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Research Article

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A novel of derivative spectrophotometry as rapid and accurate method in application of simvastatin co-crystal assay

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ABSTRACT

The objective of this study was to obtain a rapid and valid method of quantitative analysis for the determination simvastatin (SV) in combination with saccharin (SAC) and aspartame (ASP) in the co-crystal form using derivative spectrophotometric. Co-crystal preparation was conducted using solvent drops grinding (SGD). Samples of each co-crystal of ASP and SAC were dissolved in a solvent mixture of the methanol-water with a ratio (60:40); after- ward it was derived using the zero crossing order for determining level of SV. The analytical method was validated based on validation parameter requirements, including, linearity, range, accuracy, precision, limit of detection (LOD) and the limit of quantitation (LOQ). The derivate spectrometric method have exhibited the zero crossing order in the third order derivate at 251 nm, linearity studies showed the value r^2=0.9998 in a concentration range 0.25-2µg/ml. The accuracy was presented by the %recovery = 98.69%- 103.74%, and precision (%RSD) =1.67% - 2.96%. LOD and LOQ results presented a value 0.096 and 0.32µg/ml, respectively. Derivative spectrophotometric has been applied to the SV determination in co-crystal form and fulfilled all requirement of a method validation.

Keywords: Aspartame; Saccharin; Co-crystal; Simvastatin; Spectrophotometric derivative.

INTRODUCTION

SV (Figure. 1) is antihypercolesterolemia drug that it can reduce about 30% of LDL and total cholesterol in the body. In addition, SV was more tolerated by the body than other antihypercolesterolemia drugs (Kulhari *et al*., 2013), so that it became the drug of choice for most patients with antihypercolesterolemia (Aronson *et al*., 2016). Unfortunatel y, its solubility in water is very low, and it correlates with very low bioavailability (Murtaza, 2012). One potential method to improve the solubility of the SV was made in co-crystal form (Sopyan *et al*., 2016). Co-crystal is a crystal whose a structured arrangement, consists of the active substance and the crystal-forming components (co-former) with a certain stoichiometric ratio (Rodríguez, 2007). ASP and SAC (Figure. 1) are two examples of co-formers that used to increase solubility and enhance the dissolution rate of the drugs (Serajuddin, 1999). Nevertheless, it becomes troubled in Assay of drug level because of each wavelength were overlapping in the mixture.

The maximum wavelengths of the SV, SAC, and ASP in the methanol-water's combination was 240 nm, 245 nm, and 254 nm respectively, consequently, they were not allowed for determining a ternary mixture using an ordinary spectrophotometry (Saraan *et al*., 2014). It needs a selective method to determine SV in combination with ASP and SAC. Nowadays, the analysis of simvastatin in co-crystal form with SAC or ASP as co-former using derivative spectrophotometry methods have not been reported. Several studies had been conducted to determine the levels of statin derivative drugs in mixtures substance, such as HPLC with a UV detector (Sravani *et al*., 2015), HPTLC (Kumar *et al*., 2010), and Powder X-ray Diffraction (PXRD) for the quantification in co-crystal form (Sahu *et al*., 2007). These methods need complicated preparation of the samples, besides data analysis is not simple.

Derivative spectrophotometry is a technique to transform the origin spectrum of a substance into a new spectrum as a result of derivation function. Derivative spectrophotometric had been done for determination of the drug substance in the bulk and dosage form by zero crossing method (Patil *et al*., 2016) such as zero order derivative (Jeeboi *et al.*, 2016), first order derivative (Chavada *et al*, 2015), second order derivative (El - Gindy *et al*., 2003) and third order derivative (Vimal *et al.,* 2010). It was chosen as a method because it is relatively inexpensive, does not time consumes, the necessary tools readily available, and easy to operate (Şentürk *et al*., 2002).

The aim of this study was to get a valid, simple in the term and process, and a rapid quantitative analysis

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method for simvastatin assay in co-crystal form using derivative spectrophotometry without requiring the separation process.

MATERIALS AND METHODS

Materials: simvastatin was obtained from Teva with purity > 99% (Belgium), aspartame Pro Analysis was purchased from Merck (Germany), saccharin pro Analysis was obtained from Merck (Germany), and methanol pro Analysis was purchased from Merck (Germany).

Apparatus: Ultraviolet-visible Spectrophotometer (Specord 200, Analytical jena, Germany)

Preparation of SV co-crystal

Co-crystal of SV was made by solvent drops grinding method. Accurately weighted SV and co-former (ASP and SAC) using an equivalent molar ratio. The mix of the SV with each co-former was grinded and assisted by drop by drop methanol as solvent, after 10 minutes dried at room temperature for 24h.

