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Simultaneous determination of metformin hydrochloride and pioglitazone hydrochloride in binary mixture and in their ternary mixture with pioglitazone acid degradate using spectrophotometric and chemometric methods

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In this work two well known oral hypoglycemic drugs that are administered in combination for patients with type-II diabetes were simultaneously determined. Several spectrophotometric methods were developed and validated for the determination of metformin hydrochloride (MET), pioglitazone hydrochloride (PIO) and pioglitazone acid degradate (PIO Deg). Derivative, ratio derivative, isosbestic and chemometric-assisted spectrophotometric methods were developed. The first derivative (D_1) method was used for the determination of MET in the range of 5–30 $\mu g.mL^{-1}$ and PIO in the range of 10–90 $\mu g.mL^{-1}$ by measuring the peak amplitude at 247 nm and 280 nm, respectively. The concentration of PIO was calculated directly at 268 nm. The first derivative of ratio spectra (DD_1) method used the peak amplitudes at 238 nm and 248.6 nm for the determination of MET in the range of 5–30 $\mu g.mL^{-1}$.

In the isosbestic point method (ISO), the total mixture concentration was calculated by measuring the absorbance at 254.6 nm. Classical least squares (CLS), principal component regression (PCR) and partial least squares (PLS-2) were used for the quantitative determination of MET, PIO and PIO Deg. The methods developed have the advantage of simultaneous determination of the cited components without any pre-treatment. Resolution and quantitative determination of PIO degradate with a minimum concentration of 3 $\mu g.mL^{-1}$ in drug samples was done. The proposed methods were successfully used to determine each drug and the acid degradate in a laboratory-prepared mixture and pharmaceutical preparations. The results were statistically compared using one-way analysis of variance (ANOVA). The methods developed were satisfactorily applied to the analysis of the two drugs in pharmaceutical formulations.

Keywords: metformin hydrochloride; multivariate analysis; pioglitazone hydrochloride; spectrophotometry

Introduction

Oral hypoglycaemic drugs have been used since their discovery as monotherapy tablets containing a single antidiabetic agent. As the symptoms and complications of diabetis mellitus increase, patients have to use mixed therapy.[1–3] Metformin hydrochloride (MET) is chemically designated as *N*,*N*-dimethylimidodicarbonimidic diamide (Figure 1a). It is the most well known member of the biguanide group, regarded as the main compound in mixed therapies, and is always used in high doses of about 500 or 850 mg. Pioglitazone hydrochloride (PIO) is chemically designated as 5-[[4-[2-(5-Ethyl-2-pyridinyl)ethoxy]phenyl]methyl]-2,4-thiazolidinedione (Figure 1b). It is a member of the thiazolidinedione group and is used along with metformin in a dose of 15 mg per tablet.

Concerning the stability studies that have been done on MET and PIO, no degradation was observed for MET sample subjected to stress conditions like UV, light, heat, acid, base and oxidation.[4] Pioglitazone hydrochloride was completely hydrolysed when subjected to 8 hours reflux with 4 M HCl. Validated derivative spectrophotometry, thin layer densitometery (TLC), high-performance liquid chromatography (HPLC) and an ion selective electrochemical method (ISE) were established as stability-indicating methods for the determination of PIO in presence of its acid degradate (Figure 1c).[5,6] On the other hand, several spectrophotometric methods were reported for determination of MET.[7–10] However, a literature survey reveals that MET and PIO were simultaneously determined by several methods, including spectrophotometry[11], HPLC[11,12], TLC[13] LC-MS[14] and ISE[6] which was also applied as a stability-indicating method for PIO.

The aim of this work was to develop new, accurate, simple, sensitive and rapid spectrophotometric techniques for resolving the spectral overlap of MET and PIO mixture with satisfactory statistical validation measures and the use of multivariate calibration methods for the resolution and simultaneous determination of MET, PIO and PIO Deg without preliminary separation.

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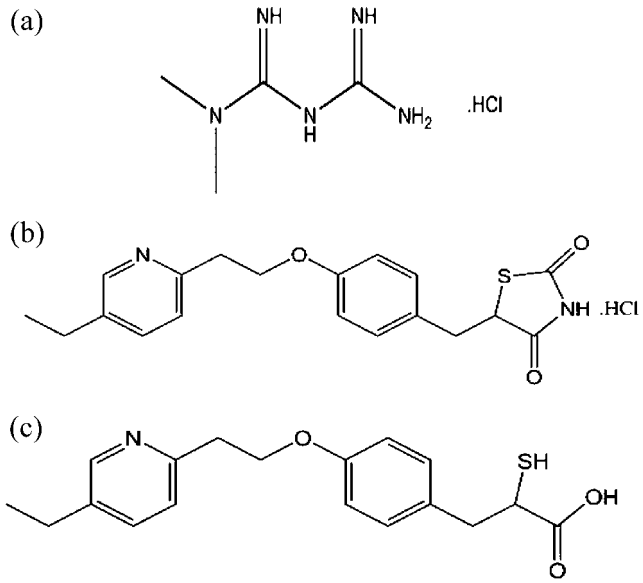

Figure 1. The chemical structures of: (a) metformin hydrochloride (MET), (b) pioglitazone hydrochloride (PIO), (c) pioglitazone acid degradate (PIO Deg).

Experimental

Instrumentation

A dual-beam Shimadzu (Kyoto/Japan) UV-Vis. spectrophotometer, model UV-1601 and UVPC personal spectroscopy software version 3.7 (SHIMADZU) were used to process absorption and derivative spectra. Classical least squares, PCR and PLS-2 analyses were carried out by using PLS-toolbox software version 2.1-PC[15] for use with MATLAB® 6.5.[16]

Materials

Pure standard

Metformin hydrochloride (MET) was kindly supplied by Chemical Industries Development Cid Co., Giza, Egypt, with a purity of 99.79 ± 0.57% according to the *British Pharmacopeia* (BP).[10]

Pioglitazone hydrochloride (PIO) was kindly supplied by Amoun Pharmaceutical Co., Cairo, Egypt, with a purity of 100.19 ± 0.84% according to the manufacturer.[17]

Pharmaceutical dosage form

Competact® tablets were labelled as containing 850 mg metformin hydrochloride and 15 mg pioglitazone hydrochloride per tablet. Batch no. 2 270 084 A, was manufactured by Takeda Global Research and Development Centre (Europe) Ltd, London, United Kingdom.

