Development and Validation of Spectrophotometric Method for Simultaneous Determination of Isopropamide and Trifluoperazine in Tablet Dosage Form

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Abstract

Aim: The aim of this study is to develop simple, sensitive, rapid, accurate, precise and economical spectrophotometric method based on simultaneous equation method for the simultaneous estimation of isopropamide and trifluoperazine in combined tablet dosage form. **Materials and Methods:** The method is based on the simultaneous equation and first-order derivative method for analysis of both the drugs using methanol:water in the ratio of 7:3 (v/v) as solvent. Isopropamide has absorbance maxima at 248.5 nm and trifluoperazine has absorbance maxima at 227.0 nm in methanol. **Results and Discussion:** The linearity was obtained in the concentration range of 5-30 µg/mL for isopropamide and 2-12 µg/mL for trifluoperazine in both the methods. The concentration of drugs was determined by using simultaneous equation method. The result of analysis has been validated statistically and by recovery studies. The results of mean recovery and other validation parameters were found within the acceptable limits. Both methods were applied to estimate the marketed formulation and found good recovery of the drug. The methods were found to be simple, sensitive, accurate and precise and were applicable for simultaneous determination of isopropamide and trifluoperazine in pharmaceutical dosage form.

Key words: Isopropamide, method development and validation, trifluoperazine, ultraviolet spectrophotometer.

INTRODUCTION

Trifluoperazine [Figure 1a] is phenothiazine chemical class drug used for short-term treatment of certain types of anxiety.[1-3] It is a typical antipsychotic drug used for treating schizophrenia and it believed to work by blockading dopamine D1 and D2 receptors in the mesocortical and mesolimbic pathways, relieving or minimizing such symptoms of schizophrenia as hallucinations, delusions and disorganized thought and speech.[4-9] Isopropamide [Figure 1b] is a long-acting quaternary anticholinergic drug used for the treatment of peptic ulcer disease, in the relief of gastrointestinal (GI) and urinary tract disorders associated with smooth muscle spasm, in rhinitis, gastritis, hyperchlorhydria, functional diarrhea, irritable or spastic colon, pyloroduodenal irritability, pylorospasm, acute nonspecific gastroenteritis, biliary dyskinesia and chronic cholelithiasis, duodenitis and GI spasm.[10] The drug works by inhibiting parasympathetic nerve impulses by selectively blocking the binding of the neurotransmitter acetylcholine to its receptor in nerve cells. The nerve fibers of the parasympathetic system are responsible for the involuntary movements of smooth muscles present in the GI tract. Inhibition here decreases acidity and motility, aiding in the treatment of GI disorders.[11] The drug may also be used to treat genitourinary spasm. Literature review reveals that there is only one spectrophotometric method for simultaneous estimation of trifluoperazine and isopropamide in the presence of trifluoperazine oxidative

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degradate.[12] There are only few spectrophotometric methods for estimation trifluoperazine individually.[13-16] A few other analytical methods have been reported with trifluoperazine and isopropamide simultaneously, individually and with other drugs.[17-22] So far no simultaneous spectrophotometric method has been reported with this combination and hence forms the basis of the present work.

MATERIALS AND METHODS

Apparatus and instrumentation

A double-beam ultraviolet (UV)/visible spectrophotometer model Teccomp UV-2301 was used to carry out spectral analysis and the data were recorded by Hitachi software. Standard and sample drugs were weighed using Denver electronic analytical balance (SI-234).

Chemicals and materials

Pure standard drugs trifluoperazine and isopropamide were obtained as gift sample from Reputed Pharmaceutical Company. Methanol was of analytical grade (Merck Specialties Private Limited, Mumbai, India). Formulations of (Stelbid; trifluoperazine - 2 mg and isopropamide - 5 mg) tablet pharmaceutical dosage form of trifluoperazine and isopropamide were procured from local market.

