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Short communication

Two derivative spectrophotometric methods for the simultaneous determination of lovastatin combined with three antioxidants

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Abstract

A zero crossing and an algorithm bivariate calibration derivative method for the simultaneous determination of lovastatin combined separately with three antioxidants (ascorbic acid, quercetin and gallic acid) in synthetic mixtures are described. The aqueous or methanolic solutions obeyed Beer's law in the concentration ranges of 3.20–17.36 μg/ml for lovastatin, 1.76–8.80 μg/ml for ascorbic acid, 1.41–7.04 μg/ml for gallic acid and 1.84–9.20 μg/ml for quercetin, for both methods, respectively. In the second derivative (^{2}D) zero crossing method measurements were carried out at 238.4 nm for lovastatin and 265.6 nm for ascorbic acid, 247.7 nm for lovastatin and 281.1 nm for quercetin, 251.8 nm for lovastatin and 267.6 nm for gallic acid. In the first derivative (^{1}D) bivariate spectrophotometric method an optimum pair of wavelengths was chosen for the determination of different binary mixtures. The proposed procedures were successfully applied to the simultaneous determination of lovastatin and different antioxidants in mixtures with high percentage of recovery, 98.3–100.4% for lovastatin, 98.3–98.6% for ascorbic acid, 99.0–99.8% for quercetin and 100.5–101.1% for gallic acid and good precision. In addition, the results from the above procedures were verified by using partial least-squares (PLS) multivariate calibration method.

Keywords: Spectrophotometric methods; Lovastatin; Antioxidants

1. Introduction

Lovastatin is a potent inhibitor of 3-hydroxy-methyl-3-glutaryl-coenzyme A (HMG-coA) reductase, the rate-controlling enzyme in cholesterol biosynthesis [1,2]. Hence, it has been proved effective in lowering the plasma cholesterol level in both animals and humans. There are numerous reports in the literature describing the susceptibility of this and other "statin" drugs to oxidative degradation [3] but the addition of quercetin, ascorbic and gallic acids, as antioxidants, has been found to stabilize them in the solid state [4]. Therefore, ascorbic acid is present as a preservative in different commercial formulations [5].

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In the European and USP 26 Pharmacopoeias [6,7] different HPLC procedures are recommended for the determination of the drug in crude and in single component formulations.

Using UV-spectrophotometry, a considerable number of methods have been reported for the quantitive determination of lovastatin or antioxidants in pharmaceutical dosage forms [8–13]. However, no method for the simultaneous UV-determinations of the above compounds appears to be available.

The principal advantage of derivative measurements is the potential reduction in the error caused by the overlap of the spectral band by interfering absorption bands of known and unknown substances. One of the most common and reliable derivative approaches for the construction of calibration graphs is the zero crossing method, developed by O'Haver [14–17]. However, the satisfactory resolution of the mixture with this technique depends on the degree of overlapping of the spectra of interest and the intensity of the bands obtained after differentiation. In the present investigation the zero crossing of lovastatin in the presence of the three antioxidants is produced at wavelength(s) where the signal(s) is not particular intense. Moreover, the signals of the two analytes, lovastatin–ascorbic acid, become less prominent as thiosulfate is added to the mixture to stabilize the latter [18].

In addition, a new bivariate algorithm [19–21] is alternatively applied to the resolution of binary mixtures using first derivative spectrophotometry.

The aim of the present study was the development of two UV-spectrophotometric, methods (a ^{2}D zero crossing and a ^{1}D bivariate procedure) for the simultaneous determinations of lovastatin combined with three different antioxidants in synthetic mixtures and in spiked pharmaceutical formulations (Mevacor®).

2. Experimental

2.1. Apparatus

A Shimadzu UV–Vis double beam Spectrophotometer model UV-2501 PC consisting of a double monochromator with a high performance double-blazed holographic grating in the aberration corrected Czerny–Turner mounting and a light source of both a 50 W halogen lamp and a D_2 lamp. The optimized operating conditions for spectrophotometric measurements were: ^{1}D, ^{2}D derivative modes, scan speed 210 nm/min, slit width 1.0 nm and sampling interval 0.1 nm.