Degraded sample

An amount of 200 mg of PIO bulk powder was accurately weighed and dissolved in 100 mL of 4 M aqueous hydrochloric acid and the solution was refluxed for 8 hours on a boiling water bath, cooled and neutralized with 4 M aqueous sodium hydroxide. The formed precipitate was filtered, washed several times with distilled water and dried. Complete degradation was confirmed using TLC plates (which were activated at 110 °C for 30 minutes) and toluene: ethyl acetate (11 : 9 V/V) as a developing system in a pre-saturated chromatographic tank. Ascending chromatography was applied and then air dried. The spots were visualized under UV light at 254 nm.[5]

Chemicals and reagents

All chemicals used throughout the work were of analytical grade and solvents were of spectroscopic grade:

- methanol (Merck, Germany);
- acetonitrile (Merck, Germany);
- perchloric acid (70%, Merck, Germany);
- formic acid (98–100%, Prolabo);
- acetic anhydride (Adwic, Egypt);
- glacial acetic acid (Adwic, Egypt);
- hydrochloric acid (Merck, Germany); 4 M aqueous solution;
- sodium hydroxide (BDH); 4 M aqueous solution;
- toluene (Adwic, Egypt);
- ethyl acetate (Adwic, Egypt).

Solutions

Stock standard solutions (0.1 mg.mL^{-1}) of MET, PIO and a stock solution (0.1 mg.mL^{-1}) of PIO Deg. were prepared by accurately weighing 25 mg of each. Then the powder was separately transferred into three 250 mL volumetric flasks, dissolved in 20 mL methanol and completed to the volume with the same solvent.

A working solution of PIO Deg (25 µg.mL^{-1}) was prepared by diluting 25 mL of the PIO Deg stock solution (0.1 mg.mL^{-1}) to 100 mL with methanol into 100 mL volumetric flask.

Laboratory-prepared mixtures

For spectrophotometric methods, solutions containing different ratios of MET and PIO, as shown in Table 1, were prepared by transferring aliquots from their stock standard solutions into a series of 10 mL volumetric flasks. The volume of each was completed to mark with methanol.

For chemometric methods, solutions containing different ratios of MET, PIO and PIO Deg were prepared, according to a multilevel multifactor experimental design,[18] by transferring different aliquots from the stock standard solutions of MET (0.1 mg.mL^{-1}), PIO (0.1 mg.mL^{-1}) and the working solution of PIO Deg (25 µg.mL^{-1}) into 10 ml volumetric flasks. The volume of each was completed to the mark with methanol.

Procedures

D_1 spectrophotometric method

Construction of the calibration curve for D_1 spectrophotometric method. Aliquots from MET and PIO stock standard solutions (0.1 mg.mL^{-1}) equivalent to 50–300 µg and 100–900 µg, respectively, were accurately measured and transferred into two separate sets of 10 mL volumetric flasks and the volumes were completed to the mark with methanol. The zero order (D_0) absorption spectra of each solution was recorded against methanol as a blank, then the first derivative (D_1) spectra were computed using scaling factor = 10 and $\Delta\lambda = 4$ nm. The peak amplitudes at 247 nm and 280 nm were measured for MET and PIO, respectively, then plotted each against its corresponding concentrations and the regression parameters were computed.

Table 1. Determination of MET and PIO in their laboratory-prepared mixtures by the proposed spectrophotometric methods

Ratio MET : PIO	D1		DD1		Isosbestic	
	MET	*PIO*	*MET at 238 nm*	*MET 248.6 nm*	*MET*	*PIO*
1 : 18	100.58	100.30	–	–	–	–
1 : 10	–	–	100.02	99.80	–	–
1 : 9	100.98	100.58	101.26	99.75	–	–
1 : 6	–	–	–	–	100.67	100.08
1 : 4	100.76	100.49	100.99	99.88	–	–
1 : 3	–	–	–	–	100.82	99.88
2 : 3	100.48	101.99	101.10	100.05	100.20	99.99
3 : 2	98.91	100.00	100.25	101.12	98.93	100.82
2 : 1	99.43	101.47	99.27	100.50	–	–
3 : 1	99.94	100.00	99.10	100.28	–	–
4 : 1	–	–	–	–	99.56	100.06
9 : 1	–	–	–	–	101.03	100.22
10 : 1	–	–	–	–	98.78	100.56
Mean±S.D.	**100.15±0.759**	**100.69±0.759**	**100.28±0.877**	**100.20±0.488**	**100.00±0.917**	**100.23±0.339**

Assay of laboratory-prepared mixtures. The absorption spectrum was recorded for each laboratory-prepared mixture, containing different ratios of MET and PIO against methanol as a blank. The peak amplitudes of D_1 spectra of the laboratory-prepared mixtures containing different ratios of MET and PIO were measured at 247 nm and 280 nm for MET and PIO, respectively. The concentrations of MET and PIO were calculated from their corresponding regression equations.

DD_1 spectrophotometric method

Construction of calibration curve for DD_1 spectrophotometric method. The previously scanned zero order (D_0) absorption spectra of MET were divided by the spectrum of PIO (20 μg.mL^{-1}), which is the best chosen divisor. The first derivative of the obtained ratio spectra (DD_1) was computed using scaling factor = 10 and $\Delta\lambda = 4$ nm. Two calibration curves were constructed relating the peak amplitudes of the DD_1 spectra at 238 nm and 248.6 nm to the corresponding drug concentrations.

Assay of laboratory-prepared mixtures. The scanned spectra of the laboratory-prepared mixtures were divided by the absorption spectrum of PIO (20 μg.mL^{-1}), then the first derivative of the ratio spectra was computed. The concentration of MET in the mixtures was calculated from the corresponding regression equations at 238 and 248.6 nm.