Preparation of standard solution

Individual standard stock solutions of trifluoperazine and isopropamide were prepared by weighing 10 mg of drugs accurately and dissolved individually in 5 mL diluent methanol:water in the ratio of 7:3 (v/v) and transferred to a 10 mL volumetric flask after sonicating for 5 min, finally volume was made up to the mark with same solvent to prepare 1000 µg/mL stock solution. Working standard solutions were prepared by proper dilution of stock solution to 10 mL to get a concentration of 100 µg/mL solution of trifluoperazine and isopropamide individually. Further concentration solutions were prepared by proper dilution with diluent.

Preparation of formulation solution

Ten formulation tablets from 2 different strips of trifluoperazine and isopropamide (Stelbid: Trifluoperazine - 2 mg and isopropamide - 5 mg) were powdered and 10 mg tablet powder was weighed accurately and was dissolved in 5 mL diluent. Solution was sonicated for 10-15 min to dissolve the drugs completely. Then, it was filtered and made up to 10 mL with same diluents to make 1000 µg/mL stock solution. As per the label claim of the two drugs, a trifluoperazine concentration of 20 µg/mL and isopropamide concentration of 8 µg/mL were obtained by subsequent dilution. The resultant solution was used for the simultaneous estimation of trifluoperazine and isopropamide in combined dosage forms.

Simultaneous equation method (method-1)

λ maximum of individual drugs were at 248.5 nm for isopropamide and 227 nm for trifluoperazine. Different aliquots of the standard solution of trifluoperazine and isopropamide were transferred into volumetric flask. The solutions were then made up to the volume with diluents, so the final concentration for trifluoperazine was in the range of 2-12 µg/mL and for isopropamide was in the range of 5-30 µg/mL. At the absorbance of these standard solutions, calibration curves were plotted at these wavelengths. Two simultaneous equations were formed using these absorptivity coefficient values.

$$C_x = A_2 a_{y1} - A_1 a_{y2} / a_{x2} a_{y1} - a_{x1} a_{y2}$$

$$C_y = A_1 a_{x2} - A_2 a_{x1} / a_{x2} a_{y1} - a_{x1} a_{y2}$$

Where,
a_{x1} = Absorptivity of trifluoperazine at 227 nm
a_{x2} = Absorptivity of trifluoperazine at 248.5 nm
a_{y1} = Absorptivity of isopropamide at 248.5 nm
a_{y2} = Absorptivity of isopropamide at 227 nm.

A_1 and A_2 are the absorbance of the diluted sample at 227 nm and 248.5 nm, respectively. Aliquots of these tablet solutions were diluted to get the concentrations of 20 µg/mL trifluoperazine and 8 µg/mL isopropamide absorbance of these solutions were measured at 227 nm and 248.5 nm, respectively and from the absorbance values, the concentration of drugs in the sample solution was determined using the simultaneous equations.

First-order derivative spectrophotometry (method-2)

In this method, trifluoperazine and isopropamide standard stock solutions were prepared and scanned in the spectrum mode from 400 nm to 200 nm. The absorption spectra obtained were derivatized from first to fourth order. First-order derivative spectra were selected for analysis of drug. From spectra of drug, the absorbance was measured at 227 nm for trifluoperazine and 248.5 nm for isopropamide and zero cross = 227.0 nm, amplitude difference (dA) with respect to wavelength difference (dλ) was measured for the respective concentration of standard and was plotted against concentrations and regression equation was calculated. All the validation tests were conducted in above prepared range.

Validation of spectrophotometric methods

The method was validated by various parameters as recommended by ICH Guidelines.[23-25]

Accuracy

To study the accuracy, percentage recoveries are to be calculated, recovery studies were carried out by standard addition method by adding the known amount of trifluoperazine and isopropamide to the pre-analyzed sample at three different concentration levels, i.e., 50%, 100% and 150% of assay concentration and percentage recoveries were calculated.

Precision

The precision of an analytical method was studied by performing repeated analysis and intermediate precision.

