Mean centering of ratio spectra and successive derivative ratio spectrophotometric methods for determination of isopropamide iodide, trifluoperazine hydrochloride and trifluoperazine oxidative degradate

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Abstract Two sensitive, selective and precise stability indicating methods for the determination of isopropamide iodide (ISO), trifluoperazine hydrochloride (TPZ) and trifluoperazine oxidative degradate (DEG) were developed and validated. Method A is a successive derivative ratio spectrophotometric one, which depends on the successive derivative of ratio spectra in two steps using 0.1 N HCl as a solvent and measuring TPZ at 250.4 and 257.2 nm, ISO at 223 and 228 nm and DEG at 210.6, 213 and 270.2 nm. Method B is mean centering of ratio spectra which depends on using the mean centered ratio spectra in two successive steps and measuring the mean centered values of the second ratio spectra at 322, 355 and 339 nm for TPZ, ISO and DEG, respectively. Factors affecting the developed methods were studied and optimized, moreover, they have been validated as per ICH guidelines and the results demonstrated that the suggested methods are reliable, reproducible and suitable for routine use with short analysis time. Statistical analysis of the two developed methods with the reported one using F- and Student’s t-test showed no significant difference regarding accuracy and precision.

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1. Introduction

Trifluoperazine hydrochloride (TPZ) chemically is 10-[3-(4-methyl-1-piperazinyl) propyl]-2-(trifluoromethyl)-10H-phenothiazine dihydrochloride (Budavaried, 2002). The structural formula of TPZ is shown below.

Trifluoperazine hydrochloride (TPZ): mol. formula, C_{21}H_{24}F_{3}N_{3}S$\cdot$2HCl; mol. wt., 480.43.

Trifluoperazine hydrochloride 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 (B.P., 2007) or using crystal violet indicator (USP, 2007).

Isopropamide iodide (ISO), chemically is γ-(aminocarbonyl)-N-methyl-N,N-bis (1-methylethyl)-γ-phenylbenzenepropanaminium iodide (Budavaried, 2002). The structural formula of ISO is shown below.

Isopropamide iodide (ISO): mol. formula, C_{23}H_{33}IN_{2}O; mol. wt., 480.43.

Isopropamide iodide is a quaternary ammonium anticholinergic drug. The official method for determination of ISO is non aqueous titration with perchloric acid (USP, 2007). The official method for the determination of ISO in tablets is ion exchange resin and the absorbance of eluant detected at 280 and 258 nm (USP, 2007).

The binary mixture of ISO and TPZ is used for their anti-emetic and anti spasmodic effects. Few analytical methods have been described for the simultaneous determination of ISO and TPZ in their binary mixture including the derivative and the derivative of ratio spectra spectrophotometry (El-Gindy et al., 2001). D^{2} spectrophotometric determination of the binary mixture after the chloroformic extraction of TPZ (El-Yazbi et al., 1991) and D^{2} spectrophotometric method for determination of the binary mixture in methanol were also stated (Hassib et al., 2002). Derivative, derivative ratio, isoabsorptive point and chemometric spectrophotometric methods have been reported for determination of the binary mixture in the presence of TPZ oxidative degradate (Abbas et al., 2010).

The aim of this work is to develop simple, sensitive, rapid and precise methods for the selective determination of isopropamide iodide, trifluoperazine hydrochloride and its oxidative degradation product. Reviewing the literature in hand, the reported spectrophotometric method (Abbas et al., 2010) can resolve the binary mixture in the presence of TPZ oxidative degradate but failed to determine the concentration of the degradate itself. The suggested work presents new spectrophotometric methods for determination of the binary mixture and the degradation product which is easily formed due to bad storage and less active than TPZ. Also, the proposed methods were able to determine the extent of degradation which can be applied for analysis of the cited drugs in quality control laboratories.

2. Experimental

2.1. Instruments

A double beam UV–visible spectrophotometer (SHIMADZU, Japan) model UV-1601 PC with quartz cell of 1 cm pathlength was connected to IBM compatible computer. The software was UVPC personal spectroscopy software version 3.7. All data analyses were performed using PLS-Toolbox 2.0 running under MATLAB®, version 6.5 (Wise and Gallagher, 1998).

