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Spectrophotometric determination of isopropamide Iodide and trifluoperazine hydrochloride in presence of trifluoperazine oxidative degradate

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Four sensitive, selective and precise stability indicating methods for the determination of isopropamide iodide (ISO) and trifluoperazine hydrochloride (TPZ) in their binary mixture and in presence of trifluoperazine oxidative degradate (OXD). Method A is a derivative spectrophotometric one, where ISO was determined by first derivative (D^1) at 226.4 nm while TPZ was determined by second derivative (D^2) at 270.2 nm. Method B is the first derivative of the ratio spectra (DD^1) spectrophotometric method, ISO can be determined by measuring the peak amplitude at 227.4 nm using 5 μg mL^{-1} of OXD as a divisor, while TPZ can be determined by measuring the peak amplitude at 249.2 and 261.4 nm using 15 μg mL^{-1} of ISO as a divisor. Method C is the isoabsorptive spectrophotometric method. This method allows determination of ISO and TPZ in their binary mixture by measuring total concentration of ISO and TPZ at their isoabsorptive point at $\lambda_{229.8}$ nm ($Aiso_1$) while TPZ concentration alone can be determined at λ_{max} 311.2 nm, then ISO concentration can be determined by subtraction. On the same basis TPZ can be determined in presence of ISO and OXD, where OXD concentration alone was determined by measuring the peak amplitude at $\lambda_{281.6}$ and $\lambda_{309.4}$ nm while total concentration of TPZ and OXD was determined at their isoabsorptive points at ($Aiso_2$ = 270.2 nm), ($Aiso_3$ = 310.6 nm) and ($Aiso_4$ = 331.8 nm) then TPZ concentration was determined by subtraction. Method D is the multivariate calibration techniques [the classical least squares (CLS), principal component regression (PCR) and partial least squares (PLS)], using the information contained in the absorption spectra of ISO, TPZ and OXD mixtures. The selectivity of the proposed methods was checked using laboratory prepared mixtures. The proposed methods have been successfully applied to the analysis of ISO and TPZ in pharmaceutical dosage form without interference from other dosage form additives and the results were statistically compared with the reported method.

Keywords: isopropamide iodide; trifluoperazine hydrochloride; trifluoperazine oxidative degradate; derivative spectrophotometry; isoabsorptive point; multivariate spectral analysis; stability-indicating method

Introduction

Trifluoperazine hydrochloride (TPZ) is 10-[3-(4-methyl-1-piperazinyl) propyl]-2-(trifluoromethyl)-10H- phenothiazine dihydrochloride.[1]

TPZ is a phenothiazine tranquilizer with anti-emetic effect. The official method for the determination of TPZ is non-aqueous titration with perchloric acid, determining the end point potentiometrically[2] or using crystal violet indicator.[3] Various spectrophotometric methods have been reported for the determination of TPZ by measurement the UV absorption at 256 nm[2] and using different reagents including bromocresol purple,[4] potassium chlorate,[5,6] and formaldehyde.[7] First and fourth derivatives UV spectrophotometry have been reported for simultaneous determination of TPZ and tranylcypromine sulfate in tablets.[8] TPZ was determined in the presence of its sulfoxide oxidative degradate by different techniques including HPLC,[9–11] spectrofluorimetry[12] and D^1, D^2 and spectrodensitometric methods.[11]

Isopropamide iodide (ISO) is γ-(aminocarbonyl)- N-methyl-N,N-bis (1-methylethyl) -γ-phenylbenzenepropanaminium iodide.[1]

ISO is a quaternary ammonium anticholinergic. The official method for the determination of ISO is non-aqueous titration with perchloric acid.[3] Various spectrophotometric methods have been reported for the determination of ISO by measurement of the UV absorption at 225 nm,[13] first and second derivatives spectrophotometry,[14] ion pair formation with methyl orange,[15] and charge transfer complexation with iodine.[16] The official method for determination of ISO in tablets is ion exchange resin and the absorbance of eluant detected at 280 and 258 nm.[3] HPLC method using C_{18} column has been reported for the simultaneous determination of ISO and phenylpropanolamine HCl in capsules.[17]

The binary mixture of ISO and TPZ is used for their anti-emetic and antispasmodic effect. Few analytical methods have been described for simultaneous determination of ISO/TPZ in their binary mixture including second derivative and second derivative

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of ratio spectra in aqueous solution.[18] D^2 spectrophotometric determination of the binary mixture in methanolic solution after chloroformic extraction of TPZ from 0.1N NaOH solution,[19] and D^2 spectrophotometric method for determination of the binary mixture in methanol also reported.[20]

The aim of this work is to develop simple, sensitive, rapid and precise methods for the selective determination of ISO/TPZ in their binary mixture and in presence of TPZ oxidative degradation product. Reviewing literature in hand, no analytical method has been reported for the simultaneous determination of both drugs mixture in presence of OXD, which is easily formed due to bad storage.

Experimental

Instruments

A double beam UV-visible spectrophotometer (SHIMADZU, Japan) model UV-1601 PC with quartz cell of 1 cm path length, connected to an IBM compatible computer. The software was UVPC personal spectroscopy software version 3.7. All data analysis was performed using PLS-Toolbox 2.0 running under MATLAB®, version 6.5.[21]

Materials

Pure standard

ISO and TPZ were kindly supplied from Kahira Pharm. & Chem. Ind. Co. (Cairo, Egypt). Their purity was found to be 99.68% and 99.58%, respectively according to the reported spectrophotometric method.[18]

Pharmaceutical dosage forms

Stellamide® tablets (Batch No. 0 610 875) is labelled to contain 6.8 mg ISO equivalent to 5 mg of isopropamide ion and 1.18 mg of TPZ equivalent to 1 mg of trifluoperazine base, manufactured by Kahira Pharm. & Chem. Ind. Co. (Cairo, Egypt).

Degraded sample of TPZ

TPZ powder was transferred into a stoppered flask, dissolved in water and 30% hydrogen peroxide solution was added. The flask was left for 8 h at room temperature and complete degradation was followed via TLC using chloroform – methanol (8 : 2, v/v) as a developing system. After complete degradation, the degradate was extracted with multiple fractions of butanol. The extract was evaporated at room temperature and the degradate powder was identified by mass spectrometry.

Chemicals and reagents

All chemicals used throughout this work were of analytical grade, and the solvents were of spectroscopic grade: butanol, chloroform and methanol (El-Nasr Pharmaceutical Chemicals Co., Abu-Zabaal, Cairo, Egypt); hydrochloric acid, 0.1N aqueous solution (El-Nasr Pharmaceutical Chemicals Co., Abu-Zabaal, Cairo, Egypt).

Standard solutions

Stock standard solutions of ISO, TPZ and OXD (1 mg mL^{-1} in methanol): 0.1 gm of ISO, TPZ and OXD were accurately weighed into three separate 100-mL volumetric flasks, 50 mL of methanol was added to each flask, shaken to dissolve then the volume was completed to the mark with methanol. Working standard solutions of ISO, TPZ and OXD (100 µg mL^{-1}): 10 mL of each of ISO, TPZ and OXD stock standard solutions (1 mg mL^{-1}) was transferred accurately into three separate 100-mL volumetric flasks, then the volume was completed to the mark with butanol (for derivative and DD^1 methods) and with 0.1N HCl (for multivariate method).

Laboratory prepared mixtures

Mixtures contain different ratios of ISO/TPZ/OXD were prepared using their respective working solutions (100 µg mL^{-1} in butanol for derivative and DD^1 methods and in 0.1N HCl for multivariate method) and from their stock standard solutions (for isoabsorptive method), as given in Tables 1, 2 and 3.

