



Journal of Advanced Pharmacy Research

Simultaneous UV Spectrophotometric Determination of Pravastatin Sodium and Pioglitazone Hydrochloride in Pharmaceutical Preparations

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Submitted on: 01-04-2017; Revised on: 28-04-2017; Accepted on: 10-05-2017

ABSTRACT

Objectives: This study aimed to develop simple UV spectrophotometric methods for simultaneous determination of pravastatin sodium and pioglitazone hydrochloride without previous separation. **Methods.** The first method is the first derivative, where the peak amplitudes of first derivative of absorption spectra were measured at 249.7 and 277 nm for pravastatin sodium and pioglitazone hydrochloride respectively. The second method is the first derivative of the ratio spectra, where the peak amplitudes were measured at 249.6 and 276.6 nm for pravastatin sodium and pioglitazone hydrochloride respectively. **Results.** The proposed methods were validated according to International Conference on Harmonization (ICH) guidelines and successfully applied for simultaneous determination of both drugs in their combined dosage form. **Conclusion.** The proposed methods are simple, rapid, economic, accurate and precise to simultaneously determine pravastatin and pioglitazone in pure form and in pharmaceutical dosage form without previous separation steps.

Keywords: Pravastatin sodium; Pioglitazone hydrochloride; First derivative; Ratio derivative.

INTRODUCTION

Pravastatin sodium (PRV), (Figure 1a), is sodium (3*R*,5*R*)-7-[(1*S*,2*S*,6*S*,8*S*,8*aR*)-1,2,6,7,8,8a-hexahydro-6-hydroxy-2-methyl-8-[(*S*)-2-methylbutyryloxy]-1-naphthyl]-3,5 dihydroxyheptanoate.^{1,2} It is a selective and competitive inhibitor of 3-hydroxy-3-methylglutaryl -coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme that converts HMG-CoA to mevalonate, a precursor of cholesterol.³ PRV is a member of the class of statins, used to treat hypercholesterolemia and related conditions and to prevent cardiovascular disease. It increases the number of hepatic low density lipoprotein (LDL) receptors on the cell surface to enhance uptake and catabolism of LDL. Secondly, PRV inhibits hepatic synthesis of very low density lipoprotein (VLDL), which reduces the total number of VLDL and LDL particles.⁴

Pioglitazone hydrochloride (PGZ), (Figure. 1b), is 5-[[4-[2-(5-Ethyl-2-pyridinyl) ethoxy]phenyl]methyl]

-2,4-thiazolidinedione hydrochloride.⁵ It is a thiazolidine-dione oral antidiabetic agent that improves insulin sensitivity. It is used in the treatment of type 2 diabetes mellitus. It is given orally as monotherapy, particularly in patients who are overweight and for whom metformin is contra-indicated or not tolerated.¹

Reviewing the literature on the new combination comprises PRV and PGZ in commercial dosage form, reveals no reported methods for simultaneous determination of both drugs. The aim of this work is to develop simple, economic, rapid, sensitive, accurate and precise UV spectrophotometric methods for simultaneous determination of PRV and PGZ without previous separation steps. These methods include; first derivative and first derivative of the ratio spectra. Although this work is old fashion, but its value arises from the lack of any published method for simultaneous determination of PRV and PGZ in their new combination.

