

# Application of TLC-Densitometric Method for Simultaneous Determination of Sacubitril and Valsartan in their Newly Approved Pharmaceutical Formulation

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**Abstract:** Entresto® (Sacubitril/valsartan) is a recently approved oral drug for the treatment of symptomatic chronic heart failure. In this study, simple, rapid and economic TLC-densitometric method has been developed for simultaneous determination of sacubitril and valsartan in their mixture. The TLC-densitometric separation process has been achieved on pre-coated silica gel (60 GF<sub>254</sub>) plates using toluene-ethylacetate-methanol mixture(4:4:2, *by volume*) as a developing system. The well separated bands have been quantified densitometrically at 260nm. Calibration graphs were found to be linear over a concentration range of 0.1–0.6µgband<sup>-1</sup> for two drugs. The proposed method has been successfully applied to the analysis of sacubitril and valsartan in Entresto® tablets.

**Keywords:** Entresto (sacubitril/valsartan), Thin layer chromatography, Densitometry.

## INTRODUCTION

Entresto® (sacubitril/valsartan) is a new FDA approved mixture for the treatment of heart failure. It contains sacubitril (SAC) **Fig. 1**, a prodrug that results in neprilysin inhibition and valsartan (VAL), **Fig. 2**, angiotensin II Type-1 receptor blocker, [1,2]. SAC is 4-[[[(1S,3R)-1-[[[1,1'-biphenyl]-4-ylmethyl]-4-ethoxy-3-methyl-4-oxobutyl]amino]-4-oxobutanoic acid. VAL is *N*-(1-oxopentyl)-*N*-[[2'-(1*H*-tetrazol-5-yl) [1,1'-biphenyl]-4-yl]methyl]-L-valine. The studied drugs are physically white powder and freely soluble in methanol [2].

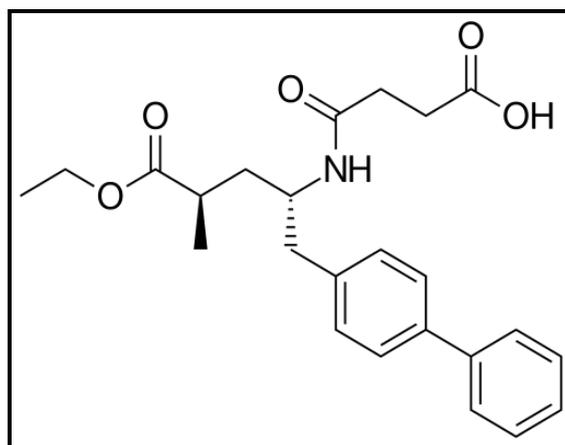


Fig. 1: Structural formula of SAC.

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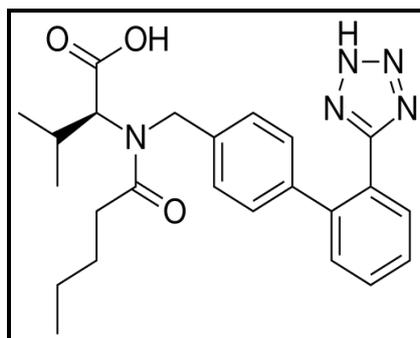


Fig. 2: Structural formula of VAL.

Different analytical techniques have been applied for pharmaceutical analysis with various applicable advantages. These techniques including either chromatographic [3-6], spectrophotometric [7- 14], spectrofluorimetric [15, 16], atomic spectrometry or application of electrochemical methods [17 - 19].

Due to the recent launch of the entresto® in the pharmaceutical market, few analytical methods have been reported to provide the simultaneous determination of SAC and VAL. The previous reported work has been proceeded through liquid chromatographic methods using UV and mass detector [20-24] or using classical mathematical spectroscopic methods [25-28]. It is not worthy to mention that, all the reported techniques recommended more expensive instruments, HPLC and UPLC, with high consumption of solvents and long analysis run time. On the other hand, the mathematical spectroscopic methods required critical recommendations in the recorded spectral data as presence of zero crossing point or computing more complicated equations involved in the mathematical processing of the zero order absorption spectra.

All these drawbacks of the previously published methods promoted the authors to select simpler, rapid, low cost and less solvents consumption analytical method. This was achieved through application of thin-layer chromatography (TLC), a rapid selective analytical tool applied for separation of multicomponent preparation [29, 30]. Also it was good for the authors that the proposed TLC technique enable nano-level quantitative analysis of the studied drugs in their newly approved pharmaceutical tablets.

