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FORCED DEGRADATION OF SACUBITRIL AND VALSARTAN: DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD

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ABSTRACT

In the present work, a new validated stability indicating HPLC method for quantitative determination of Sacubitril (SAC) and Valsartan (VAL) in tablet formulation was developed. The column was Phenomenex Luna C_{18} column (150 mm × 4.6 mm id; 5µm particle size) and the mobile phase was composed of Acetonitrile: Water in 0.1% of Trifluoroacetic acid (50:50,v/v) with a flow rate 1 ml/min. Eluents were monitored by UV detector at 242 nm. Calibration curve was linear in the concentration ranges 4.9–24.5µg/ml (R^2 value is 0.9997) for SAC and 5.1 – 25.5µg/ml (R^2 value is 0.9998) for VAL. SAC and VAL were subjected to stress conditions including acidic, alkaline, oxidation, photolysis and thermal degradation. The developed method was found to give good separation between pure drug and degraded products. The proposed method was successfully applied for the stability assay of SAC and VAL in tablet formulation and validated as per ICH guidelines.

KEYWORDS: Sacubitril, Valsartan, HPLC Method, Forced degradation studies.

INTRODUCTION

Sacubitril (SAC) (Fig.1) is chemically 4-[[(2S, 4R)-5-ethoxy-4-methyl-5-oxo-1-(4-phenyl-phenyl) pentan-2-yl] amino]-4-oxobutanoic acid. Sacubitril is an antihypertensive drug used in combination with valsartan for the treatment of heart failure.[1] Valsartan (VAL) (Fig.2) is

chemically N-(1-oxopentyl)-N-[[2'-(1H-tetrazol-5-yl) [1,1'-biphenyl]-4yl] methyl]-L-valine. It is a potent, highly selective, orally active, specific angiotensin II receptor antagonist used as a hypotensive drug.[2] On literature survey, several methods were reported for the estimation of SAC and VAL individually and in combination with other drugs.[3-10] So we have developed a novel, simple, rapid, accurate, precise and highly sensitive RP-HPLC method for estimation of SAC and VAL in bulk and tablet dosage form and validated according to ICH guidelines.

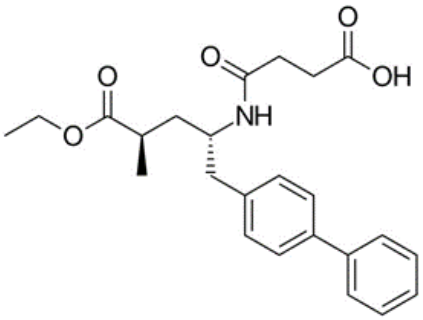

Fig. 1: Chemical structure of SAC.

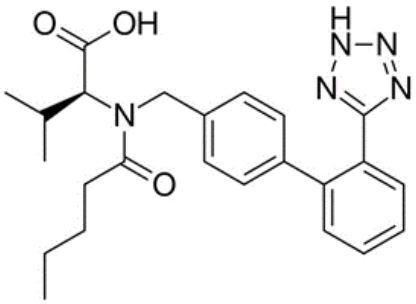

Fig. 2: Chemical structure of VAL.

MATERIALS AND METHODS

Reagents and Chemicals

Analytically pure sample of SAC and VAL was procured from Beijing mesochem technology co.ltd. (China). The pharmaceutical dosage form used in this study was a Cidmus tablets manufactured by Novartis pharmaceuticals, (Switzerland) labeled to contains 49 mg of SAC and 51 mg of VAL was purchased from welcome healthcare, Mumbai. All chemicals and reagents were of HPLC grade and were purchased from Sudhagar Biological and Chemicals Pvt. Ltd., Chennai, India.

The instrument and chromatographic conditions

Shimadzu HPLC system (Shimadzu corporation Kyoto, Japan) consisted of a pump (LC - 20AD solvent deliver module, SPD-20A UV- Visible detector) run under Lab solutions software, with manual injecting facility programmed at 20 µL capacity per injection was used. The column used was Phenomenex Luna C_{18} (150 mm × 4.6 mm, 5.0 µm particle size). Different mobile phases were tested in order to find the best condition for separation of SAC and VAL. The mobile phase contained Acetonitrile: Water in 0.1% Trifluoroacetic acid (50:50, v/v) and the flow rate was maintained at 1.0 ml/min. UV detection was carried out at 242 nm. The mobile phase and samples were filtered through a 0.45 µm membrane filter.