2.2. Materials

2.2.1. Pure standard

Isopropamide iodide and trifluoperazine hydrochloride were kindly supplied from Kahira Pharm. and Chem. Ind. Co., Cairo, Egypt. Their purity was found to be 98.95% and 101.06%, respectively according to the reported spectrophotometric method (Abbas et al., 2010).

2.2.2. Pharmaceutical formulation

Stellamide® tablets (Batch No. 0610875) are labeled to contain 6.8 mg isopropamide iodide equivalent to 5 mg of isopropamide ion and 1.18 mg of trifluoperazine hydrochloride equivalent to 1 mg of trifluoperazine base, manufactured by Kahira Pharm. and Chem. Ind. Co., Cairo, Egypt.

2.2.3. Chemicals and reagents

All chemicals used throughout this work were of analytical grade, and the solvents were of spectroscopic grade.

1. Methanol (Merck, Germany).
2. Hydrochloric acid, 0.1 N aqueous solution (Merck, Germany).

2.3. Standard solutions

(a) Stock standard solutions of ISO, TPZ and DEG (1 mg mL^{-1} in methanol).
(b) Working standard solutions of ISO, TPZ and DEG (100 μg mL^{-1}): were prepared by a suitable dilution of stock solutions of each component with 0.1 N HCl solution.

2.4. Laboratory prepared mixtures

Different mixtures containing different ratios of ISO, TPZ and DEG were prepared using their respective working solutions (100 μg mL^{-1}).
3. Procedures

3.1. Spectral characteristics of isopropamide iodide, trifluoperazine hydrochloride and trifluoperazine oxidative degradate

The absorption spectra of 25, 5 and 5 μg mL⁻¹ of ISO, TPZ and DEG, respectively, were recorded using 0.1 N HCl solution as a blank.

3.2. Successive derivative ratio spectrophotometric method

Into three separate sets of 10-mL volumetric flasks, different aliquots containing 40–220, 40–300 and 50–250 μg of TPZ, ISO and DEG, respectively, were accurately transferred from their working solutions; the volume was then completed with 0.1 N HCl. The zero order absorbance spectra of each set were then recorded in the range of 200–400 nm. For determination of TPZ, the stored spectra of TPZ were divided by the standard spectrum of 10 μg mL⁻¹ of ISO and first derivative of the produced ratio spectra was then obtained. Then these vectors (first derivative of the first ratio spectra) were divided by (d/dλ)(DEG/ISO) corresponding to the first derivative of the ratio spectra of 10 μg mL⁻¹ of each to obtain the second ratio spectra and the first derivative of these ratio spectra was then obtained using Δλ = 4. For ISO, the recorded spectra of ISO were divided by the spectrum of 10 μg mL⁻¹ of TPZ and the first derivative of these vectors was then divided by (d/dλ)(DEG/TPZ) corresponding to the first derivative of the ratio spectra of 10 μg mL⁻¹ of each and the second ratio spectra were obtained. Similarly, the recorded spectra of DEG were divided by the spectrum of 10 μg mL⁻¹ of ISO and the first derivative of these vectors was then divided by (d/dλ)(TPZ/ISO) corresponding to the first derivative of the ratio spectra of 10 μg mL⁻¹ of each and the second ratio spectra were obtained. The first derivative of these vectors was then obtained with Δλ = 4. The calibration curves were obtained by plotting the amplitudes at 250.4 and 257.2 nm for TPZ, at 223 and 228 nm for ISO and at 210.6, 213 and 270.2 nm for DEG versus the corresponding concentrations of each component.

3.3. Mean centering of ratio spectra spectrophotometric method (MCR)

Accurate aliquots equivalent to 40–220, 40–280 and 50–200 μg from each of TPZ, ISO and DEG, respectively, were transferred separately from their respective working standard solutions (100 μg mL⁻¹) into three separate series of 10-mL volumetric flasks, the volume was completed to the mark with 0.1 N HCl solution to obtain the final concentration ranges of each one. The absorption spectra of the prepared solution were measured in the range of 200–400 nm.