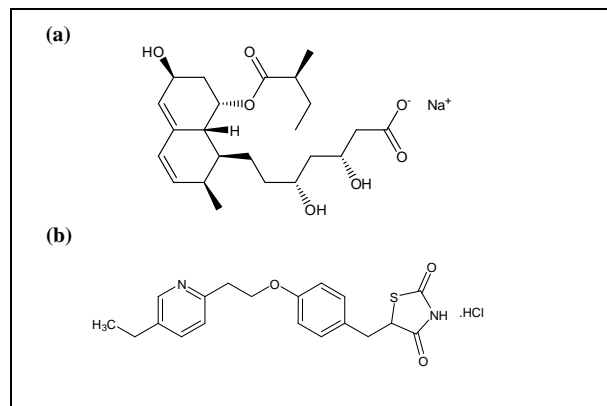


Figure 1. Structural formula of (a) PRV and (b) PGZ

MATERIALS AND METHODS

Materials

Pure materials of PRV and PGZ were obtained as gift sample from Hi Pharm Company, El-Obour City, Egypt. Their purity was found to be 99.52 and 99.61% for PRV and PGZ, respectively, according to the reported methods.^{6,7}

Pravazon® capsules containing 10 mg of each drug per capsule (B.No. APB180816), manufactured by Accent Pharma, India, marketed by UPHA Pharmaceutical Mfg, India.

Methanol, analytical grade (El-Nasr Company, Egypt).

Preparation of standard solutions

Standard solutions (100 µg/mL) of PRV and PGZ were prepared separately by transferring accurately weighed 10 mg of both drugs into two separate 100-mL volumetric flasks, then dissolved in methanol and diluted up to the mark with the same solvent.

Procedure (Construction of calibration curves)

Accurately measured aliquots equivalent to (20–200 µg) of each drug were transferred from their standard solutions (100 µg/mL) into two separate series of 10-mL volumetric flasks and the volume of each flask was diluted up to the mark with methanol, to reach the concentration range of (2 – 20 µg/mL). The absorption spectra of these solutions were measured in the range of 200 to 350 nm against methanol as a blank.

First derivative method

The first derivative corresponding to each absorption spectrum was recorded, using $\Delta\lambda = 2$ nm and scaling factor of 10. The amplitude values were measured at 249.7 and 277 nm for PRV and PGZ, respectively. The measured amplitude values versus the final drug concentrations in µg/ml were plotted to get the calibration graphs and the regression equations were derived

Ratio derivative method

The absorption spectra of PRV were divided by the spectrum of PGZ (14 µg/ml), while those of PGZ were divided by the spectrum of PRV (6 µg/ml). The first derivative of the ratio spectra were recorded, using $\Delta\lambda = 2$ nm. The amplitude values were measured at 249.6 and 276.6 nm for PRV and PGZ, respectively. The measured amplitude values versus the final drugs concentrations in µg/ml were plotted to get the calibration graphs and the regression equations were derived.

Assay of laboratory prepared mixture

Aliquots of PRV and PGZ were transferred from their standard solutions (100 µg/mL) into a series of 10-mL volumetric flasks, completed to volume with methanol to prepare mixtures in the ratio of 1:1 of PRV and PGZ. The proposed procedures described above for construction of calibration curves were then applied and the concentrations of PRV and PGZ were calculated from the corresponding regression equations.

Application to pharmaceutical formulation

The content of ten capsules were accurately weighed and mixed well. An amount of the powder equivalent to 10 mg PRV and 10 mg PGZ was weighed, dissolved in methanol by shaking for about 30 min. The solution was filtered and transferred quantitatively into 100-mL volumetric flask. The volume was then completed to the mark with methanol. Necessary dilutions were made to reach concentrations in the linearity range. The same procedures under the corresponding linearity were applied and the concentrations of PRV and PGZ were calculated from the corresponding regression equations.

RESULTS AND DISCUSSION

Many methods have been introduced for the analysis of binary mixtures among which the molecular absorption spectroscopy was the most simple, fast and applicable in almost all laboratories. Most active drugs absorb in the UV region and exhibit strongly overlapped spectra that impede their simultaneous determination. Several manipulations were performed to enable mixture resolution for example, using different order derivatives^{8,9}, derivatives of the ratio spectra^{10,11}, ratio subtraction technique¹³, and isoabsorptive method¹⁴. Many other methods which depend on measuring the absorbance at two wavelengths, include, dual wavelength method¹⁵, ratio difference method¹⁶, H-point standard addition method¹⁷, bivariate method¹⁸, and absorbance ratio method¹⁹.

