Navya sri D. et al. / Vol 4 / Issue 8 / 2014 / 449-455.

International Journal of Pharmaceutical Research & Analysis

e-ISSN: 2249 – 7781
Print ISSN: 2249 – 779X

www.ijpra.com

METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF TRIFLUOPERAZINE AND ISOPROPAMIDE IN TABLET DOSAGE FORM BY RP-HPLC

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ABSTRACT

A simple, precise, rapid, specific and accurate reverse phase high performance liquid chromatography method was developed for simultaneous estimation of Trifluoperzine and Isopropamide in pharmaceutical dosage form. Chromatographic separation was performed on Agilent zorbax SB-C18, 4.6 x 250mm, 5micros column, with mobile phase comprising of mixture of buffer (pH 6.0, adjusted with Ortho phosphoric acid, Acetonitrile in the ratio of 80:20v/v), at the flow rate 0.8ml/min. The detection was carried out at 227nm. The retention times of Trifluoperzine and Isopropamide were found to be 2.4 and 3.6mins respectively with a run time of 10mins, theoretical levels for Trifluoperzine and Isopropamide were 5194 and 6738 respectively, with a resolution of 7.6. As per ICH guidelines the method was validated for linearity, accuracy, precision, limit of detection and limit of quantitation, robustness and ruggedness. Linearity of Trifluoperzine and Isopropamide was found in the range of 30-130µg/ml and that for Isopropamide was found to be 150-250µg/ml. The correlation coefficient for Trifluoperzine and Isopropamide were 0.999 and 1 respectively. The LOD values for Trifluoperzine and Isopropamide were 2.963 and 2.9851 respectively. The LOQ values for Trifluoperzine and Isopropamide were and 9.877µg/ml and 9.9502µg/ml respectively. This demonstrates that the developed method is simple, precise, rapid, selective, accurate and reproducible for simultaneous estimation of Trifluoperzine and Isopropamide tablet dosage form.

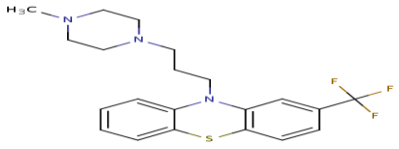
Keywords: HPLC method, Stelbid, Validation, analytical method optimization.

INTRODUCTION

Trifluoperazine is an anti emitic agent. Blocks postsynaptic mesolimbic dopaminergic D1 and D2 receptors in the brain, depresses the release of hypothalamic and hypophyseal hormones and is believed to depress the reticular activating system thus affecting basal metabolism, body temperature, wakefulness, vasomotor tone, and emesis. Prevent nausea or vomiting may also be effective against nausea, emesis, and pruritus.

Isopropamide iodide is a long-acting quaternary anticholinergic drug. It is used in the treatment of peptic ulcer and other gastrointestinal disorders marked by hyperacidity and hypermotility. Anticholinergics are a class of medications that inhibit 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 gastrointestinal tract. Inhibition here decreases acidity and motility, aiding in the treatment of gastrointestinal disorders [1-5].

Chemical name andChemical structure of Trifluoperazine



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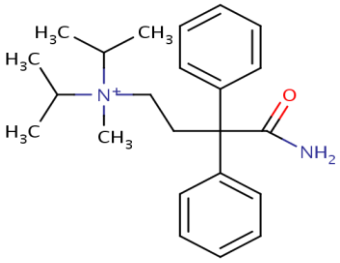
Structure 1: Trifluoperazine
10-[3-(4-Methyl-1-piperazinyl)propyl]-2-(trifluoromethyl)-10H-phenothiazine

Chemical name and Chemical structure of Isopropamide



Structure 1: Isopropamide
(3-carbamoyl-3,3-diphenylpropyl)(methyl)bis(propan-2-yl)azanium

The present study was focussed on developing a simple, economical, sensitive and specific RP-LC method for simultaneous determination of Trifluoperazine and Isopropamide in tablet dosage form [6-10].

MATERIALS AND METHODS

Reference Standards

1. Trifluoperazine % purity – 99.01%.
2. Isopropamide % purity –99.71%.

Tablet Brand Used: Stelbid

Preparation of Buffer

Take 1000mL of HPLC grade water. The pH was adjusted to 6.0 with orthophosphoric acid.