Repeatability

Six different standard mixture solutions of the same concentration of trifluoperazine and isopropamide (8:20 µg/mL) were taken and absorbance values are measured. The standard deviation and percentage relative standard deviation were also calculated.

Intraday precision

Variation of results within the same day was analyzed. Intraday precision was determined by measuring the standard mixture solution of trifluoperazine and isopropamide (8:20 µg/mL) at three different time intervals on the same day.

Interday precision

Variation of results between the days was analyzed. Interday precision was determined by measuring the standard mixture solution of trifluoperazine and isopropamide (8:20 µg/mL) on three consecutive days.

Linearity and range

The linearity of analytical method for trifluoperazine and isopropamide was determined by studying standard calibration curves. The range of analytical method was decided from the interval between upper and lower level of calibration curves by plotting the log curve.

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

Detection limit and quantitation limit were determined based on the standard deviation of y-intercepts of six calibration curves and average slope of six calibration curves were calculated.

RESULT AND DISCUSSION

Simultaneous equation method (method-1)

In this method, methanol:water in the ratio of 7:3 (v/v) was used as a solvent and drug's showed absorbance at 248.5 nm for isopropamide and 227 nm for trifluoperazine,

respectively and are describe in Figure 2. The linearity of analytical method at five concentration levels ranging from 2 - 12 µg/mL for trifluoperazine and 5-30 µg/mL for isopropamide, respectively, was determined and are presented in Table 1. The regression equation of calibration curves were y = 0.023× + 0.123 and y = 0.040× + 0.018 for isopropamide and trifluoperazine, respectively and are shown in Figure 3. The results show that an excellent correlation exists between response factor and concentration of drugs within the concentration range. The correlation coefficient (r2) was found to be 0.999 and 0.999 for both the drugs. Thus, the above data represents that simultaneous equation method obeyed Beer-Lambert's Law. The LOD was found to be 0.05 µg/mL for trifluoperazine and 0.10 µg/mL for isopropamide. LOQ was found to be 0.20 µg/mL for trifluoperazine and 0.4 µg/mL for isopropamide. The developed method was found to be accurate from percentage recovery studies and the results are shown in Table 2. The mean percentage assay shown in Table 3 was found to be 98.36% and 99.24% for trifluoperazine and isopropamide, respectively. They are obtained by comparing the results with the stated label claim. Validation results of the proposed method are presented in Table 2. The results obtained had satisfactorily fulfilled the criteria.

Table 1: Linearity results of trifluoperazine and isopropamide of method 1

Trifluoperazine		Isopropamide	
Concentration in µg/mL	Absorbance	Concentration in µg/mL	Absorbance
2	0.098	5	0.239
4	0.186	10	0.361
6	0.264	15	0.469
8	0.339	20	0.582
10	0.421	25	0.693
12	0.513	30	0.826

Table 2: Summary of validation results for method 1

Parameter	Results	
	Isopropamide	Trifluoperazine
λ max	248.5 nm	227.0 nm
Linearity	5-30 µg/mL	2-12 µg/mL
Intraday precision RSD	0.337	0.916
Interday precision RSD	0.433	0.787
Ruggedness	0.981	0.765
Recovery in %	98.45-99.80	98.17-99.80
LOD	0.10 µg/mL	0.05 µg/mL
LOQ	0.40 µg/mL	0.20 µg/mL
Formulation assay in %	99.245	98.363

RSD: Relative standard deviation, LOD: Limit of detection, LOQ: Limit of quantitation

First-order derivative spectrophotometry (method-2)