Table 1. Determination of Isopropamide Iodide and Trifluoperazine Hydrochloride in presence of Trifluoperazine oxidative degradate in laboratory prepared mixtures by the proposed derivative and first derivative of ratio spectra spectrophotometric methods

		Concentration (µg mL^{-1})			Derivative method Recovery %*		First derivative of ratio spectra method Recovery %*		
								TPZ	
OXD %	Ratio ISO : TPZ	ISO	TPZ	OXD	ISO	TPZ	ISO	at 249.2 nm	at 261.4 nm
10	5 : 1	15	2.7	0.3	99.0	101.1	98.8	101.1	100.7
20	9 : 1	22.5	2	0.5	98.5	100.5	98.5	100.5	100.5
30	4 : 1	20	3.5	1.5	98.2	101.4	98.1	100.3	99.3
40	1 : 4	5	12	8	101.2	98.9	100.6	101.1	100.8
50	3 : 2	12	4	4	101.8	98.3	98.5	101.0	99.5
60	1 : 1	10	4	6	98.2	99.5	98.0	101.5	101.3
70	2 : 1	20	3	7	96.5**	106.7**	101.4	98.2	98.2
80	3 : 2	15	2	8	92.1**	107.5**	105.9**	105.5**	95.5**
90	1 : 1	20	2	18	88.9**	110.5**	107.6**	110.0**	87.5**
Mean ± SD					99.5 ± 1.60	100.0 ± 1.25	99.1 ± 1.33	100.5 ± 1.10	100.0 ± 1.08

* Average of 3 determinations. ** Rejected values.

Table 2. Determination of Isopropamide Iodide and Trifluoperazine Hydrochloride in laboratory prepared mixtures by the proposed isoabsorptive method

	ISO/TPZ Binary mixtures						ISO/TPZ/OXD Ternary mixtures			
	Before dilution			After dilution				Recovery %*		
	Concentration ($\mu g\ mL^{-1}$)		TPZ at 311.2 nm	Concentration ($\mu g\ mL^{-1}$)		ISO at 229.8 nm		TPZ		
Ratio ISO : TPZ	ISO	TPZ	Recovery %*	ISO	TPZ	Recovery %*	OXD %	at 270.2 nm	at 310.6 nm	at 331.8 nm
5 : 1	200	40	97.4	40	8	98.7	25	98.7	99.1	99.8
4 : 1	240	60	100.0	40	10	98.8	50	101.2	98.6	101.5
1 : 4	30	120	99.7	10	40	98.8	83	99.0	100.8	98.8
1 : 1	70	70	99.6	25	25	99.8	70	100.1	100.1	100.3
2 : 3	60	90	99.6	20	30	99.7	83	100.3	99.8	100.5
3 : 2	75	50	98.9	30	20	100.1	60	99.3	98.3	100.0
2 : 1	80	40	97.7	30	15	97.8	30	100.9	100.8	101.0
Mean ± SD			99.0 ± 1.04			99.1 ± 0.80		99.9 ± 0.96	99.6 ± 1.01	100.3 ± 0.87

* Average of 3 determinations. ** Rejected values.

Table 3. The concentration of mixtures of Isopropamide Iodide, Trifluoperazine Hydrochloride and Trifluoperazine oxidative degradate used in the training and validation sets

Mixture No.	Isopropamide Iodide ($\mu g\ mL^{-1}$)	Trifluoperazine Hydrochloride ($\mu g\ mL^{-1}$)	Oxidative degradate (μgmL^{-1})
1	10	18	4
2	10	6	12
3	30	14	6
4	20	14	8
5	10	22	8
6	20	10	4
7	15	6	6
8	15	10	10
9	25	14	12
10	30	10	12
11	15	18	8
12	15	22	12
13	30	22	4
14	20	22	6
15	10	14	10
16	30	6	8
17	25	22	10
18	30	18	10
19	20	18	12
20	15	14	4
21	25	10	6
22	25	18	6
23	15	22	6
24	25	10	8
25	15	10	12
26	30	10	8

- The concentrations of mixtures used in the validation set are highlighted.

Procedures

Derivative and first derivative of ratio spectra spectrophotometric methods

Spectral characteristics of ISO, TPZ and OXD

The absorption spectra of 25, 5 and 5 $\mu g\ mL^{-1}$ of ISO, TPZ and OXD solutions, respectively, were recorded using butanol as a blank.

Construction of calibration curves

The D^1 spectra of ISO solutions in the range 5–25 $\mu g\ mL^{-1}$ were recorded using $\Delta\lambda = 4$ and scaling factor = 10, and D^2 spectra of TPZ solutions in the range 2–18 $\mu g\ mL^{-1}$ using $\Delta\lambda = 4$ and scaling factor = 100. The peak amplitude was measured at 226.4 nm for D^1 spectra of ISO and at 270.2 nm for D^2 spectra of TPZ.

The DD^1 spectra of ISO solutions were recorded using 5 $\mu g\ mL^{-1}$ of OXD as a divisor, $\Delta\lambda = 4$ and scaling factor = 10 and DD^1 spectra of TPZ solutions were recorded using 15 $\mu g\ mL^{-1}$ of ISO as a divisor, $\Delta\lambda = 4$ and scaling factor = 10. The peak amplitudes were measured at 227.4 nm for DD^1 spectra of ISO and at 249.2 and 261.4 nm for DD^1 spectra of TPZ. The calibration curves were constructed relating the peak amplitudes against the corresponding drug concentrations and the regression equations were calculated.

Analysis of laboratory prepared mixtures of ISO, TPZ and OXD

Mixtures containing different ratios of ISO/TPZ were prepared as given in Table 1, and then different aliquots of OXD in the range 10–90% of TPZ were added. The volume was completed with butanol. The concentration of both ISO and TPZ in each mixture was measured using the proposed method stated earlier.

Isoabsorptive point spectrophotometric method

Spectral characteristics of ISO, TPZ and OXD

The absorption spectra of 20 $\mu g\ mL^{-1}$ of ISO, TPZ and OXD, prepared separately, were measured and also the spectra of two binary mixtures containing 10 $\mu g\ mL^{-1}$ of ISO/TPZ and TPZ/OXD in a ratio of (1 : 1) each using methanol as a blank.

Construction of calibration curves

The zero order absorbance of each set was recorded then the absorbance was measured at $\lambda_{229.8}$ nm ($Aiso_1$) for ISO in the range 10–60 μg mL^{-1}, at $\lambda_{270.2}$ nm ($Aiso_2$), $\lambda_{310.6}$ nm ($Aiso_3$), $\lambda_{331.8}$ nm ($Aiso_4$) and λ_{max} 311.2 nm for TPZ in the range 10–100 μg mL^{-1}. The peak amplitude of the first derivative spectra at $\lambda_{281.6}$ and $\lambda_{309.4}$ nm for OXD was measured in the range 10–100 μg mL^{-1}.

Six calibration curves relating the absorbance of each curve at the selected wavelengths to the corresponding drug concentrations were constructed. Also, the calibration curves between the peak amplitudes of the first derivative of OXD at $\lambda_{281.6}$ and $\lambda_{309.4}$ nm and its corresponding concentrations were plotted using $\Delta\lambda = 4$ and scaling factor = 10. The regression equations corresponding to each calibration curve were calculated.

Analysis of laboratory prepared mixtures of ISO, TPZ and OXD

Binary mixtures containing different ratios of ISO/TPZ were prepared as given in Table 2. The absorbance of the resulting solutions at λ_{max} 311.2 nm was measured corresponding to the concentration of TPZ alone, then suitable dilution of these mixtures was made and the absorbance at ($Aiso_1$ = 229.8 nm) was measured corresponding to total concentration of ISO and TPZ in the mixture. The total concentration of ISO and TPZ was calculated using its corresponding regression equation, and the concentration of TPZ alone using its corresponding regression equation. The concentration of TPZ was subtracted, after multiplying by the dilution factor, from total concentration of the mixture yielding the actual concentration of ISO in the mixture.