2.2. Chemicals, reagents and solutions

All used chemicals were of analytical reagent grade, unless otherwise specified. Lovastatin was obtained from Merck. Sigma–Aldrich Company supplied quercetin, ascorbic and gallic acid, sodium thiosulfate and methanol. Diluents were (a) aqueous solution 0.08% w/v $Na_2S_2O_3$:methanol (50:50). This specific diluent has the property of stabilizing ascorbic acid in water for at least 2 h whereas the former improves the disintegration of mixtures and commercial tablets. Moreover, methanol ensures the dissolution of the active compound (lovastatin). (b) 95% v/v methanol was used for the analysis of lovastatin combined with gallic acid or quercetin.

2.3. Procedure

About 22.0 mg of lovastatin and the same amount of ascorbic acid reference standards were accurately weighed and transferred to separate 50 ml amber volumetric flasks. A portion of 0.08% w/v $Na_2S_2O_3$:methanol (50:50) was added to dissolve the analytes by shaking and then made up to volume with the same diluent. 10.0 ml of each standard were transferred to two different 100 ml amber volumetric flasks and diluted with 0.08% w/v $Na_2S_2O_3$:methanol (50:50). Thus two different intermediate stock solutions were obtained. Seven different portions of the latter were transferred to 25 ml amber volumetric flasks to yield three series of standard lovastatin, antioxidants and mixed solutions. The same procedure was followed for the analysis of lovastatin combined with gallic acid or quercetin using methanol 95% v/v. The three analytes (lovastatin, quercerin and gallic acid) remained stable for at least 48 h in methanolic solution.

2.4. Procedure for tablets

No less than 20 tablets (Mevacor®) were weighed and the average tablet weight was determined. The tablets were finely powdered and a portion of powder equivalent to one average tablet weight was quantitatively transferred to a 200 ml amber volumetric flask. 100 ml of 0.08% w/v $Na_2S_2O_3$:methanol (50:50) was added and the dispersion was vigorously shaken for 30 min on a mechanical shaker. Then 5 mg of ascorbic acid in dissolving solvent were added, ultrasonication followed for 10 min and the solution was diluted to volume with the same diluent and left to precipitate. Appropriate dilutions were made from the clear supernatant solution so that the concentration of each sample solution approached the concentration of that in the middle of the standard solution range. Filtration with acrodisc GHP was used to ultra clean the solutions of particles 0.45 μm or greater. The excipients and additives present in the commercial tablets were pregelatinized maize starch, microcrystalline cellulose, magnesium stearate, butylated hydroxyanisole, lactose monohydrate.

3. Results and discussion

3.1. Second derivative zero crossing spectrophotometric method

Zero crossing value means zero contribution from the spectra of co-existing compounds. Therefore, it appears to be possible to determine lovastatin combined with an antioxidant by reading the second derivative spectra of their binary mixture with no prior separation procedures [22–24].

In fact in the zero and first order derivative spectra within the range of 210–350 nm, the absorption curve of lovastatin is overlapped extensively by antioxidants. This is clearly illustrated in Fig. 1A. Fortunately, in the second derivative spectra in the same wavelength range, two zero crossing points exist on which quantitation can be based, Fig. 1C. The smoothing function and the influence of the $\Delta\lambda$ for the zero and second derivative order were tested and it was found appropriate to use the values $\Delta\lambda = 1$ and 8, respectively, in the determination of compounds. Furthermore, the spectra were smoothed through the use of 17 experimental points with a scaling factor of 100. The derivative absorbances in the second derivative spectra of the standard and sample solutions, recorded against solvent blank and measured at (a) 238.4 nm for lovastatin and 265.6 nm for ascorbic acid. (b) 247.7 nm for lovastatin and 281.1 nm for quercetin (c) 251.8 nm for lovastatin and 267.6 nm for gallic acid. The concentration range for Beer's law compliance was 3.20–17.36 μg/ml for lovastatin, 1.76–8.80 μg/ml for ascorbic acid, 1.41–7.04 μg/ml for gallic acid and 1.84–9.2 μg/ml for quercetin. The regression equations were:

$$y = 251.8(\pm 3.1)10^{-3}x - 20.8(\pm 36.1)10^{-3}\text{(lov)}$$

and

$$y = 40.6(\pm 0.6)10^{-3}x + 17.7(\pm 3.6)10^{-3}\text{(asc)}$$

$$y = 60.0(\pm 0.5)10^{-3}x + 16.9(\pm 5.2)10^{-3}\text{(lov)} \quad \text{and}$$