For determination of TPZ, the recorded spectra of TPZ were divided by the spectrum of DEG (20 μg mL⁻¹) to obtain the first ratio spectra which were then mean centered. These vectors were then divided by the mean centered ratio of 2ISO/2DEG and the mean centering of the second ratio spectra was then obtained. By the same way, the recorded spectra of ISO were divided by the spectrum of TPZ (20 μg mL⁻¹) and the obtained ratio spectra were mean centered, these vectors (mean centered ratio spectra) were divided by the mean centered (MC) ratio of (2DEG/2TPZ) to obtain the second ratio spectra which were then mean centered. For DEG, the scanned spectra of its prepared solutions were divided by the standard spectrum of 8 μg mL⁻¹ of TPZ and the obtained ratio spectra were mean centered. These vectors were divided by the mean centered ratio (MCR) of (2ISO/2TPZ) and the second ratio spectra were then mean centered.

The mean centered values of the second ratio spectra at 322, 355 and 339 nm for TPZ, ISO and DEG, respectively, were measured and plotted against the corresponding concentration of each component to construct their respective calibration equations.

3.4. Analysis of laboratory prepared mixtures

Mixtures containing different ratios of ISO and TPZ were prepared and then different aliquots of DEG in the range of 10–90% of TPZ were added. The volume was completed with 0.1 N HCl solution. The concentration of each of ISO, TPZ and DEG in each mixture was measured using the proposed methods.

3.5. Application to pharmaceutical formulation (Stellamide® tablets)

Thirty tablets of Stellamide® were weighed, 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 a 100-mL volumetric flask. 75 mL of methanol was added and sonicated for 20 min; the volume was completed with methanol and filtered. The solution was diluted with 0.1 N HCl to obtain the appropriate working solution for each method. Then the procedure of each method mentioned above was followed.

4. Results and discussion

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

Isopropamide iodide is stable and no degradation products have been observed to occur either under normal or exaggerated storage conditions (Santoro et al., 1973).

Trifluoperazine hydrochloride, 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 (Post et al., 1980) and representing 6% of TPZ metabolites, so the determination of the binary mixture in bulk powder or in their pharmaceutical formulation without the interference of DEG which is pharmacologically less active than the intact drug was an analytical task of potential.

The focus of the present work is to develop accurate, specific, reproducible and sensitive stability indicating methods for the determination of ISO and TPZ in pure form or in their pharmaceutical formulation and also the determination of TPZ oxidative degradation product.

A stability-indicating procedure may be defined as a procedure that affords the selective determination of a drug substance in the presence of its decomposition and reaction products (ICH, 2005). Different stability indicating methods

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were established for determination of certain drugs in the presence of their degradation products (Lopes and Salgado, 2009; Sharma et al., 2012).

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 irrelevant absorption. Among the techniques used to reduce or eliminate such interference or irrelevant absorption are successive derivative ratio and mean centering spectrophotometry. The suggested methods allowed the quantitative determination of ISO, TPZ and DEG without prior separation.

The zero order absorption spectra of TPZ, ISO and DEG showed severe overlapping (Fig. 1) that did not allow direct spectrophotometric determination of each of them separately. So, the aim of this work was to develop simple, sensitive and accurate analytical methods for the determination of TPZ, ISO and DEG. The first method was the successive derivative ratio spectra method which depended on the successive derivative of ratio spectra in two steps using 0.1 N HCl solution as a solvent, the second method was the newly developed mean centering of ratio spectra which was applied for determination of the studied ternary mixture without prior separation step. This method has the advantage of eliminating derivative steps and therefore signal to noise ratio is enhanced. The previously mentioned spectrophotometric methods are characterized by the ability of determination of the studied drugs and also the determination of DEG with good sensitivity.

4.2. Method development and optimization

The main step in the development of an analytical method is to improve the conditions and parameters which should be followed in development and validation. Different solvents were studied (methanol, ethanol, acetonitrile, water, 0.1 N HCl and 0.1 N NaOH), according to the sensitivity and selectivity it was found that 0.1 N HCl was the best solvent for the suggested spectrophotometric methods. The effect of divisor concentration on the method selectivity and analytical parameters such as slope, intercept and correlation coefficient of the calibration equations was tested. Different concentrations of TPZ, ISO and DEG were tested (5, 10, 15 and 20 µg mL⁻¹ for each). It was observed that changing the concentration of the divisors had a significant effect on the method selectivity, therefore 10 µg mL⁻¹ of each of TPZ, ISO and DEG was used as divisors. The value of Δλ had no considerable effect on the suggested successive derivative of ratio spectra method, when Δλ = 4 was used.

Figure 1  Zero order absorption spectra of 10 µg mL⁻¹ of ISO (—), TPZ (- - -) and DEG (…………) using 0.1 N HCl as a solvent.