Preparation of Mobile Phase

A mixture of above prepared buffer 800 mL (80%), 200 mL of HPLC grade Acetonitrile (20%) were mixed and degassed in ultrasonic water bath for 5 minutes. The mobile phase was filtered through 0.45 µ filter under vacuum.

Diluent Preparation

A mixture of HPLC grade Acetonitrile and water mixed in 20:80 ratio was prepared degassed in ultrasonic water bath for 5 minutes and filtered through 0.45 µ filter under vacuum.

Preparation of the Trifluoperazine and Acetonitrile Standard & Sample Solution

Accurately weighed and transferred 20 mg of Trifluoperazine and 50 mg of Isopropamide working standard into a 50mL clean dry volumetric flask and added about 35mL of diluent. It was sonicated to dissolve completely and made volume up to the mark with the same diluent. (Stock solution) (400, 1000 µg/mL). From this, 5 ml of the solution was pipetted into another 25ml volumetric flask and diluted up to the mark with diluent (80,200 µg/mL).

Preparation of Sample Solution

Accurately weighed and transferred tablet powder equivalent to 20mg of Trifluoperazine and 50mg of Isopropamide into a 50mL clean dry volumetric flask and added about 35mL of diluent. It was sonicated to dissolve completely and made volume up to the mark with the same diluent. (Stock solution)(400, 1000µg/ml) From this, 5 mL of the solution was pipetted into another 25ml volumetric flask and diluted up to the mark with diluent(80, 200µg/ml).

RESULTS AND DISCUSSION

Method Development and Optimization

Proper selection of the method depends upon the nature of the sample (ionic/ionisable/neutral molecule), its molecular weight and solubility. The drug selected in the present study is polar in nature. The reversed phase HPLC was selected for the separation because of its simplicity and suitability. The sensitivity of HPLC method which uses PDA detector for the proper selection of wavelength. An ideal wavelength is one that gives good response for all the drugs to be detected. The λmax was obtained at 227 nm. Different mobile phases were tried but satisfactory separation and symmetrical peaks were obtained by using a mobile phase consisting of Orthophosphoric acid (P^{H-}6) : Acetonitrile in the ratio 80 : 20.column selected is AGILENT ZORBAX ,C18, 250X4.6, 5µm and flow rate at 0.8ml/min, Injection volume is 20µl, Temperature id at 25°C.

Method validation

The method was validated for Accuracy, linearity, precision, specificity, limit of detection, limit of quantification and robustness

System Suitability Studies

(a)The column efficiency, resolution and peak asymmetry were calculated for the standard solutions The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 3 % standard.

(b) Specificity

Specificity was checked for the interference of impurities in the analysis of blank solution and injecting sample solution under optimized chromatographic conditions to demonstrate separation from impurities.

(c) Accuracy

The closeness of agreement between the true value which is accepted either conventional new value or an accepted reference value and the value found. The above prepared

solutions of Accuracy -50%, Accuracy -100% and Accuracy -150% solutions were injected. The Amount found and Amount added for Trifluoperazine and Isopropamide individual recovery and mean recovery values were calculated.

Method accuracy from recovery assays

Linearity

The linearity of this method was evaluated by Linear Regression Analysis, which was calculated by Least Square method and The calibration curve for Trifluoperazine and Isopropamide was linear over the concentration range of 40-120 and 150-250µg/ml respectively. The correlation coefficient was found to be 0.999 and 1 respectively.

Precision

The precision of an analytical method is a measure of the random error and is defined as the agreement between replicate measurements of the same sample. It is expressed as the percentage coefficient of variation (%CV) or relative standard deviation (RSD) of the replicate measurements.

$$\% \text{ RSD} = \frac{\text{Standard deviation}}{\text{Mean}} \times 100$$

Sensitivity

The sensitivity of measurement of Trifluoperazine and Isopropamide by use of the proposed method was estimated in terms of the Limit of Detection (LOD) and the Limit of Quantitation (LOQ).