$$y = 24.0(\pm 0.5)10^{-3}x + 2.3(\pm 2.5)10^{-3}\text{(querc)}$$

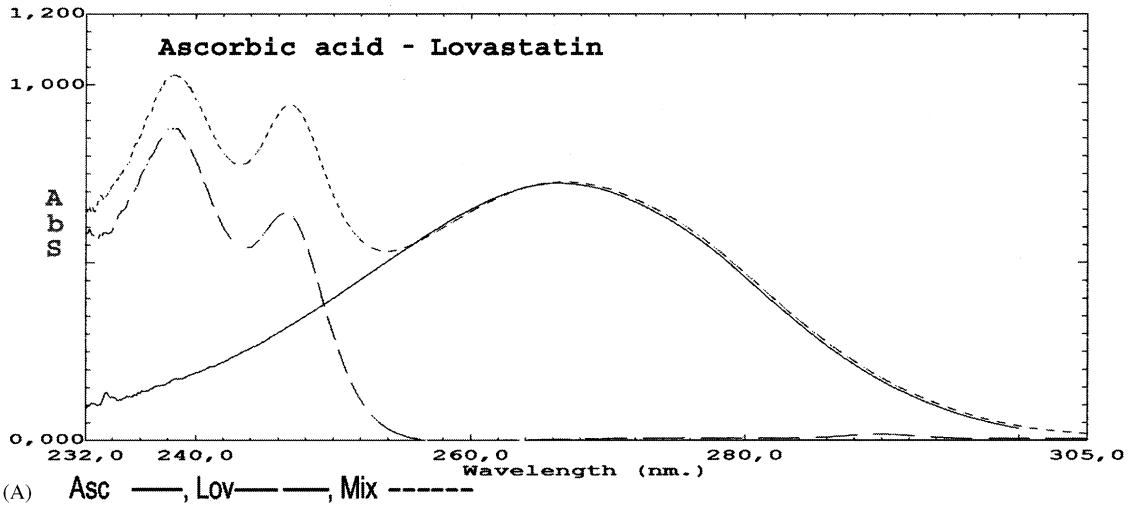
$$y = 74.5(\pm 0.2)10^{-3}x + 5.8(\pm 2.4)10^{-3}\text{(lov)} \quad \text{and}$$

$$y = 18.1(\pm 0.1)10^{-3}x - 2.2(\pm 0.6)10^{-3}\text{(gall)}$$

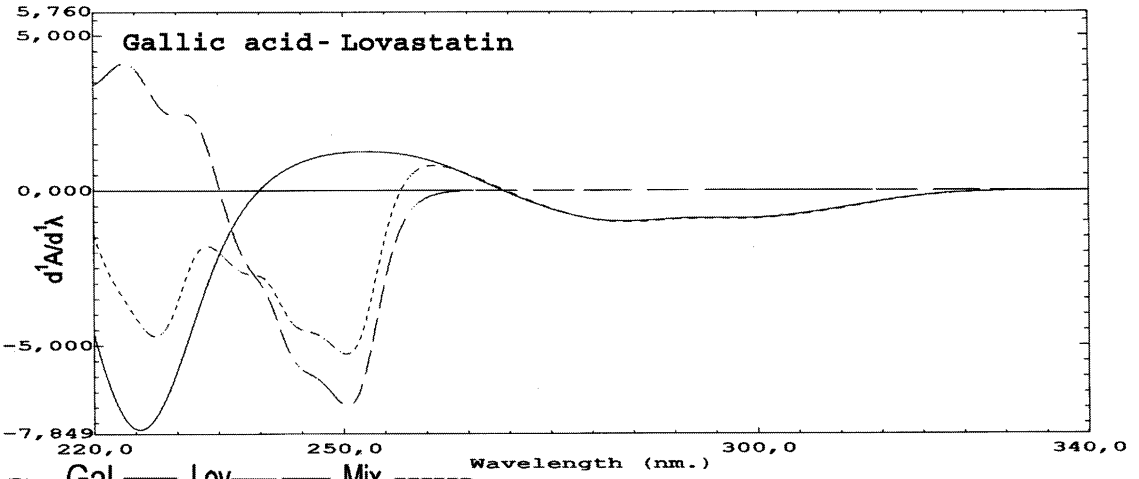
where x is the concentration in μg/ml and y is the $d^2A/d\lambda^2$ values.

The correlation coefficients of the calibration curves were 0.9997 for lovastatin and 0.9994 for ascorbic acid, 0.9998 for lovastatin and 0.9993 for quercetin, 0.9999 for lovastatin and 0.9999 gallic acid.