Mobile phase was degassed by Sonica ultrasonic cleaner (model 2200 MH) prior to use. The other instrument used are hot air oven.

PREPARATION OF STANDARD AND SAMPLE SOLUTIONS

Diluent

Mobile phase was used as the diluent.

Mobile phase

Acetonitrile: Water in 0.1% Trifluoroacetic acid (50:50, v/v) is programmed as RP HPLC method.

Preparation of stock standard solutions

Stock standard solution of SAC (490 μg/ml) was prepared by dissolving 49 mg of SAC in 100ml of diluent in 100ml volumetric flask with vigorous shaking. From this stock standard solution, Similarly, stock standard solution of VAL (510 μg/ml) was prepared by dissolving 51 mg of VAL in 100ml of diluent in 100ml volumetric flask with vigorous shaking.

Preparation of working standard solution

From above standard stock solution of SAC and VAL, 0.2 ml of SAC and VAL solutions were taken into 10 ml volumetric flask, separately and was made to the mark with the mobile phase to get 9.8 μg/ml of SAC and 10.2 μg/ml of VAL, respectively.

Preparation of sample stock solution

The average weight of 20 tablets was determined. Sample stock solution was prepared by dissolving tablet powder equivalent to 51 mg of VAL was transferred to 100 ml volumetric flask. Then 60 ml diluent was added and sonicated for 10 mins to ensure complete solubilization of drug. After sonication, volume was made up to the mark with diluent. Filter the sample stock solution with whatman filter paper.

Preparation of sample solution

From above sample stock solution of SAC and VAL, 0.2 ml was withdrawn and diluted to 10 ml using mobile phase to get concentration of 9.8 μg/ml of SAC and 10.2 μg/ml of VAL, is a sample solution.

Validation

The proposed methods were validated as per ICH guidelines.

Linearity

Different aliquots of 0.1 - 0.5 ml of SAC and VAL were transferred into series of 10 ml volumetric flasks, separately and the volume was made up to the mark with mobile phase to get concentrations 4.9–24.5µg/ml of SAC and 5.1 – 25.5µg/ml of VAL, respectively.

Accuracy

To the preanalysed sample solution, a known amount of standard stock solution was added at different levels i.e. 50, 100 and 150%. The solutions were reanalyzed by proposed method.

Precision

The reproducibility of this method was determined by analyzing tablet at different time intervals on same day in triplicates (Intra-day assay precision) and on three different days (Inter-day assay precision).

FORCED DEGRADATION STUDIES

Hydrolytic degradation under acidic condition

Pipetted out 0.2 ml of sample stock solution into a 10 ml volumetric flask and added 3 ml of 0.1 N HCl. Then, the volumetric flask was kept at 60ºC for 24 hours and then neutralized with 0.1 N NaOH and make up to 10 ml with mobile phase.

Hydrolytic degradation under alkaline condition

Pipetted out 0.2 ml of sample stock solution into a 10 ml volumetric flask and added 3 ml of 0.1 N NaOH. Then, the volumetric flask was kept at 60ºC for 24 hours and then neutralized with 0.1 N HCl and make up to 10 ml with mobile phase.

Oxidative degradation

Pipetted out 0.2 ml of sample stock solution into a 10 ml volumetric flask and added 3 ml of 0.1% w/v of hydrogen peroxide. Then, the volumetric flask was kept at 60ºC for 24 hours and then the volume was made upto the mark with mobile phase.

Photo degradation

Pipetted out 0.2 ml of sample stock solution into a 10 ml volumetric flask and expose to sunlight for 24 hrs and the volume was made up to the mark with mobile phase.

Thermal induced degradation

SAC and VAL sample was taken in petri dish and kept in Hot air oven at 60 °C for 24 hours. Then the sample was taken and diluted with diluent and injected into HPLC and analyzed.