To the best of our knowledge, there is no reported method for simultaneous determination of PRV and PGZ either in pure form or in their co-formulated pharmaceutical preparations.

In the present work, we applied two simple UV spectrophotometric methods, the first derivative and the first derivative of the ratio spectra, for simultaneous determination of PRV and PGZ in pure samples as well as in pharmaceutical preparation.

Spectral characteristics

Zero-order absorption spectra of PRV and PGZ show overlapping, **Figure 2**, which allows the analysis of PGZ in presence of PRV, but prevents the analysis of PRV in presence of PGZ. Such overlapping can be resolved by applying either the first derivative to the zero-order absorption spectra or the ratio spectra.

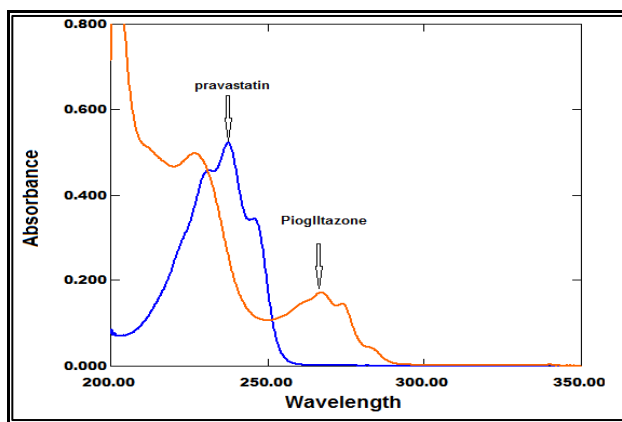


Figure 2. Zero order absorption spectra of PRV (10 ug/mL) and PGZ (10 ug/mL)

First derivative method

The first derivatives of the absorption spectra were obtained to resolve the overlapping and the amplitude values at 249.7 nm (zero crossing for PGZ) were used for determination of PRV without interference of PGZ, while the amplitude values at 277 nm (zero crossing for PRV) were used for determination of PGZ without interference of PRV (**Figures 3-5**).

Ratio derivative method

Salinas et al.¹⁰ designed a spectrophotometric method which is based on the derivation of the ratio-spectra for resolving binary mixtures. The main advantage of the ratio spectra derivative spectrophotometry is the chance of doing easy measurements in correspondence of peaks so it permits the use of the wavelength of highest value of analytical signals. Moreover, the presence of a lot of maxima and minima is another advantage which allows the determination of active compounds in the presence of other compounds and excipients which possibly interferes the assay.

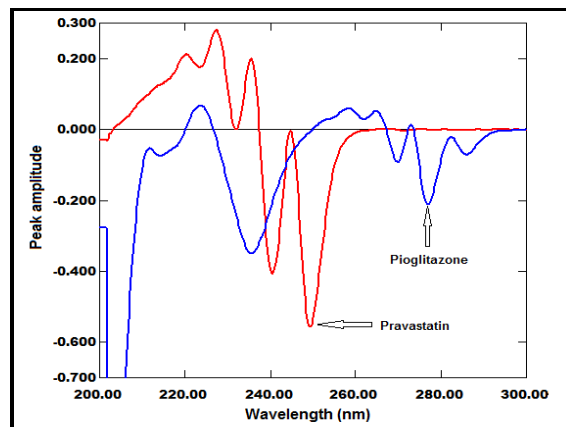


Figure 3. Overlain first derivative spectra of PRV (10ug/mL) and PGZ (10 ug/mL)