Other ternary mixtures containing different ratios of ISO/TPZ/OXD were prepared; similarly, the concentration of OXD was measured by recording the first derivative spectrum of each mixture and the peak amplitude at $\lambda_{281.6}$ and $\lambda_{309.4}$ nm was measured corresponding to the concentration of OXD alone, the concentration of OXD was calculated using its corresponding regression equations. For determination of total concentration of TPZ and OXD; the zero order spectrum was recorded and the absorbance at ($Aiso_2$ = 270.2 nm), ($Aiso_3$ = 310.6 nm) and ($Aiso_4$ = 331.8 nm) was measured corresponding to the total concentration of TPZ and OXD. The total concentration was calculated using the corresponding regression equations. The concentration of TPZ was determined by subtracting the concentration of OXD from the total concentration of the mixture.

Multivariate analysis

Spectral characteristics of ISO, TPZ and OXD

The zero order absorption spectra of 30 μg mL^{-1} of ISO and 6 μg mL^{-1} of TPZ and OXD were recorded using 0.1N HCl as a blank.

Construction of the training set

Different mixtures of ISO/TPZ/OXD in different ratios were prepared as given in Table 3. The absorbance of these mixtures was measured between 200 and 400 nm at 1 nm intervals with respect to a blank of 0.1N HCl.

The composition of the samples was randomly designed according to five level calibration design[22] in order to obtain non-correlated concentration profiles and this calibration design prepared to obey Beer's Law. Several multivariate calibration models (CLS, PCR, and PLS) were constructed using the data obtained.

Initial developed models were found to have high spectral residuals in the region below 205 and above 270 nm. As a result, this region was rejected. For CLS method, construct CLS model with non-zero intercept.

Constructing the models

To build the CLS model, the computer was fed with the absorbance and concentration matrices with non-zero intercept for the training set. The calculations were carried out to obtain the 'K' matrix. For the PCR and PLS models, the training set absorbance and concentration matrices were used together with PLS-Toolbox 2.0 software for the calculations.

Selection of the optimum number of factors to build the PCR and PLS models

The cross validation method was used, leaving out one sample at a time, to select the optimum number of factors.[23] Given a set of 16 calibration samples, the PCR and PLS calibrations were performed, and using this calibration, the concentration of the sample left out was predicted. The predicted concentrations were then compared with the actual concentrations and the root mean square error of cross validation (RMSECV) was calculated. The RMSECV was calculated in the same manner each time a new factor was added to the model. The maximum number of factors used to calculate the optimum RMSECV was selected to be 9 (half the number of samples + 1).[24] Visual inspection was used for selecting the optimum number of factors. On building the models, mean centering the data gave better results than non-scaling or scaling data using autoscale for both PCR and PLS.

Construction of the validation set

Ten different mixtures of ISO/TPZ/OXD were prepared by transferring different volumes of their working standard solutions. The developed models were applied to predict the concentration of ISO and TPZ in each mixture.

Application to pharmaceutical formulation (Stellamide® tablets)

Thirty tablets of Stellamide® were weighed, finely powdered and mixed well. An accurately weighed portion of the powder equivalent to 100 mg of ISO and 20 mg of TPZ was transferred into 100-mL volumetric flask; 75 mL methanol was added and sonicated for 20 min; and the volume was completed with methanol and filtered. The solution was diluted to obtain the appropriate working solution with the appropriate solvent for each method. Then the procedure was followed according to each method mentioned above.

Results and Discussion

ISO and TPZ are co-formulated together in a pharmaceutical formulation, so, it was necessary to determine each of them in presence of the other.

ISO is stable and no degradation products have been observed to occur either under normal or exaggerated storage conditions.[25]

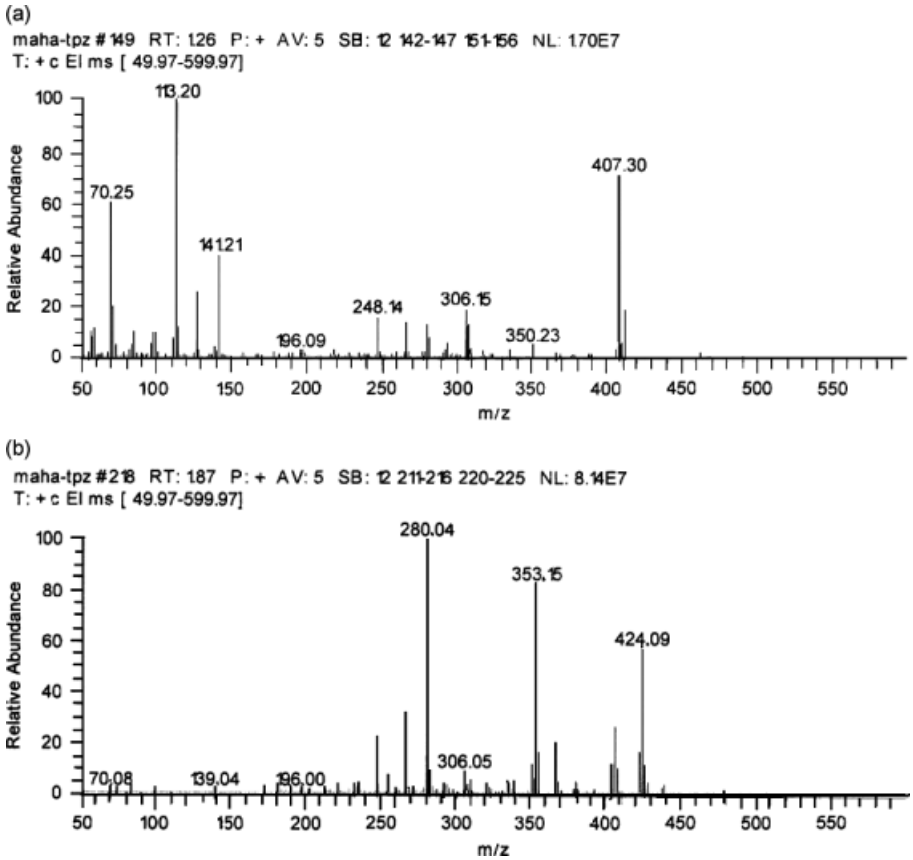
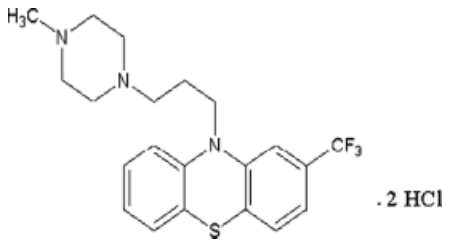
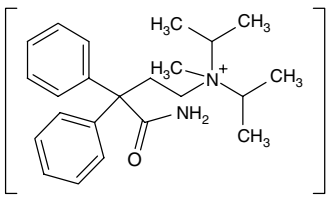


Figure 1. Mass spectra of Trifluoperazine Hydrochloride (A) and its oxidative degradate (B).



Trifluoperazine Hydrochloride (TPZ)
Mol. Formula $C_{21}H_{24}F_3N_3S.2HCl$
Mol. Wt. 480.43

Scheme 1. Structural formula of TPZ.



Isopropamide Iodide (ISO)
Mol. Formula $C_{23}H_{33}IN_2O$
Mol. Wt. 480.43

Scheme 2. Structural formula of ISO.

TPZ, being a phenothiazine derivative, is liable to oxidation with hydrogen peroxide at room temperature giving its sulfoxide oxidative degradate, which was reported to be its photodegradation product[26] and representing 6% of TPZ metabolites, so the determination of the binary mixture in bulk powder or in their pharmaceutical formulation without interference of OXD which is less active than the intact drug was an analytical task of potential.