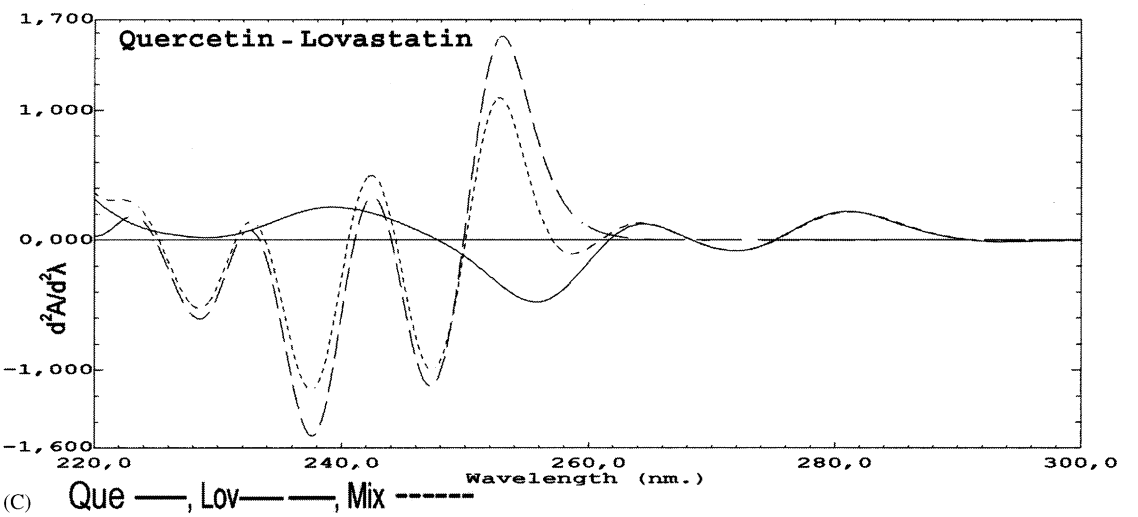
Recovery studies by using this method were performed on the synthetic mixtures prepared by adding accurately weighed amounts of drug concentration ranges. Accuracy data are presented in Table 1. A similar study was carried out to check the interference from the excipients used to the commercial dosage forms, Table 5.



(A) Asc ——, Lov— — —, Mix ------



(B) Gal ——, Lov— — —, Mix ------



(C) Que ——, Lov— — —, Mix ------

Fig. 1

3.2. Bivariate calibration spectrophotometric method

Alternatively the "bivariate calibration method" was applied to the first derivative spectra for the resolution of the binary mixtures of lovastatin and antioxidants: because in zero order "bivariate calibration method", a particular case arises when one or both of the analytes present broad bands or flat bands with no well-defined maximum. In such case similar consecutive results are expected within the range of wavelengths of the band.

In the present work the first derivative values of the two components located at five different wavelengths were selected, Fig. 1B. The optimization of the derivative spectra was based on the influence of the number of experimental points for smoothing the spectra (17 points) by a scaling factor of one hundred and the optimal $\Delta\lambda = 8$ used on the differentiation of the absorption spectra, with the object of optimizing the relation signal to noise ratio. Once the values from the regression lines of the determinants had been obtained (Table 2), the wavelength pair with the highest absolute sensitivity value was defined according to the method proposed by Kaiser [25] (Table 3). It is worth mentioning that, for the model proposed, it is necessary for the calibration curves of the two components to comply with Lambert–Beer's law at each wavelength, giving a straight line. Otherwise there will be a great error in determination, as the contribution of one of the components to the mixture will not be able to be assessed adequately. In the present investigation, most of the calibration curves at different wavelengths show a satisfactory linear regression coefficient (Table 2).

Indeed, the optimum pair of wavelengths for lovastatin–ascorbic acid was found to carry out the determination at 281.1 and 249.8 nm: the signal located for ascorbic acid is one of the most sensitive of the first derivative spectra with good linear relationship between this and the concentration. On the contrary, lovastatin does not possess a satisfactory linear relationship, probably due to the low sensitivity of its signal in this area. Following this, although the determinants of Kaiser matrices K, in which the wavelength 281.1 nm with its four pairs were considered as the most appropriate, they were rejected. Similarly, the four determinants at 235.6 nm with its pairs were not accepted (Table 3). Finally, the optimum wavelengths, according to Kaiser's method, were at 249.8 and 252.9 nm. For the relation of the mixture two calibration curves were initially considered for lovastatin and ascorbic acid, through the following system.