ISO spectrophotometric method

Construction of the calibration curve for ISO spectrophotometric method. Aliquots from PIO and MET stock solutions (0.1 mg.mL^{-1}) equivalent to 50–900 μg of PIO and 50–1000 μg of MET were separately transferred into two sets of 10 ml volumetric flasks and completed to the mark with methanol. The D_0 spectra were recorded for both drugs using methanol as a blank, then the absorbance was measured at 268 nm for PIO and 254.6 nm (A_{iso}) for MET. Two calibration curves were constructed for each drug relating the absorbance at the selected wavelength to the corresponding drug concentration and the regression equations were computed.

Assay of laboratory-prepared mixtures. The absorbances of the laboratory-prepared mixtures containing different ratios of PIO and MET were measured at 268 nm corresponding to the contents of PIO only, and at 254.6 nm (A_{iso}) corresponding to the total content of PIO and MET in the mixture. The concentration of PIO alone and the total concentration of the two drugs were calculated from their corresponding regression equations. The actual concentration of MET in each mixture was obtained by subtraction of PIO concentration from the total concentration.

Chemometric CLS, PCR and PLS-2 methods

Building the calibration models. A calibration set of 13 different laboratory-prepared mixtures of MET, PIO and PIO Deg in different ratios was prepared using multilevel multifactor experimental design.[18] The mixtures were prepared by transferring different aliquots from PIO and MET stock standard solutions (0.1 mg.mL^{-1}) and PIO Deg working solution (25 μg.mL^{-1}) into a series of 10 ml volumetric flasks. The absorption spectra of the prepared mixtures were recorded in the range 200–300 nm. The recorded spectra were then transferred to MATLAB® 6.5 for subsequent data analysis and the calibration models (CLS, PCR, PLS-2) were constructed.

Assay of validation set. The absorption spectra of the validation set that consists of nine laboratory-prepared mixtures containing different ratios of MET, PIO and PIO Deg were recorded in the range 225–300 nm. The concentrations of MET, PIO and PIO Deg were calculated using the optimized CLS, PCR and PLS-2 calibration models.

Application to pharmaceutical formulations

Ten Competact® tablets were accurately weighed, finely powdered and then the powder was analysed as follows.

For the D_1 and DD_1 method

Accurately weighed portions of the powder equivalent to 10 mg of MET and 1.0 mg of PIO were separately weighed and transferred into two 100 mL beakers, sonicated in 30 mL methanol for 10

minutes and filtered into 100 mL volumetric flasks. The residues were washed three times, using 20 mL methanol and completed to the mark with the same solvent. The prepared PIO solution was used directly for its determination by the D_1 method and 1.0 mL aliquots from the prepared MET solution were transferred to 10 mL volumetric flasks and diluted with methanol for the determination of MET.

For the isosbestic point and chemometric methods

For isosbestic point spectrophotometry, a portion of the powdered tablet equivalent to 40 mg of MET was accurately weighed. An amount of pure PIO powder was added to make the total concentration of PIO equivalent to 10 mg transferred into a 100 mL beaker and sonicated in 30 mL methanol for 10 minutes, and filtered into a 100 mL volumetric flask. The residue was washed three times, using 20 mL methanol each, and was completed to the mark with methanol. An aliquot of 1.0 mL was transferred to 10 ml volumetric flask and diluted with methanol.

For multivariate calibration a portion equivalent to 25 mg of MET was weighed. An amount of pure PIO powder was added to make the total concentration of PIO equivalent to 10 mg transferred into a 100 mL beaker and sonicated in 30 mL methanol for 10 minutes, and filtered into a 100 mL volumetric flask. The residue was washed three times each using 20 mL methanol and completed to the mark with methanol. Aliquots of 0.5–1.0 mL were transferred to 10 mL volumetric flask and diluted with methanol.

The general procedure previously described under each method was followed to determine the concentration of each of the prepared dosage form solutions.

Results and Discussion

The development of analytical methods for the determination of MET and PIO in binary mixture and in ternary mixture with PIO Deg without preliminary separation was of interest. Pioglitazone hydrochloride could be determined using zero-order spectrophotometry (D_0) by measuring the absorbance at its λ_{max} 268 nm, but MET could not be determined as its absorption spectrum overlaps that of PIO spectrum to a large extent, as shown in Figure 2. This figure also shows the overlap of MET and PIO with PIO Deg. Derivative, ratio derivative and isosbestic point spectrophotometric methods were applied to allow the resolution of the two drugs. Metformin hydrochloride was successfully determined in the presence of PIO without any preliminary separation. The great similarity in the absorption spectra of PIO and PIO Deg meant that PIO Deg could not be resolved. The resolution of the ternary mixture of MET, PIO and PIO Deg was achieved by applying multivariate calibration methods, such as CLS, PCR and PLS-2, without any preliminary separation, which introduces an advantage over the reported methods.

Derivative spectrophotometry

Derivative spectrophotometry was first suggested during the last decade and soon become a well established technique for the assay of drug in mixtures and in pharmaceutical dosage forms.[19] It is a good technique, capable of enhancing the resolution of overlapped bands.[20] It can be applied for the determination of a drug in the presence of another by selecting a wavelength where contribution of one compound is zero or almost zero

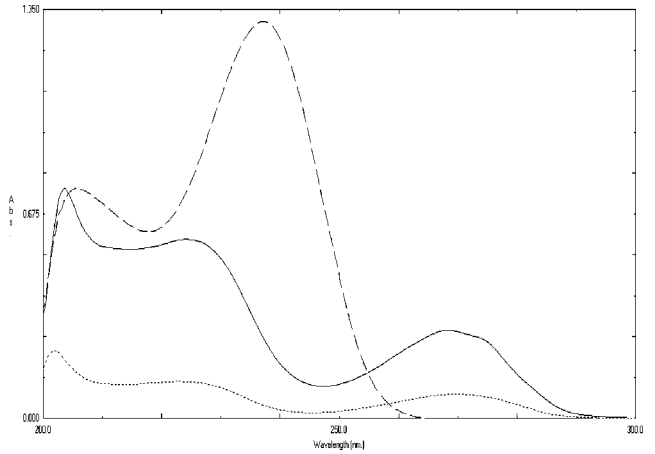

Figure 2. Zero-order absorption spectra of 15 μg/ml metformin hydrochloride (·····), 15 μg/ml pioglitazone hydrochloride (———) and 3.75 μg/ml acid degradate (- - - - .) using methanol as a blank.