## EXPERIMENTAL

### Instrumentation

CAMAG TLC Scanner 3 S/N 130319 was equipped with Linomat 5 auto-sampler and CAMAG micro syringe (100 mL) (Switzerland). The following parameters were adjusted (Scan mode: absorbance mode, Slit dimensions: 3 x 0.45 mm, Scanning speed: 20 mm/s, Result output: chromatogram and integrated peak area). Sampling was carried out on pre-coated TLC plates, silica gel (60 GF<sub>254</sub>, 20 x 10 cm), (Fluka chemie, Switzerland).

### Materials, Chemical and Standard Solutions

- Pure standard of SAC (99.5%) and VAL (99.4%) were kindly supplied by National Organization for Drug Control and Research, Giza, Egypt.
- Entresto® tablets 97/103 (Batch no. TJ027, manufactured by Novartis stein AG, Switzerland), labeled to contain 97 mg of SAC and 103 mg of VAL, were purchased from local market.
- Methanol, HPLC grade (Sigma-Aldrich, Darmstadt, Germany).
- Ethyl acetate, Hexane and toluene, analytical grade (El-Nasr Pharmaceutical Chemicals Co., Abu Zaabal, Egypt).
- Standard stock solutions of SAC and VAL (1000 µg mL<sup>-1</sup>) were prepared separately by dissolving 100 mg of each drug powder in methanol.

### Procedure

#### TLC-Densitometric Conditions

A pre-coated silica gel TLC plates were washed with methanol and dried at 60°C for 5 min in order to be activated. Samples were applied on these plates in the form of bands (6 mm length, 10 mm spacing, and 10 mm from the bottom edge of the plate). The plates were put in a chromatographic tank, previously saturated with the developing system consisted of toluene-ethyl acetate-methanol (4:4:2, *by volume*) for 30 min at room temperature. Ascending development of this developing system was proceeded and the plates were air dried and finally scanned at 260nm.

### Construction of Calibration Graph

Aliquots equivalent to 100–600  $\mu\text{g}$  of SAC and VAL were separately transferred from their standard solutions to a two set of 10-mL volumetric flasks and diluted to volume with methanol. Triplicate applications of 10  $\mu\text{L}$  from each solution were performed on the TLC plates to obtain the concentration range of 0.1–0.6  $\mu\text{g}/\text{band}^{-1}$  for each drug. The procedure under TLC-densitometric conditions was then followed. The peak area values were calculated and plotted against the corresponding concentrations of SAC and VAL in  $\mu\text{g}/\text{band}^{-1}$  to get the calibration graphs. The regression equations were finally derived.

### Application to Pharmaceutical Preparation

Ten Entresto® tablets (each tablet labeled to contain 97 mg SAC and 103 mg VAL) were weighed and finely powdered. A portion of powder equivalent to one tablet was weighed, transferred into conical flask and dissolved in 50 mL of methanol. The solution was shaken vigorously for 15 min then sonicated for about 30 min and filtered into 100-mL volumetric flask. The volume was completed to 100-mL with methanol to get a stock solution containing 970  $\mu\text{g}/\text{mL}^{-1}$  of SAC and 1030  $\mu\text{g}/\text{mL}^{-1}$  of VAL. The solution was suitably diluted with methanol to obtain sample solutions containing SAC and VAL in the concentrations ratio of 1:1.06  $\mu\text{g}/\text{mL}^{-1}$ , respectively, as in the tablet formulation. Then the procedure was completed as previously described. The concentrations of SAC and VAL have been calculated from the regression equations.

## RESULTS AND DISCUSSION

In this study, TLC analytical technique has been described for separation and simultaneous quantitation of SAC and VAL. TLC provides advantages over the previously reported techniques in terms of higher sensitivity, fast analysis times, smaller quantities of solvents.

Mobile phase for successful TLC separation process is selected and optimized based on traditional trials and error methods. It is more important and intended to get sharp symmetric peaks of the studied drugs with good resolution and more sensitive quantitative analytical process. For this purpose, we tried different solvents with recording and checking the resulted densitogram in each trial. Initially hexane–ethyl acetate (5:5, *v/v*) was tested; VAL and SAC peaks were detected close to each other and to the baseline **Fig. 3**. This was meaning poor resolution results and lower sensitivity due to the low values of the peak area. Although replacing the hexane with toluene in the applied mobile phase, toluene–ethyl acetate (5:5, *v/v*), enhanced the resolution and separation of SAC but VAL but the sensitivity still not accepted for the authors **Fig. 4**. Addition of methanol to the system, toluene–ethyl acetate–methanol (4: 4: 2, *by volume*), improved the separation of two drugs with acceptable *R<sub>f</sub>* values and improve the shape of the separated peaks with good values of the peak area **Fig. 5**.