$$(\lambda_{249.8})^1D_{LOV\ \ ASC1} = (-587.65 \times 10^{-3})[LOV] + (290.99 \times 10^{-3}) \times [ASC] - (11.22 \times 10^{-3})$$

$$(\lambda_{252.9})^1D_{LOV\ \ ASC2} = (-281.94 \times 10^{-3})[LOV] + (299.48 \times 10^{-3}) \times [ASC] + 13.01 \times 10^{-3}$$

The algorithm solution of the above system of equations allows the determination of lovastatin and ascorbic acid.

$$[ASC] = \frac{(-281.94 \times 10^{-3})(^1D_{LOV\ \ ASC1} + 11.22 \times 10^{-3}) + (-587.65 \times 10^{-3})(13.01 \times 10^{-3} - {}^1D_{LOV\ \ ASC2})}{(-281.94 \times 10^{-3})(-11.22 \times 10^{-3}) - (-587.65 \times 10^{-3})(13.01 \times 10^{-3})}$$

Fig. 1. (A) Zero order absorption spectra of lovastatin 17.36 μg/ml–ascorbic acid 8.80 μg/ml and their mixture in aqueous 0.08% w/v $Na_2S_2O_3$:methanol (50:50), (B) (^{1}D) first derivative spectra of lovastatin 16.40 μg/ml–gallic acid 7.04 μg/ml and their mixture in methanol and (C) (^{2}D) second derivative spectra of lovastatin 16.40 μg/ml–quercetin 9.20 μg/ml and their mixture also in methanol.

$$[\mathrm{LOV}] = \frac{^{1}\mathrm{D}_{\mathrm{LOV\ ASC1}} + 11.22 \times 10^{-3} - (290.99 \times 10^{-3})[\mathrm{ASC}]}{(-281.94 \times 10^{-3})}$$

The same procedure facilitates to determine the concentration of lovastatin and antioxidant in any mixture, knowing the values of the first derivative absorption spectra of the mixture at the same wavelengths.

Preparing pairs of synthetic mixtures in different concentration ranges enabled evaluation of the proposed method in three different pairs of wavelengths (a) 252.9, 249.8 for lovastatin–ascorbic acid (b) 250.5, 247 for lovastatin–quercetin (c) 250.5, 225.3 for lovastatin–gallic acid. The recovery results obtained are shown in Table 1.

3.3. Partial least squares

A multivariate calibration method was developed using the same standard solutions of lovastatin–antioxidants in order to confirm the derivative results from synthetic mixtures.

Partial least-square method (PLS) was evaluated by using a soft independent modeling of class analogy (SIMCA-P 9) software and a comparative study of the prediction capabilities of the applied chemometric approach was undertaken in the zero order spectra [26]. Further investigation has shown that zero order spectra yielded regression lines with much better linearity and better recoveries compared with the other derivative orders. The prediction error sum of squares (PRESS), and the fraction of the total variation (Q^2) of the Y's that can be predicted by a component, are good measures of the predictive power of the model and giving information about the significant of a component. Using the cross-validation method with two significant components, the following statistical parameters have been taken.

The root mean square error of prediction (RMSEP), which is the standard deviation (S.D.) of the predicted residuals (error). It is computed as:

$$\mathrm{sqrt}(\sum(\mathrm{obs} - \mathrm{pred})^2/\mathrm{N}).$$

where N is the total number of calibration samples.

The root mean square error of estimation (RMSEE), which is the S.D. of the estimated residuals (error). It is computed as:

$$\mathrm{sqrt}(\sum(\mathrm{obs} - \mathrm{estim})^2/\mathrm{N}).$$

The square of the correlation coefficient (r^2), which is an indication of the quality of fit of all data to a straight line, is presented by:

$$r^2 = \sum_{i=1}^{N}(\hat{c}_i - c_i)^2 / \sum_{i=1}^{N}(c_i - \bar{c}_i)^2$$

where $\hat{c}_i$ represent the estimated concentration, c_i is the reference concentration and $\bar{c}_i$ represents the mean of the true concentrations in the predictor set.

In order to test the performance of the proposed method, PLS was applied to the resolution of synthetic mixtures using the same concentrations of both lovastatin–antioxidant. The % Relative Standard Deviation (R.S.D.), % mean recoveries, RMSEP, RMSEE and r^2 values have been calculated and summarized in Table 4.