4.3. Successive derivative ratio spectra method

The absorption spectra of different concentrations of TPZ were recorded in the range of 200–400 nm and divided by the spectrum of the standard solution of 10 µg mL⁻¹ of ISO and the ratio spectra were obtained. First derivative of the ratio spectra was obtained with Δλ = 4 nm and scaling factor = 1. These vectors (first derivative of the ratio spectra) were then divided by (d/dΔλ)(DEG/ISO) corresponding to the derivative of the ratio of the spectra of 10 µg mL⁻¹ of each and then the first ratio spectra were obtained. The first derivative of these vectors was obtained from which TPZ was determined by measuring the peak amplitude at 250.4 and 257.2 nm, Fig. 2.

In the same way, the absorption spectra of the solutions prepared at different concentrations of ISO were recorded in the range of 200–400 nm and divided by the spectrum of the standard solution of 10 µg mL⁻¹ of TPZ and ratio spectra were obtained. First derivative of the ratio spectra was plotted with Δλ = 4 nm and scaling factor = 1. After that these vectors (first derivative of ratio spectra) are divided by (d/dΔλ)(DEG/TPZ) corresponding to the derivative of the ratio of the spectra of 10 µg mL⁻¹ of each of DEG and TPZ and then first ratio spectra were obtained. The concentration of ISO was determined by measuring the peak amplitude at 223 and 228 nm in the first derivative of ratio spectra as shown in Fig. 3.
By the same way, the concentrations of DEG were determined by measuring the peak amplitude of the first derivative of the first ratio spectra at 210.6, 213 and 270.2 nm (Fig. 4) using the spectra of 10 μg mL\(^{-1}\) of each of TPZ and ISO to obtain the first derivative of ratio spectra.

Linear correlations were obtained between peak amplitudes at 250.4 and 257.2 nm for TPZ in the concentration range of 4–22 μg mL\(^{-1}\), peak amplitudes at 223 and 228 nm for ISO in the concentration range of 4–30 μg mL\(^{-1}\) and peak amplitudes at 210.6, 213 and 270.2 nm for DEG in the concentration range of 5–25 μg mL\(^{-1}\), as given in Table 1, from which the regression equations were calculated and found to be:

\[
P_{TPZ} = 3.2848C_1 + 0.03128, \quad r = 0.9998; \quad \text{at 250.4 nm for TPZ}
\]

\[
P_{ISO} = 0.1812C_2 - 0.0110, \quad r = 0.9999; \quad \text{at 223 nm for ISO}
\]

\[
P_{DEG} = 0.2927C_3 + 0.2233, \quad r = 0.9994; \quad \text{at 210.6 nm for DEG}
\]

\[
P_{DEG} = 0.3169C_3 + 0.2445, \quad r = 0.9994; \quad \text{at 213 nm for DEG}
\]

\[
P_{DEG} = 0.1939C_3 + 0.1016, \quad r = 0.9997; \quad \text{at 270.2 nm for DEG}
\]

4.4. Mean centering of ratio spectra spectrophotometric method (MCR)

The proposed MCR method is based on the mean centering of ratio spectra; the mathematical explanation of the developed method was illustrated by Afkhami and Bahram (Afkhami and Bahram, 2006). This method was applied for resolving binary and ternary mixtures in the complex samples with unknown matrices (Afkhami and Bahram, 2005).
In order to optimize the developed MCR method, the effect of divisor concentration on the selectivity of the method has been tested. Different concentrations each of TPZ, ISO and DEG (normalized spectrum, 4, 6, 8, 14 and \(20 \frac{\text{lg}}{\text{M}}\)) were tested. It was found that the divisor had a great effect on the selectivity of the method where reproducible and good results have been obtained upon using a concentration of \(20 \frac{\text{lg}}{\text{M}}\) each of ISO and DEG (for TPZ) and \(20 \frac{\text{lg}}{\text{M}}\) each of TPZ and DEG (for ISO) as divisors. For the determination of DEG \(8 \frac{\text{lg}}{\text{M}}\) of TPZ and \(20 \frac{\text{lg}}{\text{M}}\) of ISO were found to be the best divisors.