Saturation of chromatographic tank with the developing system is an important step to ensure the homogeneity of the atmosphere and minimize the evaporation of solvent from TLC plate [31]. It was observed that the chamber saturation time of less than 25 min resulted in scattering of VAL band. Hence chamber saturation of 30 min was selected to confirm the required separation process.

With the aim of finding the best wavelength to quantify both VAL and SAC with high sensitivity and selectivity, the UV absorption spectra were recorded **Fig. 6**. It was found that at 260 nm, SAC has maximum absorbance with significant absorbance of VAL, so we choose this wavelength for analysis.

After optimization of the TLC-densitometric conditions, the plates were scanned at 260 nm, where spots were appeared at *R<sub>f</sub>* values of 0.14 and 0.41 for VAL and SAC, respectively, **Fig. 5**.

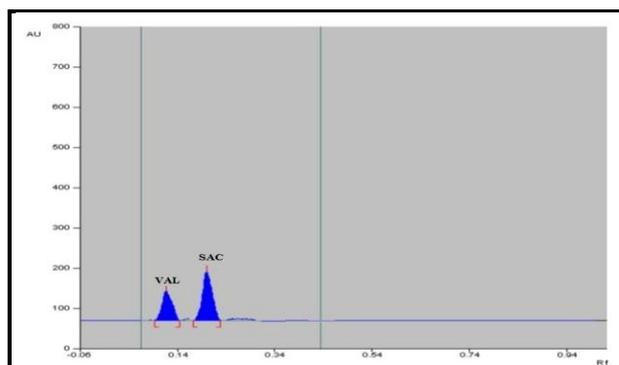


Fig. 3: TLC-densitogram of SAC ( $0.2 \mu\text{g}/\text{band}^{-1}$ ) and VAL ( $0.2 \mu\text{g}/\text{band}^{-1}$ ) using hexane–ethyl acetate (5: 5, *v/v*) as a mobile phase with UV detection at 260 nm.

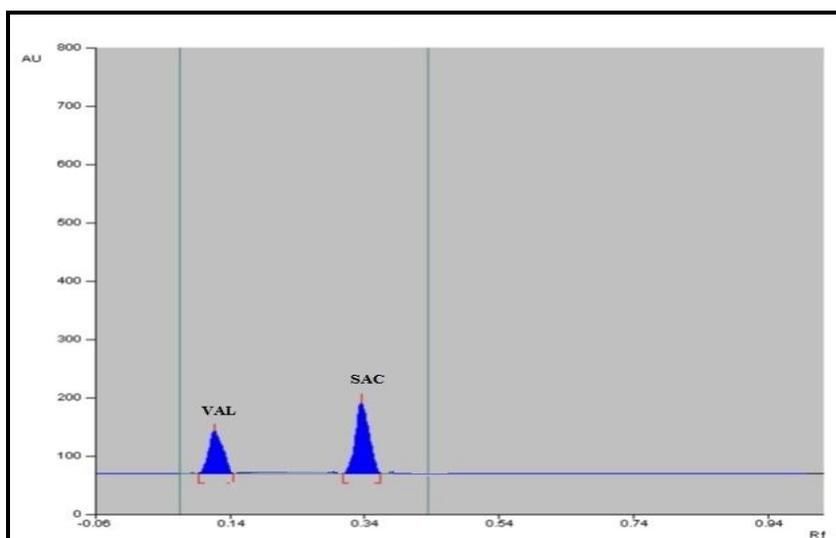


Fig. 4: TLC-densitogram of SAC ( $0.2 \mu\text{g band}^{-1}$ ) and VAL ( $0.2 \mu\text{g band}^{-1}$ ) using toluene-ethyl acetate (5: 5, v/v) as a mobile phase with UV detection at 260 nm.