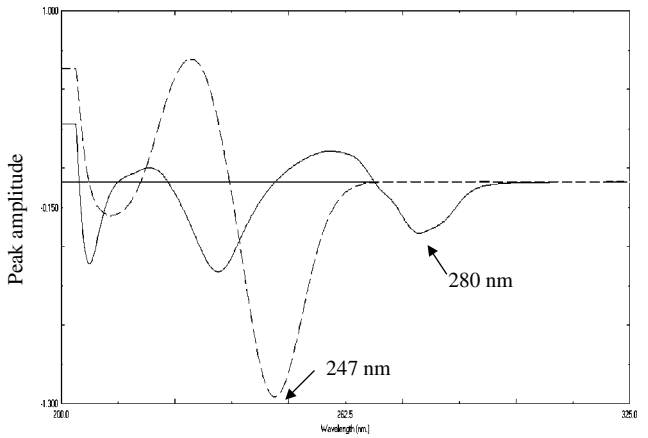

Figure 3. First derivative absorption spectra of 20 μg/ml metformin hydrochloride (- - - -) and 20 μg/ml pioglitazone hydrochloride (———) using methanol as a blank.

while the compound to be determined has a reasonable value. First derivative (D_1) showed that both MET and PIO could be determined by measuring the peak amplitude at two troughs 247 nm and 280 nm, respectively, without any interference in their measurements (Figure 3).

A linear correlation was obtained between the peak amplitude values and the corresponding concentrations for both drugs at their corresponding wavelengths. The characteristic parameters of the regression equation of the D_1 method for the determination of MET and PIO were given in Table 2.

$$P = 0.0577C + 0.0877 \; r = 0.9991 \text{ at } \lambda \text{ 247 nm for MET}$$

$$P = 0.0151C - 0.0013 \; r = 0.9990 \text{ at } \lambda \text{ 280 nm for PIO}$$

where P is the peak amplitude, C is the concentration in $\mu g.mL^{-1}$ and r is the correlation coefficient.

Derivative of ratio spectrophotometry

Another method for resolving binary mixtures without previous separation is the derivative ratio spectrophotometry (DD_1) method, which was developed by Salinas *et al.*[21] In this method

Table 2. Regression equations parameters and results of the determination of MET and PIO pure samples by the proposed spectrophotometric methods

Parameter	D1		DD1		Isosbestic	
	MET 247 nm	*PIO 280 nm*	*MET at 238 nm*	*MET at 248.6 nm*	*MET 254.6 nm*	*PIO 268 nm*
Linearity	5–30 µg/ml	10–90 µg/ml	5–30 µg/ml	5–30 µg/ml	5–100 µg/ml	5–90 µg/ml
Slope	0.0577	0.0151	0.02811	0.3699	0.005	0.0215
SE of the slope	0.000850	0.0001734	0.00216	0.003926	0.000027	0.000229
Intercept	0.0877	−0.0013	0.3546	0.4958	0.0021	0.0025
Correlation coefficient	0.9991	0.9990	0.9998	0.9995	0.9997	0.9991
Accuracy						
Mean±SD	100.04 ± 1.061	99.84 ± 0.675	99.93 ± 0.923	99.97 ± 0.916	100.35±0.730	100.00 ± 0.788
Precision						
Repeatability	100.61 ± 0.371	100.25 ± 0.465	99.82 ± 0.552	100.41 ± 0.236	99.83 ± 0.382	100.06 ± 0.788
Intermediate precision	100.10 ± 0.464	100.65 ± 0.617	100.70 ± 0.622	99.80 ± 0.316	100.45 ± 0.401	100.09 ± 0.801

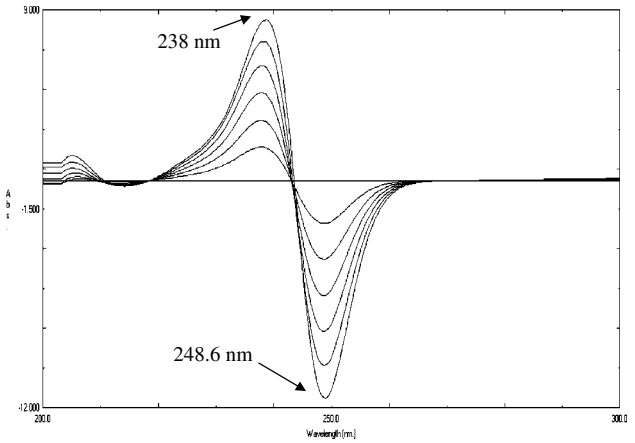

Figure 4. First derivative of ratio spectra of metformin hydrochloride 5–30 µg/ml using the spectrum of 20 µg/ml of pioglitazone hydrochloride as a divisor, methanol was used as a blank.

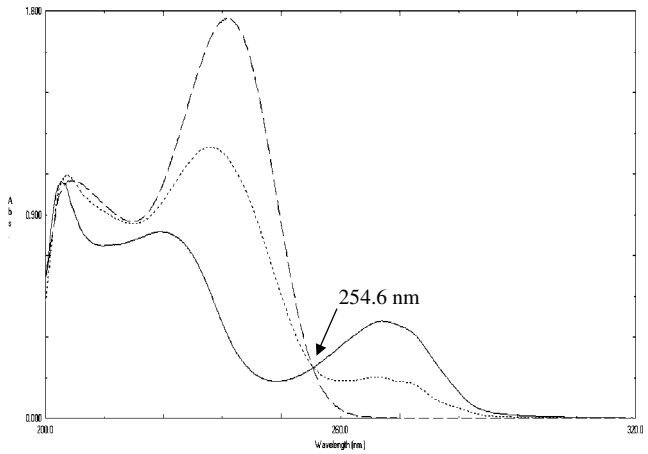

Figure 5. Zero order absorption spectra of 20 µg/ml of pioglitazone hydrochloride (———), 20 µg/ml of metformin hydrochloride (- - - -) and (1 : 1) mixture containing 10 µg/ml of each (·····) using methanol as a blank.

the absorption spectrum of the mixture is obtained and divided by the absorption spectrum of a standard solution of one of the components and then the first derivative of the ratio spectrum is obtained. This method permits the determination of components in their mixtures at the wavelengths corresponding to a maximum or minimum.