Linearity of the proposed methods was evaluated and it was evident in the range of \(4–22 \frac{\mu}{\text{g}} \frac{\text{M}}{\text{L}}\), \(4–28 \frac{\mu}{\text{g}} \frac{\text{M}}{\text{L}}\) and \(5–20 \frac{\mu}{\text{g}} \frac{\text{M}}{\text{L}}\) for TPZ, ISO and DEG, respectively (Figs. 5–7). The regression equations for the proposed methods were calculated and found to be:

\[
Y_1 = 2.2932C + 2.2722, \ r = 0.9995; \text{at } 250.4 \text{ nm for TPZ.}
\]

\[
Y_2 = 0.5579C + 0.1151, \ r = 0.9998; \text{at } 257.2 \text{ nm for ISO.}
\]

\[
Y_3 = 2.4500C^{0.7070}, \ r = 0.9997; \text{339 nm for DEG.}
\]

where \(Y_1, Y_2\) and \(Y_3\) are the peak amplitudes at the selected wavelengths, \(C_1, C_2\) and \(C_3\) are the concentrations in \(\mu \text{g} \frac{\text{M}}{\text{L}}\) and \(r_1, r_2\) and \(r_3\) are the correlation coefficients. Good linearity is evident from the high value of the correlation coefficient and the low value of intercept (Table 1).

### 4.5. Method validation

Method validation was performed according to the USP guidelines (USP, 2007) for the suggested spectrophotometric methods.

#### 4.5.1. Linearity

The linearity of the proposed methods was evaluated by analyzing different concentrations of TPZ in the range of \(4–22 \frac{\mu}{\text{g}} \frac{\text{M}}{\text{L}}\) (for both methods), of ISO in the range of \(4–30 \frac{\mu}{\text{g}} \frac{\text{M}}{\text{L}}\) (for successive ratio method) and \(4–28 \frac{\mu}{\text{g}} \frac{\text{M}}{\text{L}}\) (for MCR) and of DEG in the range of \(5–25 \frac{\mu}{\text{g}} \frac{\text{M}}{\text{L}}\) (for MCR).
successive ration method) and 5–20 \( \mu g \) mL\(^{-1}\) (for MCR). Each concentration was repeated three times. The assay was performed according to the experimental conditions previously mentioned (Table 1).

4.5.2. Accuracy

The accuracy of the results was checked by applying the proposed method for the determination of different blind samples of TPZ, ISO and DEG. The concentrations were obtained from the corresponding regression equations then the percentage recoveries were calculated.

Accuracy of the method was further assured by the use of the standard addition technique, it was performed by the addition of known amounts of pure TPZ and ISO to known concentrations of the pharmaceutical formulation; the resulting mixtures were assayed, and the results obtained were compared with the expected values, as given in Table 1. The good recoveries of the standard addition technique suggested a good accuracy of the proposed methods.

4.5.3. Precision

For evaluation of precision, repeatability of the results of three concentrations of TPZ (6, 10 and 14 \( \mu g \) mL\(^{-1}\)) and of ISO (8, 12 and 20 \( \mu g \) mL\(^{-1}\)) and of DEG (10, 14 and 18 \( \mu g \) mL\(^{-1}\)) were performed by three replicate determinations to estimate the intraday variation and seven replicate determinations on different four days to estimate the interday variation. Then the coefficient of variation at these concentration levels was calculated in Table 1.

4.5.4. Detection and quantitation limits

These approaches based on the SD of the response and the slope were used for determining the detection and quantitation limits (Table 1, where LOD = 3.3 × SD/slope and LOQ = 10 × SD/slope).

4.5.5. Selectivity

Selectivity of the methods was achieved by the analysis of different laboratory prepared mixtures of TPZ, ISO and DEG for the two spectrophotometric methods within the linearity range. Satisfactory results are shown in Table 2.

4.5.6. Stability

The working solutions of the studied drugs showed no spectrophotometric changes for 1 week when stored at room temperature (25 °C).

4.6. Statistical analysis

Table 3 shows statistical comparison of the results obtained by the proposed methods and the reported spectrophotometric method (Abbas et al., 2010). 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.

7. Conclusion

The present work introduces two different, simple, sensitive and rapid spectrophotometric methods for the determination of isopropamide iodide, trifluoperazine hydrochloride and trifluoperazine oxidative degradate.

Compared to the derivative method, MCR eliminates the derivative steps and therefore the signal-to-noise ratio is enhanced. It was also applied for resolving binary and ternary mixtures in the complex samples with unknown matrices.

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

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