RESULTS AND DISSCUSION

Method development and optimization

The HPLC procedure was optimized with a view to develop a suitable LC method for the determination of SAC and VAL in tablet dosage form. Initially, acetonitrile and water in different ratios were tried. But SAC and VAL gave broad peak. So, 0.1% of Trifluoroacetic acid was added in water and mixtures of acetonitrile and water in 0.1% of Trifluoroacetic acid in the different ratios were tried. It was found that acetonitrile : water in 0.1% of Trifluoroacetic acid in the ratio of 50:50 (v/v) gave acceptable retention times (7.505 min of SAC and 6.152 min of VAL) with flow rate of 1.0 ml/min as shown in figure 4 and also performed mobile phase black as shown in figure 3.

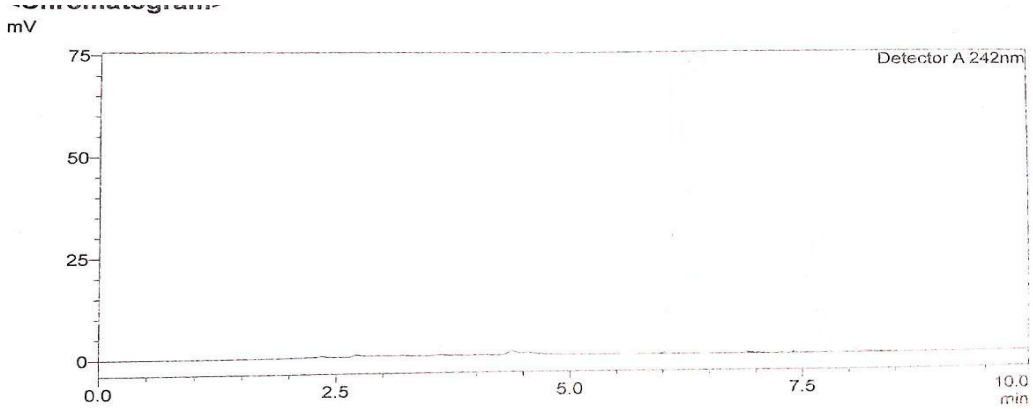

Fig 3: Chromatogram of mobile phase blank.

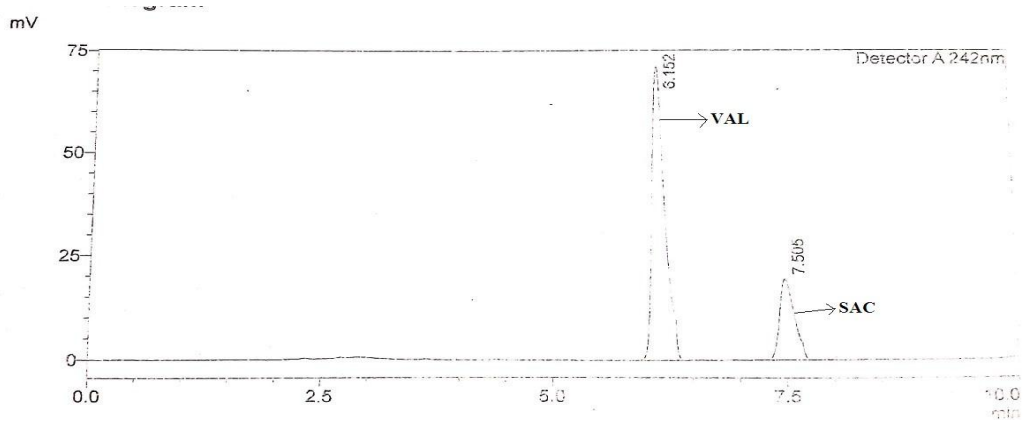

Fig 4: Chromatogram of VAL and SAC obtained at optimum chromatographic conditions.

Method validation

The described method has been validated which include parameters like system suitability, linearity, accuracy, precision, robustness, LOD (limit of detection) and LOQ (limit of quantification).

System suitability

System suitability and chromatographic parameters were validated such as tailing factor and theoretical plates was calculated. The results are given in table 1.

Table 1: System suitability parameters.

Parameters	SAC	VAL
Retention time	7.505	6.152
Peak area	244897	781028
Tailing factor (T)	1.082	1.023
Theoretical plate (N)	6876	6110
Resolution (R)	4.898	

Linearity

Linearity of this method was evaluated by linear regression analysis and calculated by least square method and studied by preparing standard solutions of SAC and VAL at different concentrations, separately in the range of 4.9–24.5 and 5.1–25.5µg/ml with correlation coefficient (r^2) of 0.9997 and 0.9998. Results are given in table 2.