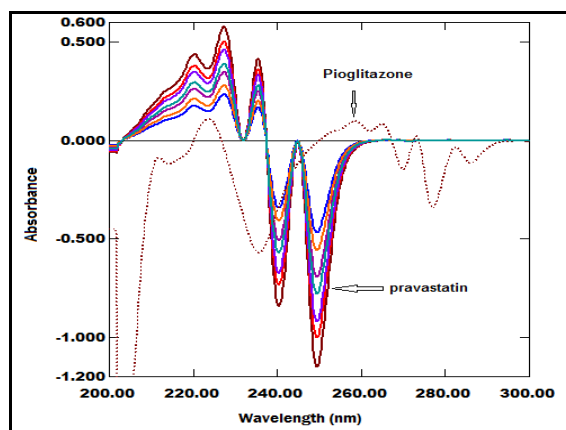


Figure 4. Overlain first derivative spectra of PRV (2 ug/mL) and PGZ (10 ug/mL)

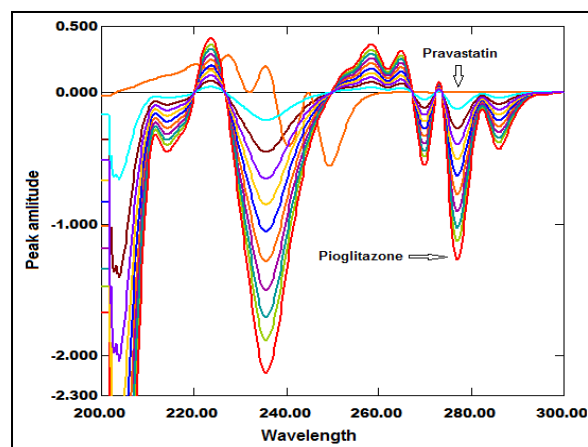


Figure 5. Overlain first derivative spectra of PGZ (2 ug/mL) and PRV (10 ug/mL).

In this method, the absorption spectra of each drug were divided by a standard absorption spectrum of the interfering drug (divisor) to get the ratio spectra. By application of the first derivative to these ratio spectra, PRV and PGZ can be quantitatively determined at 249.6 and 276.6 nm, respectively, in their binary mixture without any interference from each other (Figures 6,7). Careful choice of the divisors concentrations was of great importance, so different concentrations of the divisors were tried; the best one was 14 µg/mL of PGZ for the determination of PRV and 6 µg/mL of PRV for the determination of PGZ.

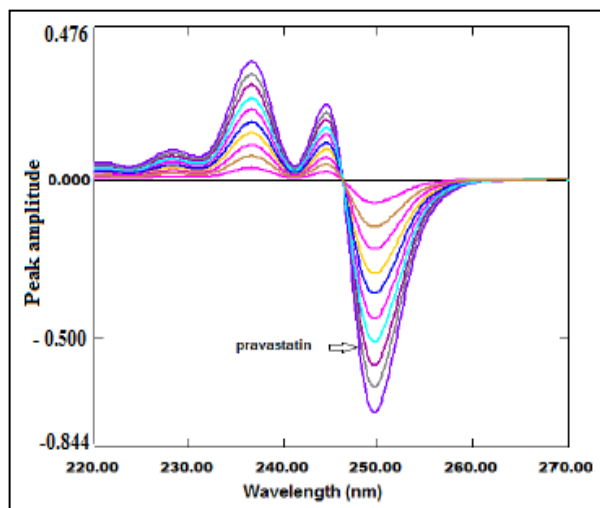


Figure 6. Overlain first derivative of the ratio spectra of PRV using a divisor of PGZ (14 µg/mL)

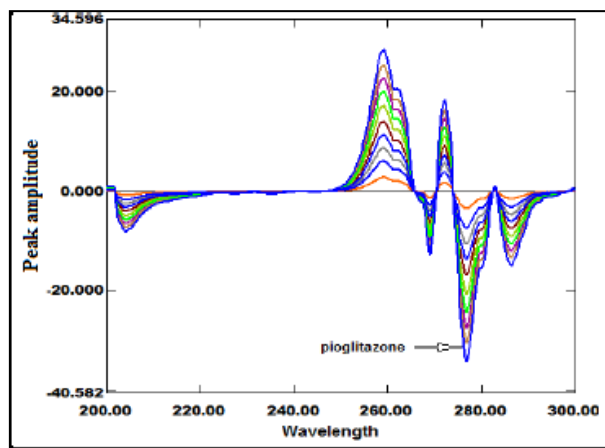


Figure 7. Overlain first derivative of the ratio spectra of PGZ using a divisor of PRV (6 µg/mL)