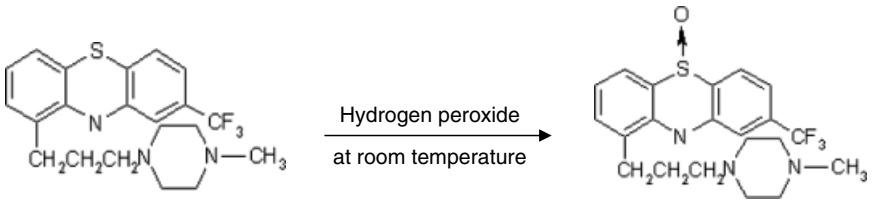
Identification of OXD

The electron impact showed mass ion peak at m/z 408 corresponding to the intact drug, while the mass ion peak of the oxidative degradate was at m/z 424 as shown in Figure 1. Therefore one can conclude that carrying out oxidation of trifluoperazine can be illustrated from Scheme 3.

Derivative spectrophotometric method

Spectrophotometry is a common technique in the field of pharmaceutical analysis. Direct UV-absorbance measurements are subjected to interference from co-formulated drugs, excipients and/or degradation products. Thus, in many cases the recorded absorption is a summation of that of the analyte and of the accompanying materials referred to irrevalent absorption. Among the techniques used to reduce or eliminate such interference or irrelevant absorption is the derivative spectrophotometry.[27–29]

The suggested method allowed the quantitative determination of ISO and TPZ in presence of OXD without prior separation. On



Trifluoperazine
Mol. Formula $C_{21}H_{24}F_3N_3S$
Mol. Wt. 407.5

Oxidative degradate
Mol. Formula $C_{21}H_{24}F_3N_3SO$
Mol. Wt. 424

Scheme 3. Oxidation pathway of Trifluoperazine hydrochloride to its oxidative degradate.

the basis of derivative spectrophotometric techniques, D^1 and D^2 techniques were applied to determine ISO and TPZ, respectively, in the presence of OXD.

In Figure 2 is showed that there was severe overlapping between ISO, TPZ and OXD which interfered with the direct determination of both drugs. Different factors affecting separation were studied to optimize resolution of drugs, including solvent effect. Different solvents were tried in order to eliminate interference, e.g., methanol, ethanol, butanol, acetonitrile, 0.05 N HCl and 0.05 N NaOH. First, second, third and fourth derivatives were obtained. It is worthwhile to mention that trials to use first, second, third and fourth derivatives were unsuccessful in methanol, ethanol, acetonitrile, 0.05N HCl and 0.05N NaOH, as no zero-crossing was obtained. The best resolution was obtained by applying D^1 for determination of ISO at 226.4 nm (corresponding to zero-crossing of TPZ and OXD) and D^2 for determination of TPZ at 270.2 nm (corresponding to zero-crossing of ISO and OXD) using butanol as a solvent. Different parameters affecting D^1 and D^2 spectra were investigated; different smoothing factor ($\Delta\lambda$) intervals and scaling factor values were tried. The best condition was obtained by using 4 nm as $\Delta\lambda$ interval for both D^1 and D^2 and scaling factor 10 and 100 for D^1 and D^2, respectively, as shown in Figures 3 and 4.

Linear correlations were obtained between peak amplitudes at 226.4 nm for D^1 spectra of ISO in the concentration range 5–25 μg mL^{-1} and peak amplitudes at 270.2 nm for D^2 spectra of TPZ in concentration range of 2–18 μg mL^{-1} from which the regression equations were calculated and found to be:

$$P.A_1 = 0.0292\,C_1 + 0.0295 \quad r = 0.9999 \quad \text{at 226.4 nm for ISO}$$
$$P.A_2 = 0.1172\,C_2 + 0.0354 \quad r = 0.9999 \quad \text{at 270.2 nm for TPZ}$$

where $P.A_1$ and $P.A_2$ are the peak amplitudes of ISO and TPZ, respectively, C_1 and C_2 are the concentration of ISO and TPZ in μg mL^{-1}, respectively, and r is the correlation coefficient.

Results described in Table 1 show that this method is selective, valid and applicable for the determination of ISO and TPZ in presence of up to 60% of OXD in different laboratory prepared mixtures.

First derivative of ratio spectra spectrophotometric method (DD^1)

Another method for resolving binary mixtures without previous separation is the derivative ratio spectrophotometry (DD^1), which was developed by Salinas *et al*.[30]

In order to improve the selectivity of the determination of ISO and TPZ in the presence of OXD, DD^1 spectrophotometric method was established. The main advantage of the method is that the whole spectrum of interfering substance is cancelled. Accordingly, the choice of the wavelength selected for calibration is not critical as in the derivative method.

The absorption spectra of ISO, TPZ and OXD were divided by the spectrum of 5 μg mL^{-1} of OXD (as a divisor), and DD^1 spectra were obtained, where ISO could be determined at 227.4 nm (corresponding to zero-crossing of TPZ and OXD). While TPZ could be determined by dividing the absorption spectra of ISO, TPZ and OXD by the spectrum of 15 μg mL^{-1} of ISO (as a divisor), and DD^1 spectra were obtained, where TPZ could be determined at 249.2 and 261.4 nm (corresponding to zero-crossing of ISO and OXD), as shown in Figures 5 and 6.

Selection of the divisor and its concentration of great importance, so different concentrations of ISO (5, 10, 15 and 20 μg mL^{-1}) and OXD (5, 10, 15 and 17 μg mL^{-1}) were tried as divisors. The best results in terms of signal-to-noise ratio, sensitivity and selectivity followed using 5 μg mL^{-1} of OXD as a divisor for determination of ISO and 15 μg mL^{-1} of ISO as a divisor for determination of TPZ.

Linear correlations were obtained between peak amplitudes at 227.4 nm for DD^1 spectra of ISO in the concentration range 5–25 μg mL^{-1} and peak amplitudes at 249.2 and 261.4 nm for DD^1 spectra of TPZ in concentration range of 2–18 μg mL^{-1} from which the linear regression equations were computed and found to be:

$$P.A_1 = 0.1469\,C_1 + 0.1106 \quad r = 0.9998 \quad \text{at 227.4 nm for ISO}$$
$$P.A_2 = 3.2166\,C_2 + 1.3230 \quad r = 0.9999 \quad \text{at 249.2 nm for TPZ}$$
$$P.A_3 = 2.9671\,C_3 + 1.1376 \quad r = 0.9999 \quad \text{at 261.4 nm for TPZ}$$

where $P.A_1$, $P.A_2$ and $P.A_3$ are the peak amplitudes of ISO and TPZ, respectively, C_1, C_2 and C_3 are the concentrations of ISO and TPZ in μg mL^{-1}, respectively, and r is the correlation coefficient.

Results obtained by applying the suggested DD^1 method in Table 1 show that neither TPZ nor ISO influence the determination of the other drug concentration in the prepared mixtures containing different ratios of both drugs and in presence of up to 70% of OXD.

Isoabsorptive point spectrophotometric method

In this work, the so-called 'isoabsorptive spectrophotometry', developed by Erram and Tipnis,[31–33] is applied for simultaneous determination of ISO and TPZ in their binary mixture, also in presence of OXD as a stability indicating procedure.

The theory of this method could be confirmed experimentally by recording the absorbance spectra of 20 μg mL^{-1} of ISO, TPZ and OXD separately, and that of a mixture containing equal concentration of ISO and TPZ (10 μg mL^{-1} of each ISO and TPZ) and a mixture containing equal concentration of TPZ and its OXD (10 μg mL^{-1} of each TPZ and OXD), as shown in Figures 7 and 8.