Table 2: Linearity data for SAV and VAL.

Drug	Concentration (µg/ml)	Area
SAC	4.9	122435
	9.8	245997
	14.7	363599
	19.6	485352
	24.5	615588
VAL	5.1	392496
	10.2	781028
	15.3	1166014
	20.4	1573025
	25.5	1944780

Accuracy

Accuracy of the proposed method was determined by performing the recovery experiment. The recovery experiment were studied by adding known amount of standard SAC and VAL to the pharmaceutical product and calculating the recovered standard amount. At 50%, 100%

and 150% standard addition level mean recovery of were found to be 99.52%, 100.10% and 99.77% for SAC and 100.15%, 100.20% and 99.89% for VAL, respectively. The results of recovery experiment are given in table 3.

Table 3: Results of the accuracy study.

Drug	Accuracy Level	Amount of drug taken (mg)	Amount of drug spiked (mg)	Recovery %	RSD% (n=3)
SAC	50	49	24.5	99.52	0.5429
	100	49	49	100.10	0.8135
	150	49	73.5	99.77	0.2849
VAL	50	51	25.5	100.15	0.1663
	100	51	51	100.20	0. 0943
	150	51	76.5	99.89	0.4713

Precision

Precision was evaluated at the repeatability and intermediate precision levels. For repeatability analysis, six independent portions of a tablet dosage form were processed through the full analytical method and results was evaluated obtained by % RSD values of 0.2152 for SAC and 0.4104 for VAL as shown in table.4.

Table 4: Precision result of the proposed method.

Sample No.	SAC		VAL	
	Peak area response	Assay (%)	Peak area response	Assay (%)
1	244897	99.55	780028	99.87
2	245366	99.74	787787	100.86
3	246286	100.11	780970	99.99
4	245284	99.71	782216	100.15
5	245802	99.92	780216	99.89
Average	245527	99.80	782243	100.15
% RSD	0.2168	0.2152	0.4111	0.4104

Robustness

Robustness study was conducted by deliberate changes in mobile phase composition and flow rate, revealed that there was no significant variation in % assay as shown in table.5.

Table 5: Robustness study

Percent assay of the drug	Mobile phase, Acetonitrile: Water in 0.1% Trifluoroacetic acid		Flow rate, ml/min	
SAC	45 : 55 (v/v)	55 : 45 (v/v)	0.9	1.1
	100.44	99.76	99.88	100.52
VAL	99.92	99.48	99.54	99.72

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ was found to be 0.4065 (µg/ml) and 1.2195 (µg/ml) for SAC and 0.3270 (µg/ml) and 0.9811 (µg/ml) for VAL estimated by using the standard formulas. The low values of LOD and LOQ illustrate that the developed method was sensitive, accurate and precise as it can detected and quantify with very low concentration. The results are given in table.6.

Table 6: LOD and LOQ data for SAC and VAL.

Drug	LOD	LOQ
SAC	0.4065 (µg/ml)	1.2195(µg/ml)
VAL	0.3270 (µg/ml)	0.9811 (µg/ml)

Forced degradation studies

Results are tabulated in table 7.

Table 7: Summary of forced degradation study.

Stress conditions	Time (h)	% Degradation of SAC	% Degradation of VAL
0.1M HCl	24	Not degraded	Not degraded
0.1M NaOH	24	Not degraded	Not degraded
0.1 % H_2O_2	24	Not degraded	Not degraded
Thermal	24	Not degraded	Not degraded
Photo	24	Not degraded	Not degraded

When stress conditions were applied to SAC and VAL, the HPLC results showed that there was no degradation occurs in acid, base, oxidative, thermal and photo as shown in figure 5-9.