Validation of the methods

The proposed methods were tested for linearity, range, limit of detection (LOD), limit of quantification

(LOQ), selectivity, accuracy and precision according to International Conference on Harmonization (ICH) guidelines.²⁰

Linearity and range

Under the described experimental conditions, the calibration graphs for the methods were constructed by plotting either the amplitudes of the first derivative of the absorption spectra (for first derivative method) or the amplitudes of the first derivative of the ratio spectra (for ratio derivative method) versus drug concentrations in µg/mL. The calibration graphs were rectilinear over the concentration range of 2-20 µg/mL for both drugs with high values of the correlation coefficient. The regression parameters are supplied in Table 1.

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

LOD and LOQ were calculated according to ICH guidelines [20] from the following equations:

$$\text{LOD} = 3.3 S_a / \text{slope}$$

$$\text{LOQ} = 10 S_a / \text{slope}$$

Where S_a is the residual standard deviation of a regression line.

LOD and LOQ values of PRV and PGZ for each method were listed in Table 1.

Selectivity

The selectivity of the methods was achieved by the analysis of different laboratory prepared mixtures of PRV and PGZ within the linearity range. Satisfactory results listed in Table 2, and the results of the standard addition technique (Table 3) prove that the proposed methods can selectively analyze each drug without any interference from the other drug or the excipients.

Accuracy

Accuracy of the proposed methods, calculated as the mean percent recovery (%R), was assessed by applying the proposed procedures for triplicate determination of three concentration levels covering the specified range for each drug (6, 10 and 16 µg/mL). The concentrations were obtained from the corresponding regression equations and the mean percent recoveries, shown in Table 1, indicate accuracy of the proposed methods. Accuracy of the methods was further assured by the use of the standard addition technique. It was performed by addition of known amounts of pure PRV and PGZ to known concentrations of the pharmaceutical preparation and the resulting mixtures were assayed, and the results obtained were compared with the expected results (Table 3). The good recoveries of the pure added PRV and PGZ suggested good accuracy of the proposed methods.

Table 1. Spectral and validation data for simultaneous determination of PRV and PGZ by the proposed methods

Parameters	PRV		PGZ	
	First derivative	Ratio derivative	First derivative	Ratio derivative
Wavelength (nm)	249.7	249.60	277	276.60
Linearity range (µg/mL)	2 — 20	2 — 20	2 — 20	2 — 20
LOD (µg/mL)	0.296	0.318	0.235	0.132
LOQ (µg/mL)	0.896	0.964	0.713	0.399
Regression equation				
Slope	0.0558	0.0363	0.0631	1.7035
Intercept	-0.0007	0.0003	0.0088	0.2102
Correlation coefficient (r)	0.9996	0.9998	0.9997	0.9997
Accuracy (%R)*	100.73	100.41	99.58	99.87
Precision (%RSD)*				
- Repeatability (intra-day)	0.745	0.436	0.851	0.578
- Intermediate precision (inter day)	1.054	0.978	1.212	0.995

* Average of three determinations of three concentration levels.

