA and SV

Parameter	VA (% degradation)	SV (% degradation)
Acidic(0.1N HCL for 2 hr)	7.77	5.92
Alkaline (0.1N NaH for 2 hr)	7.39	6.77
Hydrolytic(HPLC waters for 2hr)	6.54	12.17
Oxidative(3% H_2O_2 for 2 hr)	0.88	0.94
Photo(sun light for 24 hr)	0.83	1.64

HPLC: High Performance Liquid Chromatography

Table 6: Summary of validation parameter

Parameter	VA	SV
Calibration Range (µg/ml)	14.5-87	33.3-199.8
Optimized wavelength (nm)		
Retention Time	1.537	4.910
Precision (% RSD)	0.72-0.73	0.74-0.71
% Assay	98.71	101.80
LOD (µg/ml)	0.57	0.7310
LOQ(µg/ml)	1.73	2.215

Average of five determinations, LOD: Limit of detection, LOQ: Limit of quantification

CONCLUSION

The method enables simple, rapid, accurate, precise, specific, economical, and sensitive analysis of VA and SV in combined bulk and tablet dosage form. This method was validated following ICH guidelines. The method can, therefore, be used for routine quality control analysis VA and SV in bulk and tablet dosage form.

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CONFLICT OF INTEREST

Authors declare that they have no conflict of interest exists in this investigation.

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