In order to optimize the DD_1 method that was developed, the influence of different variables was studied. These variables included divisor concentration, $\Delta\lambda$ and smoothing factor. The careful choice of the divisor and the working wavelengths were of great importance, so four different concentrations of PIO (10, 20, 30 and 40 µg.mL^{-1}) were tried as divisors. It was found that minimum noise and better selectivity were obtained when using 20 µg.mL^{-1} of PIO spectrum. Two calibration curves were constructed at the two wavelengths, 238 nm and 248.6 nm, representing the relationship between the peak amplitudes of DD_1 and the corresponding concentrations (Figure 4). The characteristic parameters of the regression equation of DD_1 method for the determination of MET were given in Table 2:

$$P = 0.2811C + 0.3546 \quad r = 0.9998 \text{ at } 238 \text{ nm}$$

$$P = 0.3699C + 0.4958 \quad r = 0.9995 \text{ at } 248.6 \text{ nm}$$

where P is the peak amplitude, C is the concentration of the drug in µg.mL^{-1} and r is the correlation coefficient.

Isosbestic spectrophotometric method

Erram and Tipnis[22–25] developed the isosbestic spectrophotometric method. The method was used for the simultaneous determination of MET and PIO in their binary mixtures. At the isosbestic point the mixture of drugs acts as a single component and gives the same absorbance value as pure drug. Thus, by measuring the absorbance value at the chosen isosbestic point, 254.6 nm (A_{iso}) (Figure 5), the total concentration of both PIO and MET could be calculated, while the concentration of PIO in MET and PIO mixture could be calculated, without any interference, at 268 nm. Thus the concentration of MET could be calculated by simple subtraction.

A linear correlation was obtained between the absorbance values against the corresponding concentrations of both drugs at their corresponding wavelengths. The characteristic parameters of the regression equation of ISO method for the determination of

Table 3. Concentrations of MET, PIO and PIO Deg in the calibration and validation sets

Sample number	MET (μg.ml^{-1})	PIO (μg.ml^{-1})	PIO Deg (μg.ml^{-1})
1	12	15	3.00
2	18	12	3.38
3	18	13.5	3.75
4	15	18	3.38
5	13.5	15	3.38
6	13.5	13.5	4.13
7	16.5	13.5	4.50
8	18	16.5	4.13
9	16.5	18	3.75
10	15	16.5	4.50
11	18	15	4.50
12	18	18	3.00
13	12	18	4.13
14	12	16.5	3.75
15	15	12	4.13
16	16.5	15	4.13
17	16.5	16.5	3.38
18	13.5	16.5	3.00
19	13.5	12	3.75
20	15	13.5	3.00
21	10	10	0
22	0	10	0

The shaded samples are those of the validation set.

MET and PIO were given in Table 2:

$$A_{iso} = 0.005C + 0.0021 \text{ r} = 0.9997 \text{ at 254.6 nm for total MET and PIO}$$

$$A = 0.0215C + 0.0025 \text{ r} = 0.9991 \text{ at 268 nm for PIO}$$

where A_{iso} is the absorbance at isosbestic point 254.6 nm, A is the absorbance at 268 nm, C is the concentration of the drug in μg.mL^{-1} and r is the correlation coefficient.

The previously proposed methods were applied for the determination of PIO and MET in bulk powder. Satisfactory results were obtained (Table 2).

The laboratory-prepared mixtures were analysed by the proposed spectrophotometric methods. Table 1 proves the validity of the proposed methods for the determination of the studied drugs in their laboratory-prepared mixtures.

CLS, PCR and PLS-2 methods

Multivariate calibration methods are very useful in the analysis of complex spectra because of the simultaneous inclusion of many spectral wavelengths instead of using one single wavelength, which greatly improves the precision and predictive ability of these methods.[26] The quality of multicomponent analysis results is dependent on the wavelength range and spectral mode used.[27] In this work, spectral resolution was assayed with absorbance spectra for CLS, PCR and PLS-2 methods. The spectra were measured at 0.1 nm intervals over the range of 200–300 nm where there are absorption characteristics of the three components. Three different methods, CLS, PCR and PLS-2, were applied for the determination of MET, PIO and PIO Deg in their laboratory prepared mixtures and in pharmaceutical preparation.

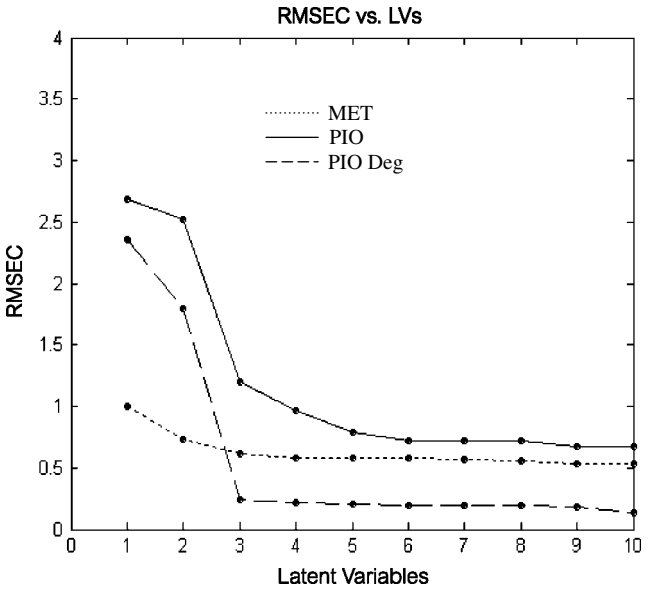

Figure 6. Root mean square error of calibration (RMSEC) plot of the cross validation results of the training set as a function of the number of principle components used to construct the PCR calibration.