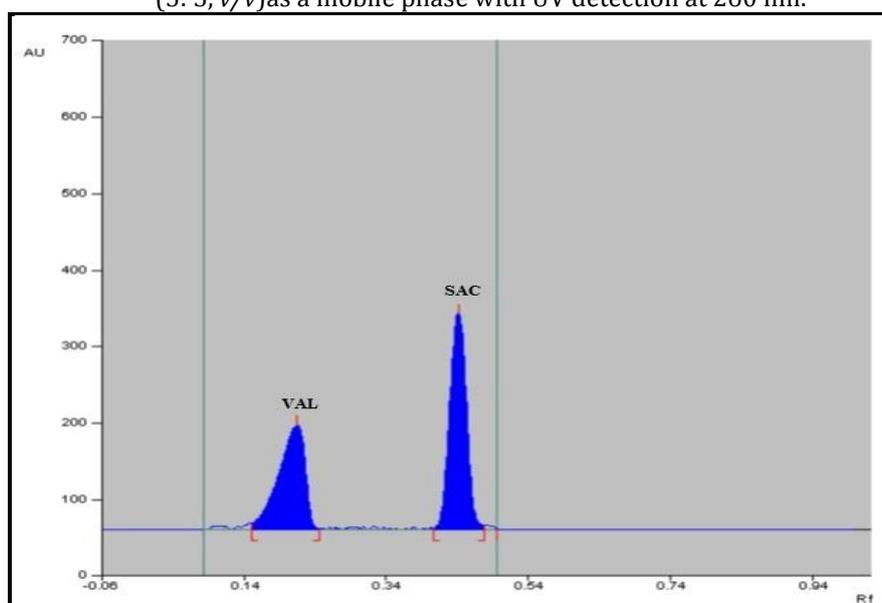


Fig. 5: TLC-densitogram of SAC ( $0.2 \mu\text{g band}^{-1}$ ) and VAL ( $0.2 \mu\text{g band}^{-1}$ ) using toluene-ethyl acetate-methanol (4: 4: 2, by volume) as a mobile phase with UV detection at 260 nm.

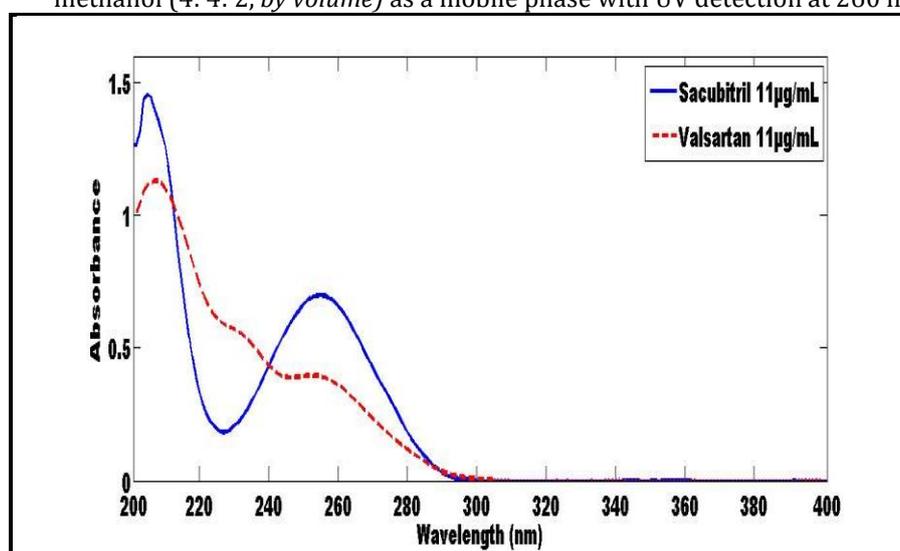


Fig. 6: Absorption spectra of SAC and VAL.

### Method Validation

Validation of the described methods was performed in a compliance with International Conference of Harmonization (ICH) guidelines [32].

- **Linearity**

Under the optimum TLC-densitometric conditions, calibration graphs for SAC and VAL were constructed by plotting the peak area values of the separated bands versus the drugs concentrations in  $\mu\text{g}/\text{band}^{-1}$ . The regression plot was found to be linear over the range of 0.1–0.6  $\mu\text{g}/\text{band}^{-1}$  for SAC and VAL. Values of slopes, intercepts and coefficient of determination ( $r^2$ ) are presented in **Table 1**.

- **Limit of detection (LOD) and limit of quantitation (LOQ)**

Residual standard deviation of the regression line ( $S_a$ ) and slope were used for calculation the LOD ( $3.3 S_a / \text{slope}$ ) and LOQ ( $10 S_a / \text{slope}$ ), The obtained results are presented in **Table 1**.