3.4. Statistics

The two proposed methods were validated as to precision (reported as the R.S.D.%), linearity (evaluated by regression equations), detection and determination limits and accuracy (bias%). The limit of detection (LOD) and limit of quantitation (LOQ) of the procedure are also as shown in Table 6, which were calculated according to the following criterions. (a) Zero crossing method:

Table 1
Recovery results in synthetic binary mixtures of (a) ascorbic acid–lovastatin (b) quercetin–lovastatin (c) gallic acid–lovastatin, applying the zero crossing and bivariate method

Ascorbic acid					Lovastatin				
Added (μg/ml)	Zero crossing		Bivariate		Added (μg/ml)	Zero crossing		Bivariate	
	Found (μg/ml)	Bias%	Found (μg/ml)	Bias%		Found (μg/ml)	Bias%	Found (μg/ml)	Bias%
(*a*)									
1.76	1.68	−4.8	1.69	−3.9	3.47	3.43	−1.2	3.45	−0.7
2.64	2.55	−3.5	2.73	+3.5	5.21	5.22	+0.2	5.20	−0.1
4.40	4.29	−2.5	4.42	+0.4	8.68	8.52	−1.8	8.63	−0.6
5.28	5.18	−1.8	5.27	−0.1	10.42	10.21	−2.0	10.37	−0.5
6.16	6.10	−0.9	5.94	−3.6	12.15	11.91	−2.0	11.92	−1.9
7.92	7.94	+0.3	7.67	−3.2	15.62	15.28	−2.2	15.34	−1.8
8.80	8.91	+1.3	8.55	−2.8	17.36	17.10	−1.5	17.87	+1.8
Mean%recovery		98.3	98.6		Mean%recovery		98.5		99.5
%R.S.D.	2.0		2.6		%R.S.D.		0.8		1.1
Quercetin					Lovastatin				
Added (μg/ml)	Zero crossing		Bivariate		Added (μg/ml)	Zero crossing		Bivariate	
	Found (μg/ml)	Bias%	Found (μg/ml)	Bias%		Found (μg/ml)	Bias%	Found (μg/ml)	Bias%
(*b*)									
1.84	1.82	−1.1	1.79	−2.6	3.28	3.16	−3.6	3.27	−0.3
3.68	3.86	+4.8	3.68	0.0	6.56	6.40	−2.4	6.49	−1.1
4.60	4.48	−2.6	4.47	−2.8	8.20	8.38	+2.2	8.54	+4.2
6.44	6.41	−0.4	6.52	+1.2	11.48	11.97	+4.3	11.35	−1.1
9.20	9.05	−1.6	9.14	−0.6	16.40	16.37	−0.2	16.42	−0.1
Mean%recovery		99.8		99.0	Mean%recovery		100.1		100.4
%R.S.D.		2.6		1.6	%R.S.D.		2.9		2.0
Gallic acid					Lovastatin				
Added (μg/ml)	Zero crossing		Bivariate		Added (μg/ml)	Zero crossing		Bivariate	
	Found (μg/ml)	Bias%	Found (μg/ml)	Bias%		Found (μg/ml)	Bias%	Found (μg/ml)	Bias%
(*c*)									
1.41	1.38	−2.3	1.42	+0.8	3.28	3.18	−2.9	3.26	−0.6
2.82	2.90	+2.8	2.86	+1.3	6.56	6.49	−1.1	6.57	+0.1
3.52	3.63	+3.0	3.56	+1.0	8.20	8.13	−0.8	8.23	+0.4
4.93	5.05	+2.4	4.91	−0.4	11.48	11.20	−2.4	11.25	−2.0
7.04	7.03	−0.2	7.01	−0.4	16.40	16.22	−1.1	16.47	+0.4
Mean%recovery		101.1		100.5	Mean%recovery		98.3		99.7
%R.S.D.		2.0		0.7	%R.S.D.		0.8		0.9

Mean of three replicates.

Table 2
Correlation data of calibration curves to ^{1}D spectrum obtained for the two component (a) lovastatin–ascorbic acid, (b) lovastatin–quercetin and (c) lovastatin–gallic acid, at the selected wavelengths and considered as sensitivity parameters in Kaiser's matrix