The zero-order absorption spectra of isopropamide and trifluoperazine are represented in Figure 2. The close overlap of the absorption spectra of isopropamide and trifluoperazine prevents the correct use of zero-order absorption measurements for their simultaneous determination in binary mixtures. On the other hand, the first-order spectrum did not suffer any interference at the determination wavelength of isopropamide (248.5 nm) and trifluoperazine (227.0 nm) as expected. As per the ICH guidelines, the method validation parameters checked were linearity, accuracy, precision, LOD and LOQ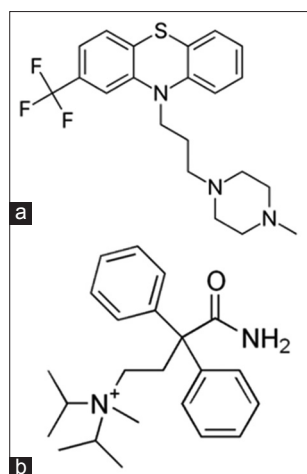. The calibration curve [Figure 4] was obtained with six concentrations of the standard solution. A critical evaluation of proposed method was performed by statistical analysis of data where slope, intercept and correlation coefficient are shown in Table 3. Linearity for isopropamide and trifluoperazine was observed in the concentration range of 5-30 µg/mL for isopropamide and 2-12 µg/mL for trifluoperazine. The linearity graphs were shown in Figure 5a and b. The correlation coefficient for isopropamide and trifluoperazine were found to be 0.998 and 0.999 respectively. The results of intraday, interday and ruggedness precisions of the proposed method were conducted with six repetitive analyses and the percentage relative standard deviation has been found below 2% for both drugs. The recovery was performed at three levels, 50, 100 and 150% for isopropamide and trifluoperazine standard concentration. Results are found to be 99.04-99.75% for isopropamide and 98.50-99.60% for trifluoperazine. The LOD was 0.10 µg/mL and 0.05 µg/mL and the values LOQ

Figure 1: (a and b) Chemical structure of trifluoperazine and isopropamide

Table 3: Linearity results of trifluoperazine and isopropamide of method 2

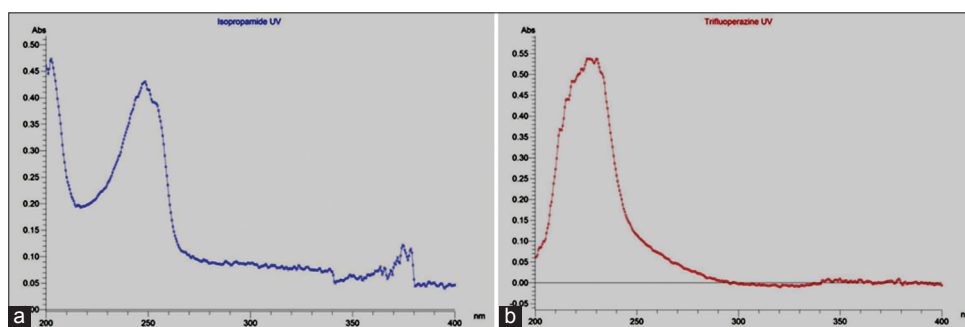| Trifluoperazine		Isopropamide	
Concentration in µg/mL	Absorbance	Concentration in µg/mL	Absorbance
2	0.00125	5	0.00569
4	0.00351	10	0.01358
6	0.00603	15	0.01986
8	0.00813	20	0.02803
10	0.01028	25	0.03461
12	0.01268	30	0.04128

Figure 2: (a and b) Scanning spectra of isopropamide and trifluoperazine
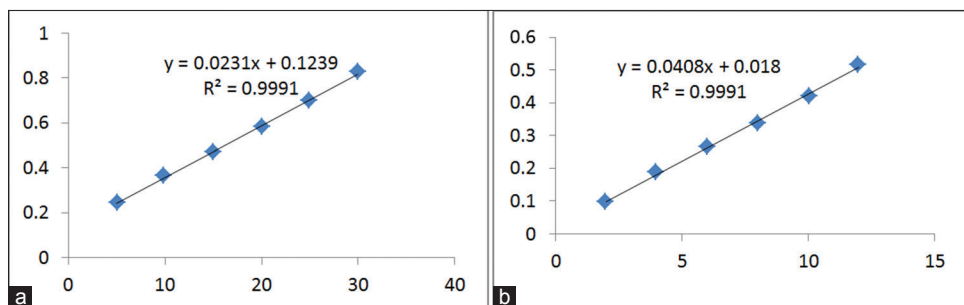