Table 2. Determination of PRV and PGZ in laboratory prepared mixtures by the proposed methods

Method	Concentration taken (µg/mL)		Concentration found (µg/mL)		% Recovery	
	PRV	PGZ	PRV	PGZ	PRV	PGZ
First derivative	5	5	4.94	5.05	98.80	101.00
	7	7	7.09	6.89	101.29	98.43
	10	10	10.06	10.1	100.60	101.00
	12	12	11.89	12.09	99.08	100.75
	Mean ± %RSD				99.94 ± 1.195	100.29 ± 1.246
Ratio derivative	5	5	5.02	5.04	100.40	100.80
	7	7	6.95	6.98	99.29	99.71
	10	10	10.03	9.89	100.30	98.90
	12	12	11.91	12.01	99.25	100.08
	Mean ± %RSD				99.81 ± 0.627	99.87 ± 0.792

Precision

Precision of the proposed methods, calculated as percent relative standard deviation (%RSD) of the percent recoveries, was checked by applying the proposed procedures for triplicate determination of three concentration levels covering the specified range for each drug (6, 10 and 16 µg/mL) in the same day (intra-day analysis) for repeatability and on three different days (inter day analysis) for intermediate precision. The results in **Table 1** indicate precision of the method.

Application to pharmaceutical formulation:

The proposed methods were applied for the determination of PRV and PGZ in their combined pharmaceutical formulation, Pravazon® capsules. Satisfactory results were obtained in good agreement with the label claim, and the results of the standard addition technique indicate no interference from excipients and additives (**Table 3**).

Table 3. Determination of PRV and PGZ in Pravazon® capsules by the proposed methods and application of standard addition technique

Proposed methods	Pravazon® capsules		Standard addition technique							
	% Recovery* ± %RSD		Taken (µg/mL)		Pure added (µg/mL)		Pure found* (µg/mL)		% Recovery	
First derivative	PRV	PGZ	PRV	PGZ	PRV	PGZ	PRV	PGZ	PRV	PGZ
	101.12 ± 1.155	100.64 ± 0.988	5	5	5	5	4.98	4.93	99.60	98.60
					7	7	7.11	7.06	101.57	100.86
					10	10	10.06	9.96	100.60	99.60
	Mean ± %RSD								100.59 ± 0.980	99.69 ± 1.135
Ratio derivative	100.32 ± 0.781	99.56 ± 0.873	5	5	5	5	5.06	4.98	101.20	99.60
					7	7	7.03	7.04	100.43	100.57
					10	10	10.07	9.91	100.70	99.10
	Mean ± %RSD								100.78 ± 0.388	99.76 ± 0.750

* Average of three determinations

Table 4. Statistical comparison between the results obtained by the proposed methods and the reported methods for the determination of PRV [6] and PGZ [7] in pure powder form

Parameters	First derivative		Ratio derivative		Reported methods	
	PRV	PGZ	PRV	PGZ	PRV ^(a)	PGZ ^(b)
n	5	5	5	5	5	5
Mean %R	100.31	100.29	100.25	100.19	99.52	99.61
%RSD	1.319	1.297	1.116	1.002	1.086	1.297
Student's t-test (2.306) ^(c)	1.032	0.916	1.043	0.892	—	—
F value (6.388) ^(c)	1.498	1.594	1.072	1.054	—	—

^(a) Depends on measuring the UV absorbance of PRV at 240 nm.

^(b) Depends on measuring the UV absorbance of PGZ at 224.4 nm.

^(c) The values in the parenthesis are tabulated values of t and F at (p= 0.05).

Statistical analysis

Table 4 showed statistical comparison of the results obtained by the proposed methods and the reported methods for PRV⁶ and PGZ⁷ in their pure form. The calculated t and F values were less than the theoretical ones indicating that; there was no significant difference between the proposed and the reported methods with respect to accuracy and precision.

CONCLUSION

The present work describes the first spectrophotometric methods for the assay of the new combination containing PRV and PGZ. The proposed methods are simple, rapid, economic, accurate and precise and can be used for simultaneous determination of PRV and PGZ in pure form and in pharmaceutical dosage form without previous separation steps.

Conflict of Interest

The authors declare that they don't have any conflict of interest.

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