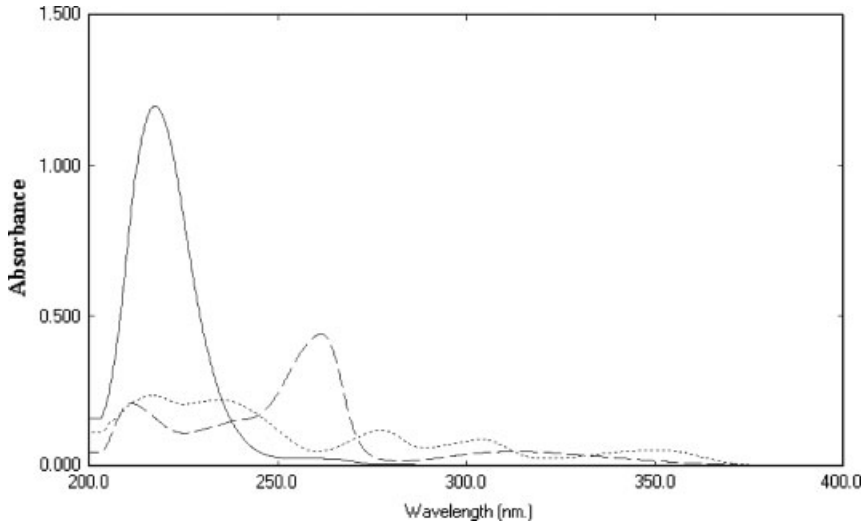


Figure 2. Zero order absorption spectra of 25 μg mL^{-1} of Isopropamide Iodide (———), 5 μg mL^{-1} of Trifluoperazine Hydrochloride (- - - -) and Trifluoperazine oxidative degradate (· · · ·) using butanol as a solvent.

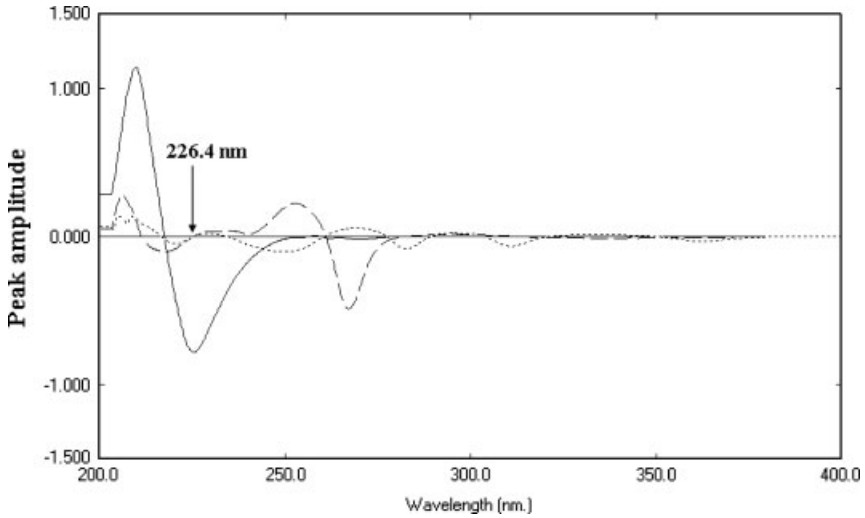


Figure 3. First derivative absorption spectra of 25 μg mL^{-1} of Isopropamide Iodide (———), 5 μg mL^{-1} of Trifluoperazine Hydrochloride (- - - -) and Trifluoperazine oxidative degradate (· · · ·) using butanol as a solvent.

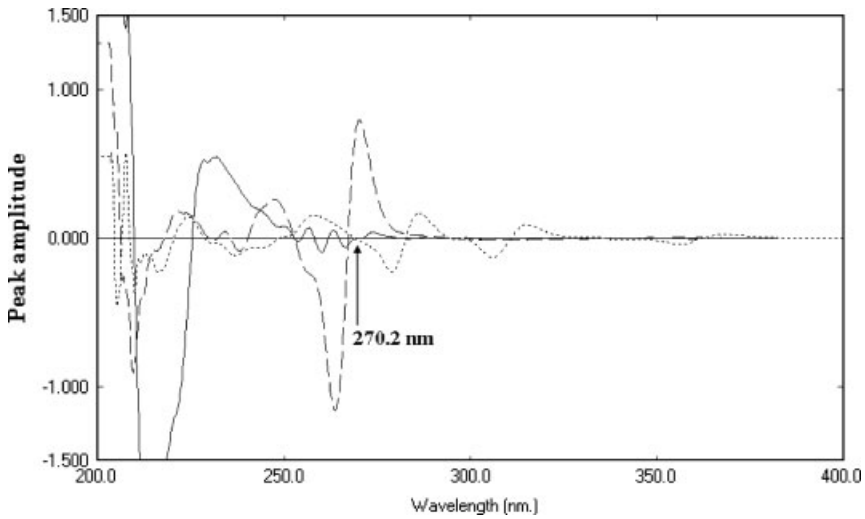


Figure 4. Second derivative absorption spectra of 25 μg mL^{-1} of Isopropamide Iodide (———), 5 μg mL^{-1} of Trifluoperazine Hydrochloride (- - - -) and Trifluoperazine oxidative degradate (· · · ·) using butanol as a solvent.

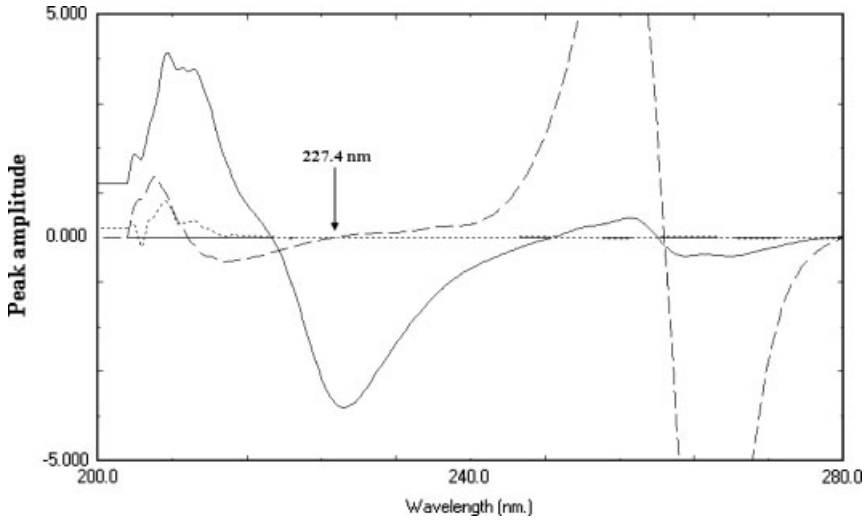


Figure 5. First derivative of ratio spectra of 25 μg mL^{-1} of Isopropamide Iodide (———), 5 μg mL^{-1} of Trifluoperazine Hydrochloride (- - - -) and 10 μg mL^{-1} of Trifluoperazine oxidative degradate (· · · ·) using 5 μg mL^{-1} of Trifluoperazine oxidative degradate as a divisor and butanol as a solvent.