Determination of maximum wavelength for SV, ASP and SAC

A certain amount of stock solution with a concentration of 200µg/ml simvastatin was pipetted then gradually diluted using a solvent mixture of methanol :water (60:40) in order to obtain a final concentration of 14.33 and 23.89mM. Each of this solution was measured at a wavelength range between 200-300 nm with a solvent mixture of methanol:water (60:40) as a blank, in order to obtain the maximum wavelength (Figure. 2)

Determination of SV wave length (zero crossing)

SV, ASP and SAC with each concentration 10µg/ml were created absorbance spectrum absorption. Furthermore, the results of each spectrum were derived mathematically in the wavelength interval of 200 -300 nm from zero order to the third order. The spectrum of each substance from each derivative order was overlapped. It has be done to known the order and wavelength measurement of simvastatin, in which ASP and SAC have shown absorbance=0 (Figure. 3).

Preparation of calibration curve

Preparation of calibration curve was made by a series of the mixed solution containing SV, ASP, and SAC. Variation of simvastatin concentration was made (0.25; 0.75; 1.25; 1.7; 2) µg/ml with a concentration of ASP and SAC were constant at 0.5 and 0.3µg/ml. The solution mixture was measured on the order and the wavelength measurement of SV, whereas the absorbance of ASP and SAC=0, respectively, in three times. (n=3)

Validation of analysis method

Measurment of linearity

The linearity studies had carried out by calculating the coefficient correlation (r) which was in linear regression of a simvastatin standardization curve.

Determination of Accuracy and precision

Accuracy and Precision were obtained by measuring the concentration of three variations of a simulated sample created by mixing SV, ASP and SAC then dissolved in a solvent mixture of methanol :water (60:40). Simvastatin's concentration was (0.6; 1; 1.5µg/ml), whereas the Aspartame's and Saccharin's concentrations were fixed at 0.5 and 0.3µg/ml. Each variation concentration was measured on SV wavelength measurement respectively in six times and then calculated %recovery and relative standard deviation (%RSD). The intermediate result of precision expreseed by Intraday and interday precision. Intraday precision was determined by assay of the SV equivalent to 0.8µg/ ml in co-crystal samples within the day (n=3). The inter day precision was determined by analyzing a same concentration of sample daily for three days.

Calculation of the LOD and LOQ

The value of the limit of detection and limit of quantitation were calculated statistically using linear regression line of SV calibration curve using formula below:

$$Residual\ Standard\ Devitaion\ (Sy/x) = \sqrt{\frac{\sum_i (Yi - Yi')^2}{n-2}}$$

$$Limit\ of\ Detection\ (LOD) = \frac{3\mathrm{Sy/x}}{b}$$

$$Limitb\ of\ quantitasi\ (LOQ) = \frac{10\mathrm{Sy/x}}{b}$$

RESULTS

Determination of maximum wavelentgh (SV, ASP and SAC)

SV spectrum has presented maximum absorption at a wavelength of 240 nm with the absorbance value of 0.5285 (Figure. 2).

Measurement wavelengths of SV (zero crossing)

Each spectrum of substance had been obtained previously from 200-300 nm, subsequently it was derivated to dA/ dl in order to obtain a zero-crossing. Zero crossing of a mixture (three substances) was obtained at 251 nm in third order derivative (Figure. 3).

Preparation of calibration curve

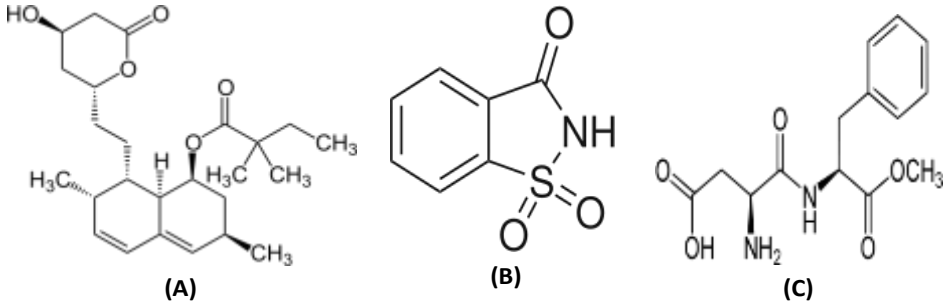
The measurement of the absorbance of the fifth variation of concentration performed on the calibration curve, the best linear regression equation produced was y=0.0017x – 0.00004, and r =0.9998 in a concentration range 0.25- 2µg/ml (Figure. 4).