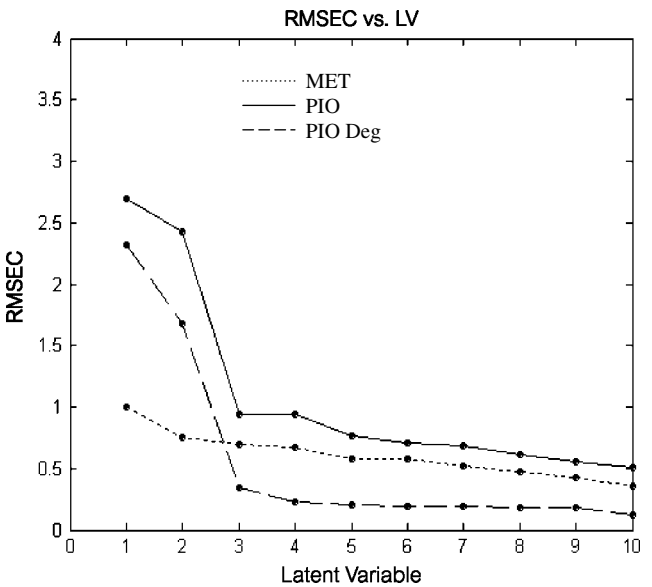

Figure 7. RMSEC plot of the cross validation results of the training set as a function of the number of principle components used to construct the PLS-2 calibration.

Experimental design of the calibration and validation sets

Brereton[18] constructed multilevel-multifactor design in which the levels (L) were the concentrations used and the number of experiments was L^2. For the calibration and validation sets, different mixtures of MET, PIO and PIO Deg were prepared in the laboratory. The concentration range for both MET and PIO is 10.0–15.0 μg.mL^{-1}, whereas for PIO Deg the concentration range is 0–4.5 μg.mL^{-1} (Table 3). The spectra of the prepared mixtures were recorded in the range of 200–300 nm and the spectral data acquisition was taken with 0.1 nm intervals, thus producing 1001 data points per spectrum. In order to decrease the initial number of wavelengths; every tenth wavelength was selected, thus the

Table 4. Statistical parameters of the linear relationship between the calculated and the true concentrations of MET, PIO and PIO Deg In the calibration set obtained by the proposed chemometric methods

Statistical parameter	MET			PIO			PIO Deg		
	CLS	*PCR*	*PLS-2*	*CLS*	*PCR*	*PLS-2*	*CLS*	*PCR*	*PLS-2*
Slope	0.9951	0.9933	0.9902	0.9587	0.9696	1.0072	1.0213	0.9028	0.9283
SE of slope	0.016	0.011	0.015	0.014	0.013	0.0097	0.005	0.016	0.008
Intercept	0.012	0.053	0.104	0.564	0.385	−0.062	−0.011	0.366	0.218
SE of intercept	0.230	0.164	0.221	0.186	0.184	0.133	0.643	0.059	0.030
Correlation coefficient (r)	0.9995	0.9997	0.9996	0.9994	0.9994	0.9997	0.9999	0.9997	0.9999
SE of regression	0.105	0.075	0.100	0.092	0.092	0.076	0.005	0.0169	0.009

Table 5. Percentage recoveries of MET, PIO and PIO Deg. in the validation set

	Concentration			Recovery %								
Sample number	MET	PIO	Deg	MET CLS	PCR	PLS-2	PIO CLS	PCR	PLS-2	PIO Deg. CLS	PCR	PLS-2
1	12	15	3.00	101.36	100.58	98.03	101.24	98.05	101.78	98.07	102.10	98.68
2	18	13.5	3.75	99.43	100.02	100.27	102.04	98.32	98.19	101.95	101.88	102.20
3	15	18	3.38	100.85	101.61	101.24	98.04	98.27	98.31	102.91	101.21	100.84
4	13.5	13.5	4.13	98.64	98.14	98.54	101.92	101.73	101.07	98.47	99.32	98.88
5	18	16.5	4.13	99.61	99.72	99.95	99.59	101.07	101.46	101.29	100.87	99.44
6	15	16.5	4.50	98.49	99.01	98.92	99.42	101.53	101.52	99.05	102.20	98.89
7	18	15	4.50	99.23	100.25	100.53	98.43	101.47	101.27	101.71	102.07	98.00
8	16.5	16.5	3.38	98.98	99.52	99.80	101.77	98.87	98.71	101.40	101.24	101.48
9	15	13.5	3.00	101.61	98.73	99.43	101.78	99.44	99.71	102.33	102.27	101.73
Mean				99.80	99.73	99.64	100.51	99.86	100.22	100.80	101.46	100.01
SD				1.176	1.044	1.014	1.536	1.569	1.492	1.785	0.947	1.555
RSD%				1.178	1.047	1.018	1.528	1.571	1.489	1.771	0.933	1.555
RMSEP				0.166	0.147	0.140	0.229	0.229	0.223	0.067	0.065	0.055

produced spectral data matrix has 22 rows representing different samples and 101 columns representing wavelengths (22 × 101). Thirteen samples were chosen and used for calibration and nine were used as an external validation set.

CLS model

The training set was used for constructing the CLS model (absorptivity at different wavelengths). In the CLS method, all the components in the calibration samples must be known unlike PCR and PLS-2 methods that could be used to determine the components under investigation even in the presence of unknown components (interfering substance).[28] The absorbance matrix of the calibration samples (13 × 101) and their corresponding concentration matrix (13 × 3) were used to find the absorbitivity matrix (k-matrix). The k-matrix was further used for predicting the concentration of the three components in the validation and pharmaceutical formulation samples.