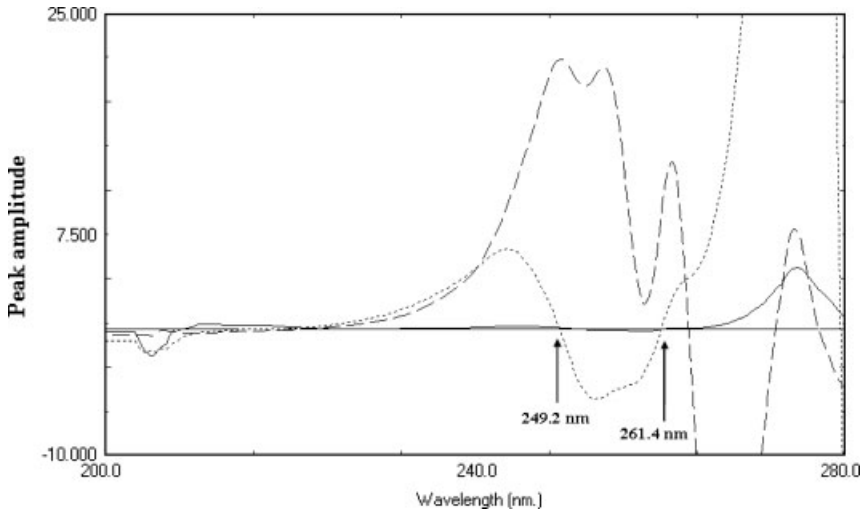


Figure 6. First derivative of ratio spectra of 25 μg mL^{-1} of Isopropamide Iodide (———), 5 μg mL^{-1} of Trifluoperazine Hydrochloride (- - - -) and 10 μg mL^{-1} of Trifluoperazine oxidative degradate (· · · ·) using 15 μg mL^{-1} of Isopropamide Iodide as a divisor and butanol as a solvent.

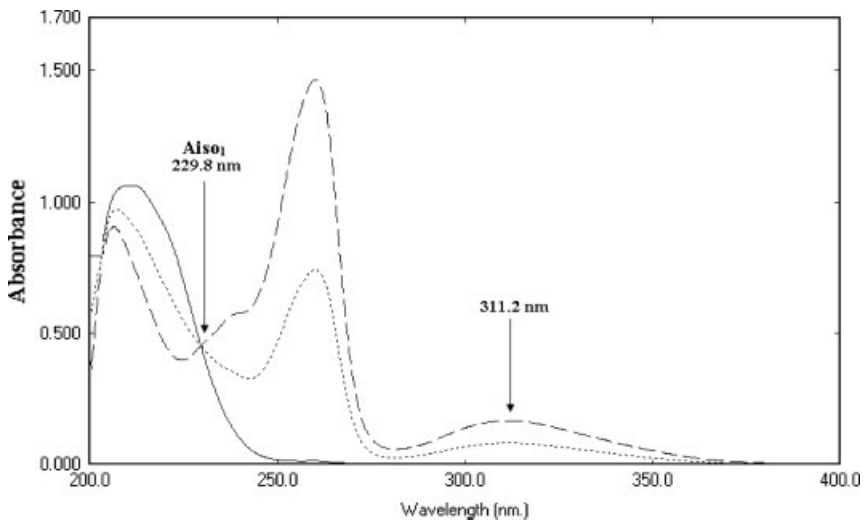


Figure 7. Zero-order absorption spectra of 20 μg mL^{-1} of Isopropamide iodide (———), 20 μg mL^{-1} of Trifluoperazine Hydrochloride (- - - -) and a (1 : 1) mixture contains 10 μg mL^{-1} of each (· · · ·) using methanol as a blank.

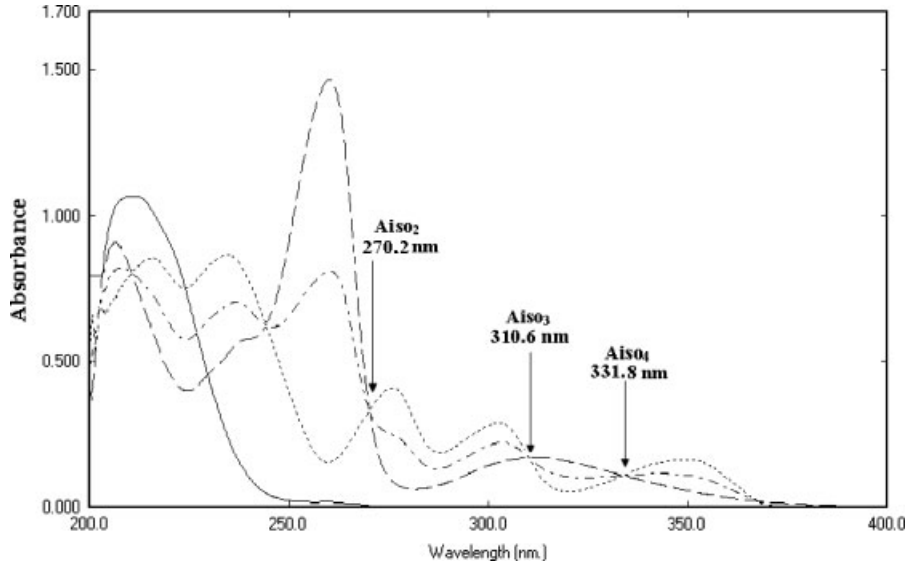


Figure 8. Zero-order absorption spectra of 20 μg mL^{-1} of Isopropamide Iodide (———), 20 μg mL^{-1} of Trifluoperazine Hydrochloride (- - - -), 20 μg mL^{-1} of Trifluoperazine oxidative degradate (· · · ·) and a (1 : 1) mixture contains 10 μg mL^{-1} of each Trifluoperazine and its degradate (-.-.-.-) using methanol as a blank.

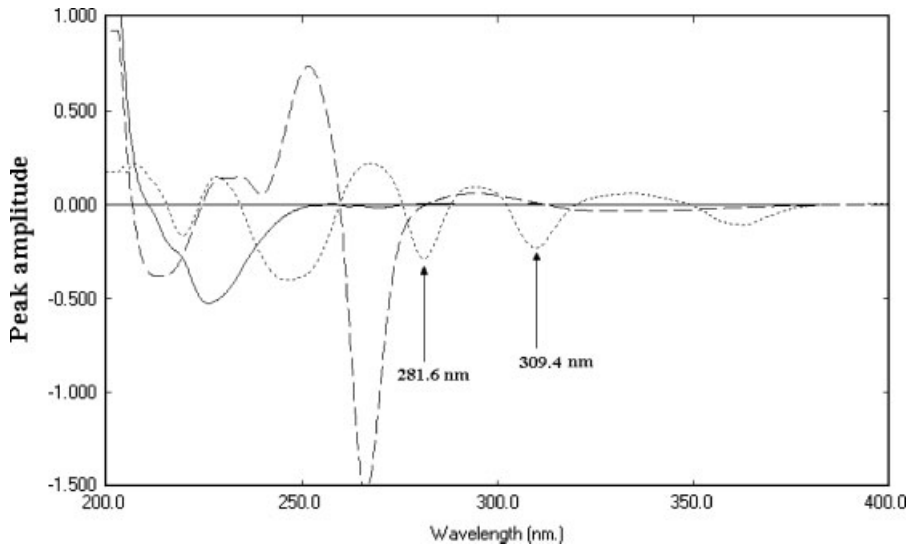


Figure 9. First derivative absorption spectra of 20 μg mL^{-1} of Isopropamide Iodide (———), 20 μg mL^{-1} of Trifluoperazine Hydrochloride (- - - -) and 20 μg mL^{-1} of Trifluoperazine oxidative degradate (· · · ·) using methanol as a blank.

In Figure 7, it can be seen that the mixture and the pure drugs have different absorbance spectra; they possess the same absorbance at their isoabsorptive points. These data allows us to conclude, according to the theory, that the mixture of drugs acts as a single component and gives the same absorbance value as pure drugs at their isoabsorptive points. Thus, by measuring the absorbance value at the chosen isoabsorptive points, the total concentration of the mixture could be calculated as explained by the theory previously; by applying the suggested procedure the absorbance at $\lambda_{229.8}$ nm ($Aiso_1$) for ISO was obtained over different ranges, while the concentration of TPZ in ISO/TPZ mixture could be calculated at its λ_{max} 311.2 nm without any interference from ISO. The absorbance at $\lambda_{270.2}$ nm ($Aiso_2$), $\lambda_{310.6}$ nm ($Aiso_3$) and $\lambda_{331.8}$ nm ($Aiso_4$) for TPZ was obtained. The concentration of OXD in ISO/TPZ/OXD mixture could be determined from the first derivative spectra at $\lambda_{281.6}$ and $\lambda_{309.4}$ nm as in Figure 9. Thus by applying this procedure the absorbance at λ_{max} 311.2 nm for TPZ and the peak amplitude of the first derivative spectra at $\lambda_{281.6}$ and $\lambda_{309.4}$ nm for OXD were obtained. Thus the concentration of either ISO or TPZ could be calculated by subtraction.