- **Accuracy**

Accuracy was calculated as a mean percent recovery of three determination for three concentration levels (0.2, 0.3, 0.4  $\mu\text{g}/\text{band}^{-1}$ ) and the results are presented in **Table 1**. Moreover, standard addition technique was applied to assess the accuracy and there was no interference from excipients (**Table 2**).

- **Precision**

Precision was calculated as a relative standard deviation of three determination for three concentration levels (0.2, 0.3, 0.4  $\mu\text{g}/\text{band}^{-1}$ ) within one day for repeatability and on three successive days for intermediate precision and the results are presented in **Table 1**.

- **Specificity**

The specificity is the ability to assess the analyte of interest in the presence of the related compounds expected to be present as matrix components or blank compositions. The blank and placebo were tested to confirm that no interference at the  $R_f$  values of SAC and VAL in the densitograms. Comparison of TLC densitograms of SAC and VAL with blank and sample matrix, without active ingredients, confirmed that there were no significant bands with similar or near  $R_f$  values of the studied drugs. There was a baseline ramp up to  $R_f$  values of 0.08 in the placebo densitogram but not interfere with the analysis of the studied drugs. The densitograms of SAC, VAL, blank and placebo using the described method as shown in **Fig. 7**.

Moreover, the results of the standard addition technique (**Table 2**) prove that the tablet excipients do not interfere with any of two separated drugs.

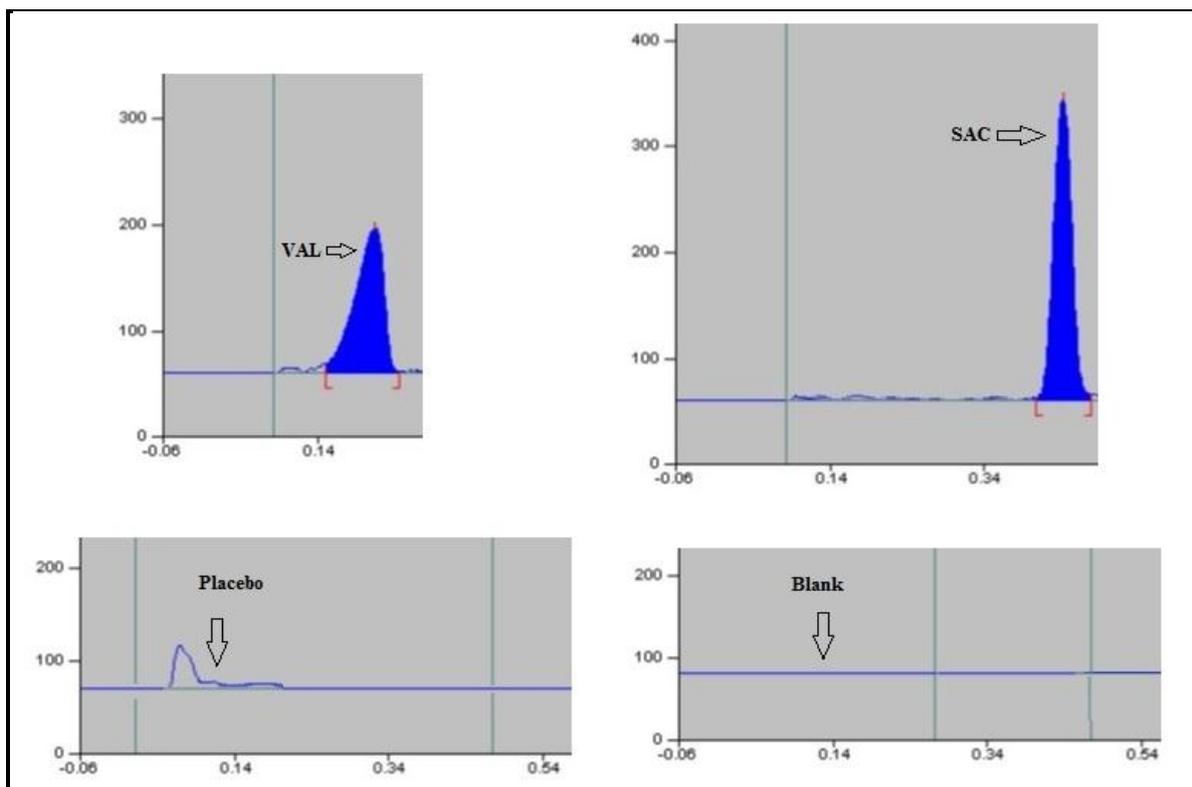


Fig. 7: TLC-densitogram of SAC and VAL, placebo and blank by the described method.