nm	Lovastatin			Ascorbic acid		
	Slope $\times 10^{-3} \pm$S.D. $\times 10^{-3}$	Intercept $\times 10^{-3} \pm$S.D. $\times 10^{-3}$	Correlation coefficient	Slope $\times 10^{-3} \pm$S.D. $\times 10^{-3}$	Intercept $\times 10^{-3} \pm$S.D. $\times 10^{-3}$	Correlation coefficient
(*a*)						
235.6	323.89 ± 3.22	110.49 ± 37.35	0.9998	131.85 ± 10.74	− 158.17 ± 62.37	0.9838
240.8	− 378.80 ± 4.35	80.24 ± 49.77	0.9997	195.45 ± 6.36	− 186.04 ± 36.74	0.9974
249.8	− 587.65 ± 3.37	21.84 ± 38.05	0.9999	290.98 ± 3.65	− 33.06 ± 20.95	0.9996
252.9	− 281.94 ± 1.83	18.98 ± 20.87	0.9999	299.48 ± 3.35	− 5.98 ± 19.23	0.9997
281.1	0.67 ± 0.67	14.05 ± 6.97	0.4901	− 375.10 ± 3.93	45.20 ± 23.17	0.9997
nm	Lovastatin			Quercetin		
	Slope $\times 10^{-3} \pm$S.D. $\times 10^{-3}$	Intercept $\times 10^{-3} \pm$S.D. $\times 10^{-3}$	Correlation coefficient	Slope $\times 10^{-3} \pm$S.D. $\times 10^{-3}$	Intercept $\times 10^{-3} \pm$S.D. $\times 10^{-3}$	Correlation coefficient
(*b*)						
223.6	238.65 ± 1.65	− 36.49 ± 16.63	0.9999	− 132.35 ± 4.75	− 25.06 ± 24.13	0.9980
230.7	143.35 ± 1.63	− 35.46 ± 16.96	0.9997	− 92.50 ± 7.85	− 39.76 ± 39.87	0.9895
247	− 348.72 ± 1.22	− 35.66 ± 12.25	0.9999	191.70 ± 3.26	− 28.55 ± 16.58	0.9996
250.5	− 396.92 ± 1.22	− 43.69 ± 12.13	0.9999	167.24 ± 3.23	− 15.47 ± 16.36	0.9995
393.2				− 256.70 ± 6.46	20.97 ± 32.74	0.9990
nm	Lovastatin			Gallic acid		
	Slope $\times 10^{-3} \pm$S.D. $\times 10^{-3}$	Intercept $\times 10^{-3} \pm$S.D. $\times 10^{-3}$	Correlation coefficient	Slope $\times 10^{-3} \pm$S.D. $\times 10^{-3}$	Intercept $\times 10^{-3} \pm$S.D. $\times 10^{-3}$	Correlation coefficient
(*c*)						
223.6	238.65 ± 1.64	− 36.49 ± 16.64	0.9999	− 1035.69 ± 11.54	9.10 ± 47.24	0.9998
225.3	221.58 ± 1.78	− 28.89 ± 17.73	0.9999	− 1102.02 ± 9.42	48.89 ± 38.64	0.9999
230.7	143.35 ± 1.63	− 35.46 ± 16.97	0.9997	− 767.83 ± 8.94	70.35 ± 36.73	0.9997
250.5	− 396.92 ± 1.25	− 43.69 ± 12.12	0.9999	182.90 ± 2.03	− 57.45 ± 8.74	0.9998
252.5	− 342.90 ± 1.17	− 50.24 ± 10.14	0.9999	194.20 ± 10.44	− 106.04 ± 42.34	0.9944

Table 3
Values of the selectivity matrix determinants calculated according to Kaiser's method ($K \times 10^{-3}$) for the mixture of (a) lovastatin and ascorbic acid (b) lovastatin and quercetin and (c) lovastatin and gallic acid

λ (nm)	235.6	240.8	249.8	252.9	281.1
(*a*)					
235.6	0	− 113 259.6	− 171 727.0	− 134 172.0	121 579.5
240.8		0	− 4609.7	58 361.8	− 141 986.9
249.8			0	**93 950.5**	− 220 232.6
252.9				0	− 105 555.0
281.1					0
λ (nm)	223.6	230.7	247	250.5	393.2
(*b*)					
223.6	0	3102.8	403.9	12 620.5	61 261.5
230.7		0	4776.4	12 741.2	36 797.9
247			0	**− 17 769.6**	− 89 516.4
250.5				0	− 101 889.4
393.2					0
λ (nm)	223.6	225.3	230.7	250.5	252.5
(*c*)					
223.6	0	33 508.9	34 776.5	367 437.0	308 795.4
225.3		0	12 161.2	**396 886.8**	334 855.1
230.7			0	278548.4	235 452.6
250.5				0	14 364.9
252.5					0

LOD = 3 s/m and LOQ = 10 s/m, respectively, where s, is the standard deviation of the blank (n = 5) and m is the slope of the corresponding calibration curve. (b) Bivariate method: LOD is the analyte concentration giving a signal, equal to the blank signal (in the corresponding two wavelengths for which measurements were taken) plus 3s, where s, is the standard deviation of the blank (n = 5).