Calibration curves were constructed for the above linear relations relating the absorbances and the peak amplitudes to the corresponding concentrations. The linear relationships were obtained in different ranges. The corresponding regression equations were computed and found to be:

$Aiso_1 = 0.0220\,C + 0.0170$	$r = 0.9999$	at $\lambda_{229.8}$ nm
$Aiso_2 = 0.0124\,C + 0.1545$	$r = 0.9999$	at $\lambda_{270.2}$ nm
$Aiso_3 = 0.0077\,C + 0.0181$	$r = 0.9999$	at $\lambda_{310.6}$ nm
$Aiso_4 = 0.0052\,C + 0.0214$	$r = 0.9999$	at $\lambda_{331.8}$ nm

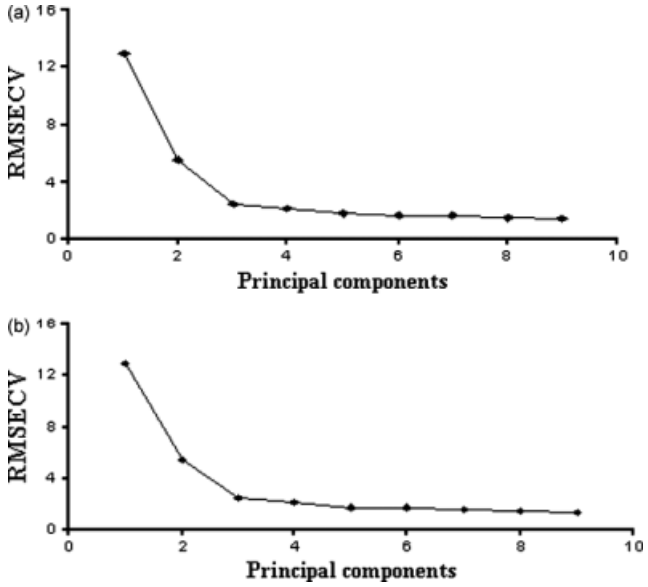


Figure 10. (A) RMSECV plot of a training set prediction using cross validation (principal component regression model). (B) RMSECV plot of a training set prediction using cross validation (partial least squares model).

$A_{max} = 0.0077\,C + 0.0181$	$r = 0.9999$	at λ_{max} 311.2 nm
$P_{281.6} = 0.0129\,C + 0.0273$	$r = 0.9999$	at $\lambda_{281.6}$ nm
$P_{309.4} = 0.0103\,C + 0.0186$	$r = 0.9999$	at $\lambda_{309.4}$ nm

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

In order to demonstrate the validity and applicability of the proposed method, recovery studies were performed by analyzing laboratory prepared mixtures of ISO/TPZ and ISO/TPZ/OXD which prepared in different ratios. Results obtained are shown in Table 2.

Multivariate calibration method

Chemometrics is the art of processing data with various numerical techniques in order to extract useful information.[23] It is the application of mathematical and statistical methods to design optimum procedures and to provide maximum chemical information through analysis of chemical data.

In this method, different chemometric approaches were applied for the determination of ISO and TPZ in presence of OXD, including CLS, PCR and PLS. These multivariate calibrations were useful in spectral analysis because the simultaneous inclusion of many spectral wavelengths instead of single wavelength greatly improved the precision and predictive ability.[34]

The first step in the simultaneous determination of three components by multivariate calibration methods involved constructing the calibration matrix for ternary mixture. The calibration set was obtained by using the absorption spectra of a set of 16 mixtures of ISO, TPZ and OXD with different ratios of each component; their concentrations are given in Table 3. Better results were obtained on rejecting the spectral region above 270 nm and below 205 nm.

In this study, the 'leave one out' cross-validation method was used and the RMSECV values of different developed models were compared. Three factors were found suitable for both PCR and PLS as shown in Figure 10. Table 3 shows different concentrations of ISO/TPZ/OXD used in the validation set. To validate the prediction ability of the suggested models, they were used to predict the concentration of ISO and TPZ in laboratory-prepared mixtures containing different ratios of them and OXD, satisfactory results were obtained as shown in Table 4.

For evaluation of the predictive abilities of the developed models, several diagnostic tools were used: predictive versus actual concentration plot (model and sample diagnostic); concentration residuals versus actual concentration plot (model and sample diagnostic; and root mean square error of prediction (RMSEP) (model diagnostic), the predicted concentrations of the validation samples were calculated.

The suggested methods are valid and applicable for the analysis of ISO and TPZ in their pharmaceutical formulation (Stellamide® tablets). Furthermore, the validity of the proposed method was assessed by applying the standard addition technique, which showed accurate results and there was no interference from tablet excipients (lactose, cellulose, crosscarmellose sodium, gelatin, magnesium stearate, titanium dioxide and hydroxyl propyl methyl cellulose) as shown in Table 5.

Method validation was performed according to USP guidelines[3] for all the proposed methods. Table 6 shows results of accuracy, repeatability and intermediate precision of the methods. Other regression equation parameters are shown in

Table 4. Results of assay validation parameters of the proposed multivariate method for the determination of Isopropamide Iodide and Trifluoperazine Hydrochloride

Validation parameters	Isopropamide Iodide			Trifluoperazine Hydrochloride			Oxidative degradate		
	CLS	PCR	PLS	CLS	PCR	PLS	CLS	PCR	PLS
Mean ± SD	100.3 ± 0.85	100.4 ± 1.08	100.4 ± 1.07	100.2 ± 0.73	100.3 ± 0.72	100.3 ± 0.72	99.9 ± 2.65	100.0 ± 2.54	100.0 ± 2.44
RMSEP	0.21	0.33	0.35	0.20	0.20	0.20	0.73	0.68	0.65
Predicted versus actual concentration plot									
							–	–	–
a-Slope	1.0078	1.0219	1.0224	1.0070	1.0082	1.0081			
b-Intercept	−0.0747	−0.2793	−0.2867	−0.0662	−0.0634	−0.0600			
c-Correlation coefficient (r)	0.9998	0.9997	0.9997	0.9999	0.9999	0.9999			

Table 5. Determination of Isopropamide Iodide and Trifluoperazine Hydrochloride in their pharmaceutical formulation by the proposed methods and application of standard addition technique

Stellamide® tablets claimed to contain 5 mg ISO and 1 mg TPZ/tablet (Batch No. 0610875)	Technique	Component		Taken (μg mL^{-1})	Found %* ± SD	Standard addition technique Mean ± SD **
	Derivative spectrophotometric method		ISO	5	102.0 ± 1.23	100.3 ± 1.45
			TPZ	4	99.50 ± 1.050	100.7 ± 1.80
	First derivative of ratio spectra spectrophotometric method		ISO	5	102.6 ± 1.10	100.9 ± 0.93
		TPZ	at 249.2 nm	4	100.3 ± 1.11	99.8 ± 1.57
			at 261.4 nm		100.4 ± 1.11	100.2 ± 1.69
	Isoabsorptive method		ISO	20	102.0 ± 0.76	100.2 ± 0.78
		TPZ	at 311.2 nm	50	100.9 ± 0.70	100.4 ± 1.15
			at 270.2 nm		100.6 ± 0.80	100.4 ± 1.10
			at 310.6 nm		100.8 ± 0.79	100.4 ± 0.84
			at 331.8 nm		100.8 ± 0.773	100.4 ± 0.94
	Multivariate method	ISO	CLS	10	102.2 ± 1.52	99.67 ± 1.073
			PCR		101.5 ± 1.34	99.7 ± 1.05
			PLS		101.3 ± 1.31	99.9 ± 0.87
		TPZ	CLS	8	99.3 ± 1.48	99.8 ± 1.32
			PCR		100.1 ± 1.37	99.5 ± 1.46
			PLS		99.8 ± 1.33	99.4 ± 1.27

* Average of 6 determinations.
** Average of 3 determinations.