- **Robustness**

The method was found to be robust, as it wasn't appreciably influenced by minor deviation in experimental parameters, e.g.: changing methanol volume in the developing system  $\pm 2\%$  and changing saturation time  $\pm 2$  min. These proved by smaller values of RSD as shown in **Table 1**.

- **System suitability**

Parameters including capacity factor (K), tailing factor (T) and resolution factor (R) were calculated to determine if the operating system were performed properly. The obtained values were in the acceptable ranges as shown in **Table 3**.

Table 1: Regression and validation data for determination of SAC and VAL by the proposed method.

Parameter	SAC	VAL
Slope	45263.857	65087.057
Intercept	433.400	3876.087
Coefficient of determination ( $r^2$ )	0.9996	0.9997
Range ( $\mu\text{g band}^{-1}$ )	0.1–0.6	0.1–0.6
Accuracy (mean %R) *	99.79	99.66
Repeatability (%RSD) *	1.046	1.179
Intermediate precision (%RSD) *	1.179	1.488
LOD ( $\mu\text{g band}^{-1}$ )	0.012	0.011
LOQ ( $\mu\text{g band}^{-1}$ )	0.036	0.035
Robustness (%RSD)	Methanol volume $\pm 2\%$	0.835
	Saturation time $\pm 2$ min	0.689

\* Average of three determinations for three concentrations repeated three times.

Table 2: Determination of SAC and VAL in Entresto<sup>®</sup> tablets by the proposed method and application of standard addition technique

Entresto <sup>®</sup> tablets		Standard addition technique							
% Recovery* $\pm$ %RSD		Pharmaceutical ( $\mu\text{g band}^{-1}$ )		Pure added ( $\mu\text{g band}^{-1}$ )		Pure found ( $\mu\text{g band}^{-1}$ )		% Recovery**	
SAC	VAL	SAC	VAL	SAC	VAL	SAC	VAL	SAC	VAL
100.11 $\pm$ 1.170	100.46 $\pm$ 0.861	0.1	0.1	0.1	0.1	0.099	0.100	99.22	100.21
				0.2	0.2	0.201	0.203	100.54	101.68
				0.3	0.3	0.302	0.303	100.74	101.11
Mean $\pm$ %RSD								100.17	101.00
								$\pm 0.825$	$\pm 0.731$

\*Average of five determinations.

\*\*Average of three determinations.

Table 3: System suitability testing parameters of the developed TLC–densitometric method

Parameters	SAC	VAL	Reference value [33]
Capacity factor (K)	1.439	6.143	1-10
Tailing factor (T)	0.842	1.173	< 2
Resolution factor (R)	3.857		> 2

#### Application to Pharmaceutical Formulations

The described method was applied for determination of SAC and VAL in Entresto<sup>®</sup> tablets. Satisfactory results were obtained in good agreement with the label claim. Standard addition technique was applied, and the results indicate no matrix interference. Statistical analysis of the obtained results and those obtained by the reported method [5] by applying *t*-test and *F*-test at 95% confidence level indicate no significant differences, as shown in **Table 4**.

Table 4: Statistical comparison of the results obtained by applying the proposed method and the reported method [5].

Parameters	Proposed method		Reported method*	
	SAC	VAL	SAC	VAL
N**	5	5	5	5
$\bar{X}$ ***	100.11	100.46	100.15	99.75
%RSD	1.170	0.861	1.212	1.371
Variance	1.372	0.748	1.472	1.869
<i>t</i> -test (2.306)****	0.050	0.974		
<i>F</i> -test (6.388)****	1.073	2.499		

\* HPLC determination on C<sub>18</sub> column using mobile phase consists of acetonitrile: methanol: water (pH 3.0 adjusted with Ortho-phosphoric acid) (30: 50: 20, *by volume*).

\*\* Number of experiments.

\*\*\* The mean of percent recovery of pharmaceutical preparation.

\*\*\*\* The values in parenthesis are tabulated values of "t" and "F" at (P = 0.05).

## Conclusion

In this work, simple, fast and economic TLC-densitometric method has been successfully applied for determination of SAC and VAL in their mixtures and in their pharmaceutical formulation without prior separation. The major advantage of the applied method above all reported liquid chromatographic methods is that several samples can be run simultaneously using a small quantity of mobile phase, thus lowering the analysis time and cost per analysis with high sample throughput. On the other hand it doesn't need extra mathematical processing steps as in spectroscopic methods.

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