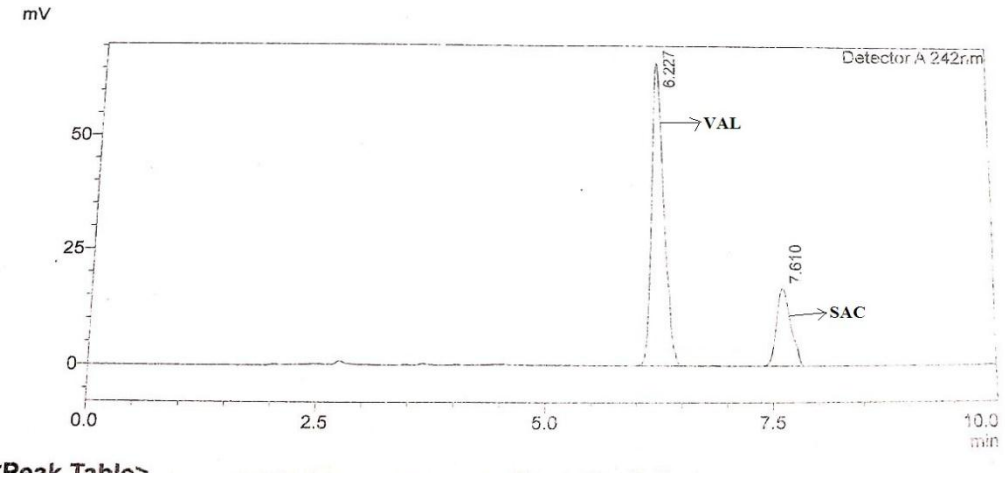

Fig. 5: Typical chromatogram of VAL and SAC forced degradation study–Acid degradation.

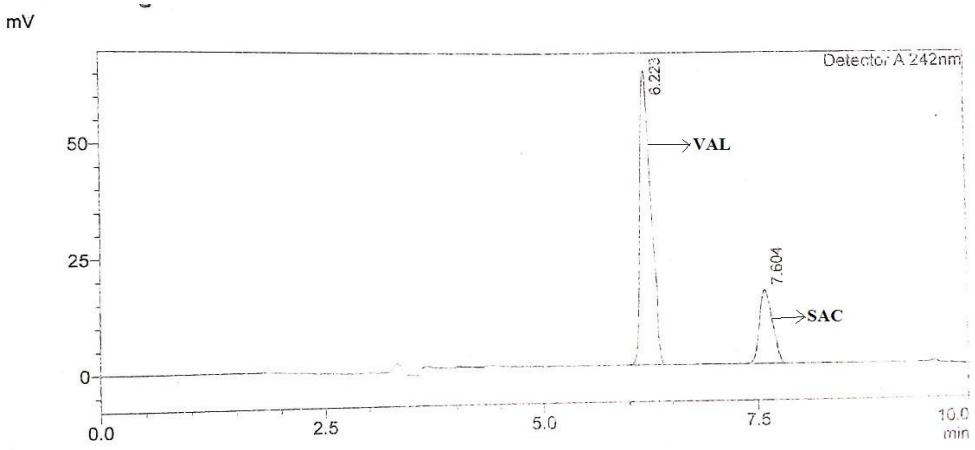

Fig. 6: Typical chromatogram of VAL and SAC forced degradation study–Base degradation.

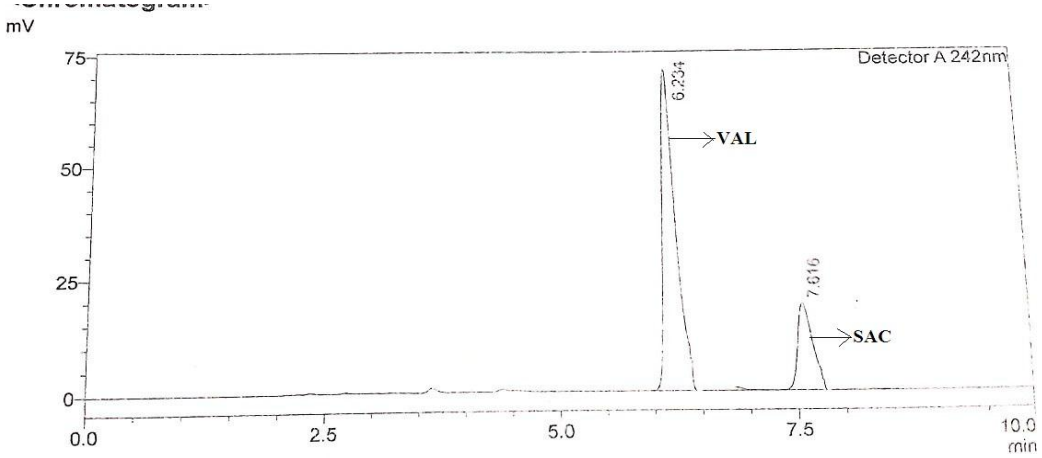

Fig. 7: Typical chromatogram of VAL and SAC forced degradation study–Oxidative degradation.