Table 1: Calculation of SV maximum wavelength

Wavelength Calculation	
Diena (heteroanular)	214 nm
3 ring residue	15 nm
1 exocyclic bonding	5 nm
Total maximum	234 nm

Table 2: Molar extinction of SV

Molar Extinction Value of Simvastatin at 240 nm			
No	**Concentration (M)**	**(A)**	**e ($M^{-1}.cm^{-1}$)**
1	14,33	0,5411	22648,2
2	23,89	0,2768	23176,6
	Average	0,4089	22912,75



(A) **(B)** **(C)**

Figure 1: Structures of SV (A), SAC (B) and ASP (C)

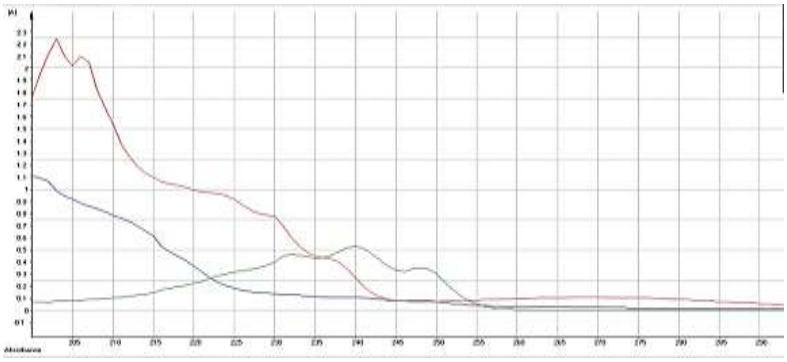


Figure 2: Spectrum overlay of zero order from SV (-), Asp (-) and Sac (-)

Table 3: linaerity of calibration curve

Replication	Value		
	Intercept	Gradien	Correlation coefficient
1	-4 x 10^{-5}	0.0017	0.9998
2	-2 x 10^{-5}	0.0017	0.9978
3	+2 x 10^{-5}	0.0018	0.9988

Table 4: Results studies of accuracy and precision

Replication (n=6)	% Recovery (µg/ml)		
	0.6	1	1.5
1	101.96	98.43	102.22
2	98.69	96.47	102.22
3	98.69	102.35	104.84
4	95.42	98.43	106.14
5	95.42	102.35	102.22
6	101.96	96.47	104.84
X	98.69	99.08	103.74
% RSD±SD	2.96±0.017	2.7±0.026	1.6±0.026

Table 5: Result studies of intermediet precision

Precision	% Label claim estimated* (Mean ± % RSD)
Intra day	0.7941± 0.7291
Inter day	0.7649± 1.4039

Table 6: Calculation of LOD and LOQ

NO	X	Y	Yi	$(Y-Yi)^2$
1	0.25	0.00040	0.000385	2.25×10^{-10}
2	0.75	0.00123	0.001235	2.77×10^{-12}
3	1.25	0.00213	0.002085	2.34×10^{-9}
4	1.75	0.00296	0.002935	1.00×10^{-9}
5	2	0.00343	0.003360	5.37×10^{-9}
Sy/x				5.46×10^{-5}
LOD				0.096
LOQ				0.32

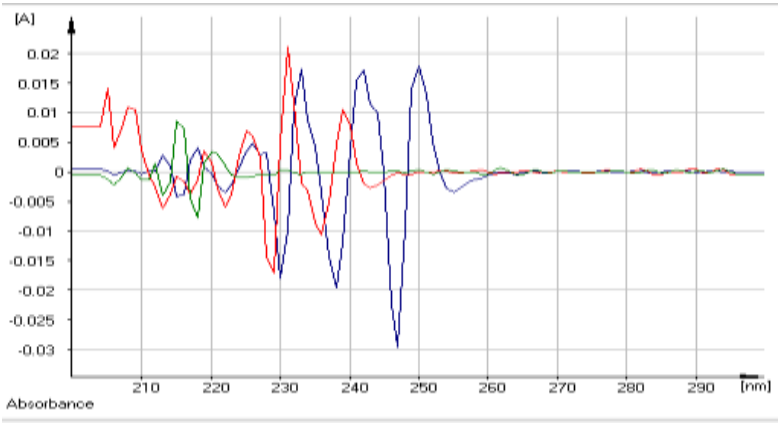


Figure 3: Spectrum overlay of third order derivative from SV (-), Sac (-) and Asp (-)