PCR and PLS-2 models

In order to build the PCR and PLS models, the raw data of the calibration samples were mean centred[29] as a pre-processing step and the 'leave-one-out' cross-validation method was used.[30] The appropriate selection of the number of factors to be used for building the model is crucial for achieving correct quantitation in PCR and PLS-2 calibrations. To choose the optimum number of

Table 6. Statistical parameters of the linear relationship between the predicted and the true concentrations of MET, PIO and PIO Deg in the validation set obtained by the proposed chemometric methods

Statistical parameter	MET			PIO			PIO Deg		
	CLS	*PCR*	*PLS-2*	*CLS*	*PCR*	*PLS-2*	*CLS*	*PCR*	*PLS-2*
Slope	0.9844	0.9965	1.0443	0.8761	0.9539	0.9088	0.9492	1.0355	0.9235
SE of slope	0.014	0.014	0.004	0.015	0.015	0.028	0.025	0.018	0.016
Intercept	0.140	−0.011	−0.773	1.966	0.533	1.336	0.249	−0.078	0.308
SE of intercept	0.230	0.225	0.069	0.232	0.230	0.435	0.095	0.070	0.061
Correlation coefficient (Q^2)	0.9990	0.9989	0.9999	0.9993	0.9996	0.9987	0.9984	0.9989	0.9993
SE of regression	0.085	0.079	0.025	0.053	0.047	0.084	0.028	0.022	0.018

Table 7. Determination of MET and PIO in Competact® tablets by the proposed methods

Competact® tablets 15 mg PIO and 85 0 mg MET/ tablet B.No2270084A		DD_1 Method			Multivariate Calibration			
	Mean ± SD	at 238 nm mean±SD	at 248.6 nm mean±SD	Isosbestic point mean±SD	CLS mean ± SD	PCR mean±SD	PLS-2 mean ± SD	Reported* method[11] Mean ± SD
MET	99.82 ± 0.670	100.32 ± 0.438	99.63 ± 0.329	100.30 ± 0.529	101.51 ± 0.485	99.97 ± 1.081	99.52 ± 0.356	100.37 ± 0.278
PIO	100.08 ± 0.898	–	–	99.89 ± 1.042	99.03 ± 1.786	99.77 ± 0.145	99.30 ± 1.081	101.07 ± 0.116

* Second derivative spectrophotometry at 227.55 nm for PIO and 257.25 for MET in mixture of methanol – acetonitrile (30: 70 v/v).

Table 8. Statistical analysis of the results obtained by applying the proposed methods for the determination of MET and PIO compared to reported method* [11]

	D_1 method		DD_1 method		Isosbestic		Multivariate Calibration						Reported[11] method*	
							CLS		*PCR*		*PLS-2*			
Value	MET	PIO	MET at 238 Nm	MET at 248.6 nm	MET	PIO	MET	PIO	MET	PIO	MET	PIO	MET	PIO
Mean	100.04	99.84	99.93	99.97	100.35	100.00	99.77	100.00	99.99	100.16	99.97	100.18	99.88	99.77
SD	1.061	0.675	0.923	0.916	0.730	0.788	1.136	1.148	1.188	1.125	1.235	1.050	0.741	0.537
RSD%	1.061	0.676	0.924	0.916	0.727	0.788	1.139	1.148	1.188	1.232	1.235	1.052	0.742	0.538
n	6	6	6	6	8	6	13	13	13	13	13	13	6	6
Variance	1.126	0.456	0.852	0.839	0.533	0.621	1.290	1.318	1.411	1.266	1.525	1.103	0.549	0.288
Student's t test	2.052	0.591	0.104	0.187	1.030	2.156	0.208	0.462	0.193	0.799	0.147	1.812	–	–
	(2.228)	(2.228)	(2.228)	(2.228)	(3.97)	(5.05)	(2.113)	(2.113)	(2.113)	(2.113)	(2.113)	(2.113)		
F value	2.051	1.583	1.552	1.528	1.184	0.591	2.350	4.576	2.570	4.395	2.778	3.830	–	–
	(5.05)	(5.05)	(5.05)	(5.05)	(2.179)	(2.228)	(4.68)	(4.68)	(4.68)	(4.68)	(4.68)	(4.68)		

The values in parentheses are the corresponding theoretical values of t and F at (P = 0.05).
* Second derivative spectrophotometry at 227.55 nm for PIO and 257.25 for MET in mixture of methanol – acetonitrile (30: 70 v/v).

Table 9. One-way ANOVA testing of the proposed methods used for determination of MET and PIO in their mixture

Methods	MET n	MET Mean	MET SD	MET SE	PIO n	PIO Mean	PIO SD	PIO SE
D_1	7	100.15	0.758	0.286	7	100.69	0.759	0.287
DD_1 at 238 nm	7	100.28	0.877	0.331	–	–	–	–
DD_1 at 248.6 nm	7	100.20	0.488	0.184	–	–	–	–
Isosbestic point	7	100.00	0.917	0.347	7	100.23	0.339	0.128
CLS	9	99.80	1.176	0.392	9	100.51	1.536	0.512
PCR	9	99.73	1.014	0.348	9	99.86	1.569	0.523
PLS-2	9	99.64	1.014	0.338	9	100.22	1.492	0.497

ANOVA of methods used for MET determination

Source	DF	Sum of squares	Mean square	F valve
Between exp.	6	2.999	0.500	**0.564**
Within exp.	48	42.545	0.886	

$F_{critical} = 2.3$

ANOVA of methods used for PIO determination

Source	DF	Sum of squares	Mean square	F valve
Between exp.	4	3.289	0.822	**0.489**
Within exp.	36	60.523	1.681	

$F_{critical} = 2.57$

significant latent variables, F statistics[31] were used. The optimum number of LVs described by the constructed models was found to be five factors for both PCR and PLS as shown in Figures 6 and 7.

The predictive ability of the developed models was evaluated by plotting known concentrations versus predicted concentrations for each compound for each of the developed models. The statistical parameters of the regression equations are represented in Table 4. A good linear relationship was observed for each compound, as indicated by their correlation coefficients.

In order to assess the predictive ability of each of the developed models, it was applied on an external validation set for determination of the three components. The recoveries, mean recoveries, standard deviation, relative standard deviation and RMSEP values are summarized in Table 5. The following equation gives the RMSEP:

$$RMSEP = \sqrt{\frac{\sum (y_r - y_p)^2}{n}}$$

where, y_r and y_p are the true and predicted values, respectively, and n is the number of samples used in validation.