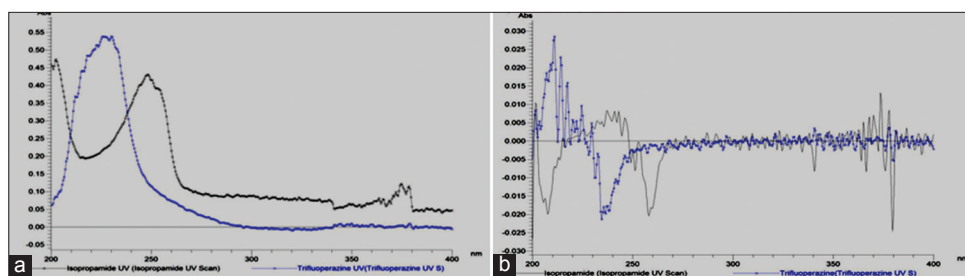Figure 3: (a and b) Linearity graph of isopropamide and trifluoperazine of method 1

Figure 4: (a and b) Overlay of scanning spectra of isopropamide and trifluoperazine
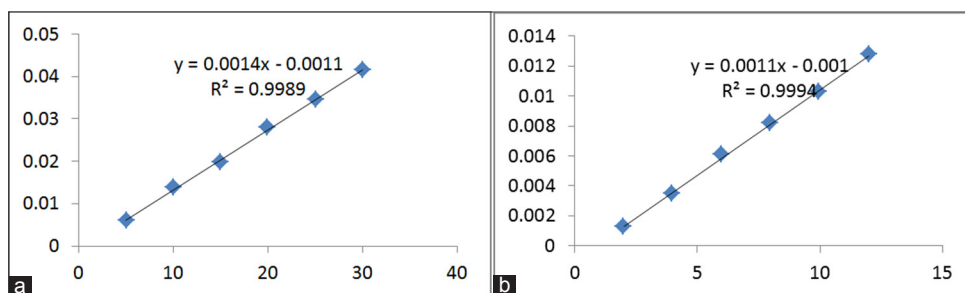

Figure 5: (a and b) Linearity graph of isopropamide and trifluoperazine for method 2

Table 4: Summary of validation results for method 2

Parameter	Results	
	Isopropamide	Trifluoperazine
λ max	248.5 nm	227.0 nm
Linearity	5-30 µg/mL	2-12 µg/mL
Intraday precision RSD	1.290	1.341
Interday precision RSD	0.636	1.681
Ruggedness	0.282	1.463
Recovery in %	99.04-99.75	98.50-99.60
LOD	0.10 µg/mL	0.05 µg/mL
LOQ	0.40 µg/mL	0.20 µg/mL
Formulation assay in %	99.625	98.250

RSD: Relative standard deviation, LOD: Limit of detection, LOQ: Limit of quantitation

were 0.4 µg/mL and 0.20 µg/mL for isopropamide and trifluoperazine, respectively. Validation results with the proposed method are presented in Table 4. These low values indicate the good sensitivity of the proposed methods. The applicability of the proposed methods for the determination of isopropamide and trifluoperazine in commercial dosage forms was examined by analyzing marketed products. It is evident that there is good agreement between the amount estimated and those claimed by the manufacturers. Percent label claims are very close to 100, with low value of standard deviation.

CONCLUSION

The developed methods were suitable for simultaneous estimation of isopropamide and trifluoperazine in tablet formulation. The developed method is economic, specific, and sensitive. Recovery studies showed that there is no interference of excipients. Hence, this method can be used in quality control and routine analysis of the finished product.

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