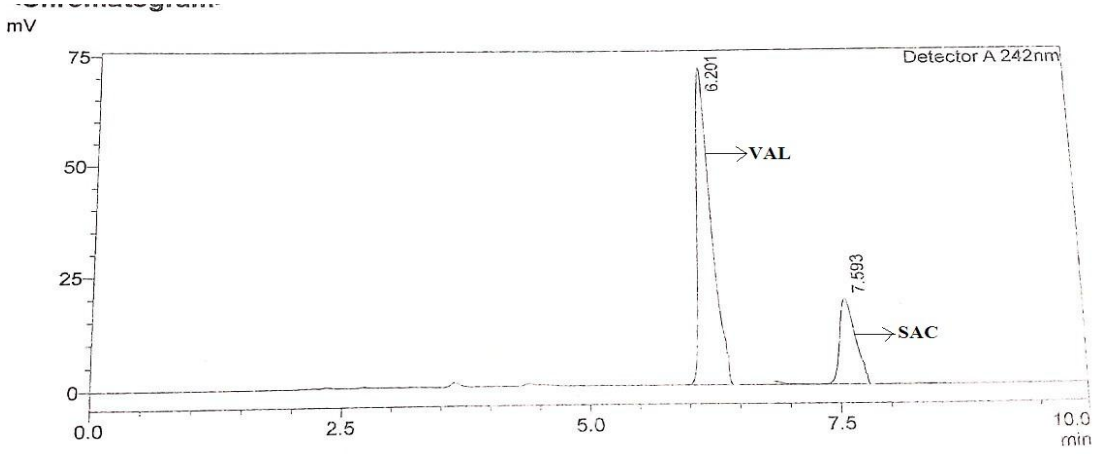

Fig. 8: Typical chromatogram of VAL and SAC forced degradation study–Thermal degradation.

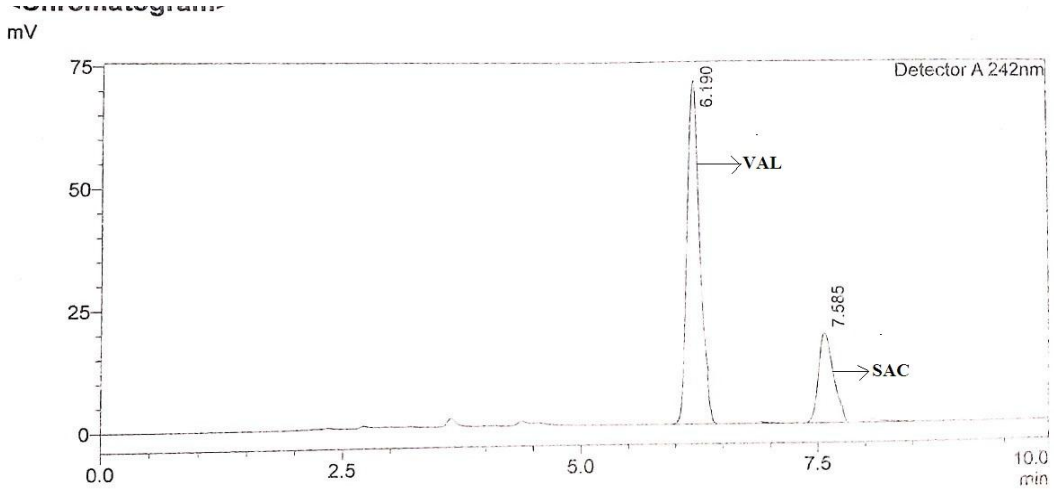

Fig. 9: Typical chromatogram of VAL and SAC forced degradation study– Photo degradation.

CONCLUSIONS

The stability-indicating RP–HPLC method was developed, validated according to ICH guidelines and was applied for the determination of SAC and VAL in tablet formulation. The results obtained from validation studies revealed that, the developed method was found to be rapid, simple, accurate, precise, specific, selective and economical. Results obtained from the force degradation, indicates that there was no degradation occurs in acid, base, oxidative, thermal and photo. The proposed method has the ability to separate the drug in tablet dosage form and could be used as a stability-indicating method for the determination of SAC and VAL either in bulk powder or in tablet formulation.

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