Table 6 presents statistical parameters of the regression equations obtained when plotting the predicted against the actual concentrations for the three components in the validation set.

The high ratio of MET to PIO in their pharmaceutical preparation creates a big problem in their analysis. This problem was overcome with different techniques depending on the method used, either by direct determination of both drugs in different portions of powdered tablets or by fortification of the sample by spiked PIO. So the proposed methods were successfully applied to the analysis of MET and PIO in its pharmaceutical formulation as shown in Table 7.

The results obtained by applying the proposed methods for the determination of MET and PIO in bulk powder were statistically compared with the reported method.[11] The t-value and F-value were less than the theoretical ones, which indicates that there is no significant difference between the proposed methods and the reported one regarding both accuracy and precision, as shown in Table 8.

The results obtained by applying the proposed methods for the determination of MET and PIO in laboratory-prepared mixtures were compared statistically using a one-way ANOVA. The calculated F-value compared with the critical one indicating no significant difference between the proposed methods (Table 9).

Conclusion

The proposed spectrophotometric methods are simple, sensitive, precise and could be easily applied in quality-control laboratories for the simultaneous determination of PIO and MET. The proposed CLS, PCR and PLS-2 methods provide simple, accurate and reproducible quantitative analysis for the determination of MET, PIO and PIO Deg in pharmaceutical dosage form without interference from excipients. The chemometric methods used in this work can be performed easily with adequate software support and provide a clean example of the high resolving power of that technique. The proposed methods could be used in the routine analysis of the studied drugs either in their pure bulk powders or in dosage form in quality-control laboratories without any preliminary separation step.

References

[1] H. P. Rang, M. M. Dale, J. M. Ritter, P. K. Moore, *Pharmacology*, 5 edn, Churchill Livingstone: London, **2003**, pp. 380.
[2] D. G. Grahame-Smith, J. K. Aronson, *Oxford Textbook of Clinical Pharmacology and Drug Therapy,* 3 edn, Oxford University Press: New York, **2002**, pp. 324.
[3] A. G. Gilman, *Goodman and Gilman's, The Pharmacological Basis of Therapeutics*, 10 edn, McGraw-Hill inc New York, **2001**, pp. 1701.
[4] A. A. Sakalgaonkar, S. R. Mirgane, B. R. Arbad, *Int. J. Appl. Env. Sci.* **2008**, *3*(2), 169.
[5] M. R. El-Ghobashy, A. M. Yehia, A. A. Mostafa, *Bull. Fac. Pharm. Cairo Univ.* **2008**, *46*(2), 25.
[6] M. R. El-Ghobashy, A. M. Yehia, A. A. Mostafa, *Analytical Letters* **2009**, *42*(1), 123.
[7] M. G. El-Bardicy, S. Z. El-Khateeb, A. S. A. Ahmed, H. N. Assaadi, *Spectrosc. Lett.* **1989**, *22*(9), 1173.
[8] A. Aruna, K. Nancey, *Indian Drugs* **2000**, *37*(11), 533.
[9] S. Ashour, R. Kabbani, *Analytical Letters*, **2003**, *36*(2), 361.
[10] *The British Pharmacopoeia*, Her Majesty's Stationary Office: London, **2005**, pp. 1292.
[11] M. B. Shankar, V. D. Modi, D. A. Shah, K. K. Bhatt, R. S. Mehta, M. Geetha, B. J. Patel, *J. AOAC Int.*, **2005**, *88*(4), 1167.
[12] B. L. Kolte, B. B. Raut, A. A. Deo, M. A. Bagool, D. B. Shinde, *J. Chromatogr. Sci.*, **2004**, *42*(1), 27.
[13] A. Gumieniczek, H. Hopkala, A. Berecka, D. Kowalczuk, *J. Planar Chromatogr. Mod. TLC*, **2003**, *16*(4), 271.
[14] M. Wang, I. R. Miksa, *J. Chromatography B*, **2007**, *856*, 318.
[15] B. M. Wise, N. B. Gallagher, PLS_Toolbox 2.1, Eigenvector Research Inc., **2001**.
[16] Matlab 6.5, Mathworks Inc., **1999**.
[17] Amoun Pharmaceutical Co., HPLC manufacturer's procedure. Personal communication, **2007**.
[18] R. G. Brereton, *Analyst*, **1997**, *122*, 1521.

[19] S. Altinös, D. Tekeli, *J. Pharm. Biomed. Anal.*, **2001**, *24*(3), 507.
[20] D. Bonazzi, R. Gotti, V. Andrisano, V. Cavrini, *J. Pharm. Biomed. Anal.*, **1997**, *16*(3), 431.
[21] F. Salinas, J. J. B. Nevado, M. A. Espinosa, *Talanta*, **1990**, *37*(3), 347.
[22] S. V. Erram, H. P. Tipnis, *Indian Drugs*, **1993**, *30*(7), 319.
[23] S. V. Erram, H. P. Tipnis, *Indian Drugs*, **1993**, *30*(8), 371.
[24] S. V. Erram, H. P. Tipnis, *Indian Drugs*, **1993**, *30*(9), 462.
[25] S. V. Erram, H. P. Tipnis, *Indian Drugs*, **1993**, *30*(11), 555.
[26] Y. Ni, X. Gong, *Anal. Chem. Acta*, **1997**, *354*, 163.
[27] M. Blanco, J. Coello, F. Gonzales, H. Iturriaga, S. Maspoch, *J. Phar. Sci.*, **1993**, *82*, 834.
[28] D. M. Haaland, E. V. Thomas, *Anal. Chem.*, **1990**, *62*, 1091.
[29] T. Pharm-Gia, Q. P. Duong, *Math. Comput. Modell.*, **1989**, *12*, 1613.
[30] R. G. Brereton, *Analyst*, **2000**, *125*(11), 2125.
[31] D. M. Haaland, E. V. Thomas, *Anal. Chem.*, **1988**, *60*, 1193.