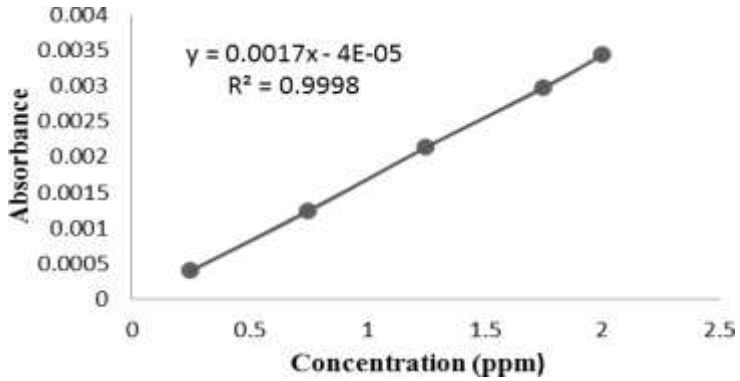


Figure 4: Calibration curve of simvastatin

Validation of analysis method

Validation of analysis method used to prove that the method used was appropriate for the desired purpose, accordingly, the method analysis have to fulfilled the recent requirements, such as:linearity, range, accuracy, precision, LOD and LOQ.

Linearity

Based on the calculation of correlation coefficient (r) from the best linear regression equation, linearity parameter was set at r=0.9998 in a concentration range 0.25- 2µg/ml.

Accuracy and Precision

Accuracy value was expressed as %recovery. It is method generated an average of the %recovery in the range 98.69%- 103.74% (Table. 4). The results of precision studies (%RSD) of the method was 1.67%- 2.96% (Table. 4). The intermediate result of precision expreseed by Intraday and interday precision (Table. 5).

LOD and LOQ

Based on an approach to the calculation of standard deviation of the instruments response and gradients,

the LOD and LOQ of the method were 0.096µg/ml and 0.32µg/ml respectively (Table. 6).

DISSCUSION

The maximum wavelength of the SV was observed at 238 nm in zero order. The simvastatin's maximum wavelength, calculated theoretically by Woodward Fischer rules (Table. 1), would be found at 234 nm (Yadav, 2013). The example of difference in maximum wavelength was myrcene molecule whose maximum wavelength calculation results in 219 nm, if it was measured at 224 nm. This shift could be caused by several factors, such as, because the solvent used as well as co-planarity or conformation of molecules conjugated system, The value of the molar extinction of simvastatin obtained in this research had an average value of 22912.75 M-1.cm^{-1} (Table. 2). The resulting value in the calculation of molar extinction simvastatin was greater than 10.000 M^{-1}cm^{-1}, accordingly, it is shown that the chromophore system of the SV was quite good and can be analyzed quantitatively using UV-Vis spectrophotometry. The spectrum SV has a peak absorption at a wavelength of 238 nm, 246 nm, and 258 nm, in which ASP had 4 absorption peaks at a wavelength 247 nm, 252 nm, 258 nm, and 264 nm. SAC's spectrum showed the absorption peak of 230 nm, 237 nm, and 270 nm. Based on a maximum wavelength of each substance, the assay of simvastatin in the co-crystal could not be done at its absorption peak because overlapping spectrum with both co-formers. The weak non-covalent interaction between SV and its co-former would be disconnected because their interaction with the solvent and would be returned to their respective substances (Snyder *et al.*, 1997). Therefore, assay of simvastatin in the co-crystal form was done by spectrophotometric method using derivative zero crossing techniques (El -Gindy *et al.*, 2003). This value of correlation cooeficient was greater than (r > 0.99) that has fulfilled the requirement of validation parameter for (r) (Table. 3). The accuracy has been showed good accuracy. Accordingly, the value of acceptable accuracy requirement for samples in the concentrations range 0.1–10µg/ml must be into 80-110% (Snyder *et al.*, 1997). The (% RSD) produced indicating a good precision because its value was allowed for samples with concentrations 0.1- 10µg/ ml is <11%. The intermediate precision showed a good value %RSD < 2% (Table. 5) (Snyder *et al.*, 1997). The value of LOD and LOQ of the method have indicated that the method was strong enough to determination of SV in cocrystal form with ASP and SAC as coformer.

CONCLUSION

The derivatives spectrophotometric method using zero crossing in the third derivate has been applied to determination of simvastatin in the co-crystal form and validation studies had exhibited the acceptable value requirement of the validation parameters.

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