Table 6, which shows good linear relationship for the method as revealed by the correlation coefficient. Descriptive statistics of the regression showed low values of standard error of intercept and slope which revealed high accuracy with minimum deviations and low scattering of the calibration points.[35]

Table 7 shows statistical comparison of the results obtained by the proposed methods and the reported spectrophotometric method.[18] The calculated *t*- and F-values are less than the theoretical ones indicating that there is no significant difference between the proposed methods and the reported method with respect to accuracy and precision. One-way ANOVA was applied for the comparison of these methods showing no significant difference between the proposed methods and the reported method for both ISO as $F_{calculated}$ (0.857) < $F_{tabulated}$ (2.251) and TPZ as $F_{calculated}$ (1.483) < $F_{tabulated}$ (2.064).

Conclusion

The present work introduces four different, simple, sensitive and rapid spectrophotometric methods for the determination of ISO and TPZ in binary mixture and in presence of OXD and in their pharmaceutical preparation.

The proposed derivative and first derivative of ratio spectra methods were found to be more sensitive and selective than the published spectrophotometric methods and have the advantage of determination of ISO and TPZ in the presence of OXD.

Isoabsorptive point method has the advantage of being simple, rapid and could be considered as a stability-indicating method for determination of TPZ in the presence of its oxidative degradate in addition to the determination of the binary mixture of ISO and TPZ. It has the advantage over derivative methods of determination of OXD concentration which is useful for quality control analysis.

In this work, chemometric methods present simple, selective, accurate and economical procedures for the simultaneous determination of ISO and TPZ and would be useful for the stability investigation of TPZ in pharmaceutical analysis and quality control for medicinal manufacturers. These chemometric methods were useful in spectral analysis because the simultaneous inclusion of many spectral wavelengths instead of single wavelength greatly improved the precision and predictive ability. The multivariate methods have the advantage of small relative prediction errors obtained by the smaller number of latent variables, which verify their ability to get accurate results; these methods are less dependent on data manipulation than other spectrophotometric methods and as a single operation are less prone to human error.

The proposed methods have the advantage over other published methods of analyzing the binary mixture in the presence of OXD which is easily formed and could be useful for the stability investigation of TPZ and for checking the extent of degradation in pharmaceutical formulations due to their simplicity, accuracy and sensitivity.

Table 6. Results of assay validation parameters of the proposed methods for the determination of Isopropamide Iodide, Trifluoperazine Hydrochloride and Trifluoperazine oxidative degradate

	Derivative method		DD[1] method			Isoabsorptive method						
				TPZ		ISO	TPZ				OXD	
Parameters	ISO	TPZ	ISO	at 249.2 nm	at 261.4 nm	at 229.8 nm	at 311.2 nm	at 270.2 nm	at 310.6 nm	at 331.8 nm	at 281.6 nm	at 309.4 nm
Range (µg mL^{-1})	5–25	2–18	5–25	2–18		10–60	20–160				10–100	
Linearity												
Slope	0.0292	0.1172	0.1469	3.2166	2.9671	0.022	0.0077	0.0124	0.0077	0.0052	0.0129	0.0103
Intercept	0.0295	0.0354	0.1106	1.323	1.1376	0.017	0.0181	0.1545	0.0181	0.0214	0.0273	0.0186
Correlation coefficient (r)	0.9999	0.9999	0.9998	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999
Accuracy (mean ± SD)	100.0 ± 0.92	100.0 ± 1.16	99.9 ± 0.91	99.9 ± 1.11	99.8 ± 1.15	99.9 ± 0.80	100.1 ± 0.69	99.9 ± 1.13	100.1 ± 0.69	99.1 ± 0.70	99.9 ± 1.03	99.5 ± 0.90
Specificity and Selectivity	99.5 ± 1.60	100.0 ± 1.25	99.1 ± 1.33	100.5 ± 1.10	100.0 ± 1.08	99.1 ± 0.8	99.0 ± 1.04	99.9 ± 0.96	99.6 ± 1.01	100.3 ± 0.87	99.1 ± 1.34	99.3 ± 1.16
Precision (RSD%)												
Repeatability*	0.66	0.71	0.58	0.72	0.70	0.61	0.73	0.63	0.73	0.66	0.75	0.76
Intermediate precision*	0.90	0.89	0.85	0.90	0.86	0.82	0.85	0.79	0.90	0.81	0.90	0.90
LOD**	1.01	0.45	1.12	0.51	0.45	2.05	4.08	4.54	5.01	5.11	1.78	2.05
LOQ**	2.88	1.2	2.5	1.08	1.13	5.11	9.45	8.98	9.88	10.03	3.45	4.01

* The intraday precision (n=3), average of three different concentrations repeated three times within day. The interday precision (n=3), average of three different concentrations repeated three times in three successive days.
** Limit of detection and quantitation are determined via calculations.[3]
LOD = (SD of the response/slope) × 3.3; LOQ = (SD of the response/slope) × 10.

Table 7. Statistical comparison of the results obtained by the proposed methods and the reported method for the determination of pure Isopropamide Iodide and Trifluoperazine Hydrochloride

	Derivative method		DD[1] method			Isoabsorptive method				Multivariate method						Reported method*[18]	
				TPZ		ISO	TPZ			ISO			TPZ				
Items	ISO	TPZ	ISO	at 249.2 nm	at 261.4 nm	at 229.8 nm	at 310.6 & 311.2 nm	at 270.2 nm	at 331.8 nm	CLS	PCR	PLS	CLS	PCR	PLS	ISO	TPZ
Mean	100.0	100.0	99.9	99.9	99.8	99.9	100.1	99.9	99.1	100.3	100.4	100.4	100.2	100.3	100.3	99.7	99.6
SD	0.92	1.16	0.91	1.11	1.15	0.80	0.69	1.13	0.70	0.85	1.08	1.07	0.73	0.72	0.72	1.16	1.20
RSD %	0.92	1.16	0.91	1.11	1.15	0.80	0.69	1.13	0.71	0.85	1.08	1.07	0.73	0.72	0.72	1.16	1.20
n	10	10	10	10	10	10	8	8	8	10	10	10	10	10	10	8	8
Variance	0.85	1.35	0.83	1.23	1.32	0.64	0.48	1.28	0.50	0.72	1.17	1.14	0.53	0.52	0.52	1.35	1.44
Student's t-test	0.736	0.662	0.534	0.643	0.395	0.369	1.125	0.463	0.999	1.289	1.419	1.442	1.313	1.604	1.628		
	(2.120)	(2.120)	(2.120)	(2.120)	(2.120)	(2.120)	(2.145)	(2.145)	(2.145)	(2.120)	(2.120)	(2.120)	(2.120)	(2.120)	(2.120)		
F-value	1.62	1.07	1.65	1.18	1.09	2.14	3.06	1.13	2.99	1.88	1.17	1.18	2.72	2.79	2.81		
	(3.29)	(3.29)	(3.29)	(3.29)	(3.29)	(3.29)	(3.79)	(3.79)	(3.79)	(3.29)	(3.29)	(3.29)	(3.29)	(3.29)	(3.29)		

* Second derivative spectrophotometric determination of ISO at 230.2 nm and of TPZ at 270.4 nm in aqueous solution.
- Figures between parenthesis represent the corresponding tabulated values of t and F at P = 0.05.

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