After the determination of the two component mixtures, lovastatin–antioxidant, a comparative statistical study (paired-*t*-test and regression lines test) [27,28] of these two methods was carried out (Table 7). On comparing the results it can be observed that there is no statistically significant differences $t_{statist} < t_{critical}$ ($P = 0.05$) between the two methods, except for the determination of lovastatin in gallic or quercetin mixture. On the

Table 4
Statistical parameters and recovery results using PLS algorithm

	r^2	RMSEE	RMSEP	%Mean recovery	%R.S.D.
Lovastatin	0.9998	0.0832	0.5759	96.3	1.3
Ascorbic acid	0.9999	0.0428	0.0638	101.4	1.7
Lovastatin	0.9991	0.1995	0.5015	101.2	3.7
Quercetin	0.9998	0.0425	0.1441	100.6	3.7
Lovastatin	0.9997	0.1212	0.1106	100.8	1.5
Gallic acid	0.9999	0.0228	0.0784	101.6	1.0

Table 5
Results for lovastatin and spiked ascorbic acid obtained from commercial formulations

Lovastatin					Ascorbic acid				
Labeled amount (mg/tablet)	Zero crossing		Bivariate		Labeled amount (mg/tablet)	Zero crossing		Bivariate	
	Found (%)	%R.S.D. (n = 5)	Found (%)	%R.S.D. (n = 5)		Found (%)	%R.S.D. (n = 5)	Found (%)	%R.S.D. (n = 5)
10	98.54	1.32	98.81	2.05	5	98.32	1.56	98.59	1.42
20	99.12	1.71	99.26	1.64	5	99.17	1.83	99.86	2.18
40	101.76	1.86	102.11	1.68	5	102.16	2.14	101.00	2.24

Table 6
LOD and LOQ μg/ml for the analytes, by the two proposed methods

	Zero crossing		Bivariate	
	LOD	LOQ	LOD	LOQ
Lovastatin	0.218	0.727	0.238	0.795
Ascorbic acid	0.296	0.987	0.479	1.595
Lovastatin	0.885	2.949	0.423	1.409
Quercetin	0.491	1.637	0.235	0.783
Lovastatin	0.241	0.804	0.354	1.180
Gallic acid	0.332	1.105	0.185	0.615

other hand, the use of the regression line statistical study has shown that the calculated slopes and intercepts do not differ significantly from the ideal values of 1 and 0, respectively, and thus there is no evidence for systematic differences between the two methods.

4. Conclusion

The two methods enable the quantitation of mixture lovastatin–antioxidants, even in the presence of different excipients, with good accuracy and precision, either in laboratory prepared samples or in spiked pharmaceutical dosage forms.

Moreover, comparing the results obtained, the bivariate technique may be competitive and, in some cases even superior (in that results are more accurate) to commonly used zero crossing derivative spectrophotometric procedure. Finally, it was concluded that the short analysis time and low costs are the main advantages of these two UV derivative spectrophotometric methods for the determination of lovastatin in combination with antioxidants in routine analysis. High percentage recovery shows that the methods are free from interferences from the excipients and additives commonly used in the formulations of drugs. Although derivative spectroscopy has been used for a long time in pharmaceutical analysis this procedure is simple and practical in application in routine laboratories. Using the described methodology more complicated calculations such as PLS are not necessary.

Table 7
Statistical parametric results from the comparison obtained between the two proposed methods

	t-test			Regression line			
	$t_{critical}$	$t_{statist}$	Pearson correlation	Intercept	Slope	Correlation coefficient	Sy/x
Lovastatin	2.45	1.38	0.9999	0.0643	0.9975	0.9999	0.080
Ascorbic acid		0.66	0.9981	−0.4018	1.0915	0.9982	0.177
Lovastatin	2.78	3.55	0.9987	0.0835	1.0107	0.9998	0.116
Quercetin		0.05	0.9998	0.1823	0.9634	0.9987	0.161
Lovastatin	2.78	3.03	0.9999	0.0069	1.0115	0.9999	0.067
Gallic acid		0.57	0.9992	0.0117	1.0026	0.9992	0.099

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