Limit of detection (LOD) and Limit of quantification (LOQ) were estimated from the signal-to-noise ratio. The detection limit was defined as the lowest concentration level resulting in a peak height of three times the baseline noise. The quantification limit was defined as the lowest concentration level that provided a peak height with a signal -to noise ratio higher than 10. The LOD and LOQ values for Trifluoperazine and Isopropamide were reported in the Table 8.

Robustness

The Robustness of the method was determined under different conditions including change in flow rate, temperature. The chromatograms were recorded and the results of the chromatograms are given below.

DISCUSSION

RP-HPLC method was developed for simultaneous estimation of Trifluoperazine and Isoprpamide in tablet dosage form. Chromatographic separation was performed on agillent zorbax sb-c18, with mobile phase comprising of mixture of buffer (pH6.0, adjusted with Orthophosphoric acid), Acetonitrile in the ratio of 80:200v/v, at the flow rate 0.8ml/min. The detection was carried out at 227nm.

Table 1. Observation of System Suitability Parameters

S. No	Parameter	Trifluoperazine	Isopropamidee
1	Retention time	2.400	3.623
2	Theoretical plates	5194	6738
3	Tailing factor	1.57	1.40
4	Area	4347057	6342343
5	Resolution	7.46	

Table 2. Observation of standard chromatogram

ID	Name	Retention	Area	USP Plate	USP	USP
1	Trifluoperazine	2.400	434757	5194	1.57	
2	Isopropamide	3.623	6342343.3	6738	1.40	7.60

Table 3. Observation of sample chromatogram

ID	Name	Retention Time (min)	Area (µV*sec)
1	Trifluoperazine	2.403	4031892
2	Isopropamide	3.617	6533221

Table 4. Accuracy Observation of Trifluoperazine

Trifluoperazine						
Spiked Level	**Sample Weight**	**Sample Area**	**µg/ml added**	**µg/ml found**	**% Recovery**	**% Mean**
50%	1204.40	2017955	39.642	39.77	100.33	100.36
50%	1204.40	2020800	39.642	39.83	100.48	
50%	1204.40	2017170	39.642	39.76	100.30	
50%	1204.40	2022790	39.642	39.87	100.58	

50%	1204.40	2015988	39.642	39.74	100.24	
50%	1204.40	2015806	39.642	39.73	100.23	
100%	2408.70	4020482	79.280	79.24	99.96	100.06
100%	2408.70	4028999	79.280	79.41	100.17	
100%	2408.70	4024012	79.280	79.31	100.04	
150%	3613.10	6036487	118.922	118.98	100.05	100.03
150%	3613.10	6039886	118.922	119.05	100.11	
150%	3613.10	6031323	118.922	118.88	99.96	
150%	3613.10	6039847	118.922	119.05	100.11	
150%	3613.10	6030619	118.922	118.86	99.95	
150%	3613.10	6035320	118.922	118.96	100.03	

Table 5. Accuracy Observation of Isopropamide

Isopropamide						
Spiked Level	**Sample Weight**	**Sample Area**	**µg/ml added**	**µg/ml found**	**% Recovery**	**% Mean**
50%	1204.40	3266665	99.704	99.68	99.98	100.00
50%	1204.40	3267669	99.704	99.72	100.01	
50%	1204.40	3268204	99.704	99.73	100.03	
50%	1204.40	3268566	99.704	99.74	100.04	
50%	1204.40	3263387	99.704	99.58	99.88	
50%	1204.40	3269157	99.704	99.76	100.06	
100%	2408.70	6535012.00	199.400	199.42	100.01	100.00
100%	2408.70	6538209.00	199.400	199.52	100.06	
100%	2408.70	6530293.00	199.400	199.28	99.94	
150%	3613.10	9801022	299.104	299.09	99.99	100.07
150%	3613.10	9809441	299.104	299.34	100.08	
150%	3613.10	9802843	299.104	299.14	100.01	
150%	3613.10	9816473	299.104	299.56	100.15	
150%	3613.10	9807050	299.104	299.27	100.06	
150%	3613.10	9816287	299.104	299.55	100.15	

Table 6. Represents the regression data including, linearity range, slope, correlation coefficient:

S.NO	**Parameter**	**Trifluoperazine**	**Isopropamide**
1.	Linearity Range	40-120µg/ml	100-300µg/ml
2.	Correlation coefficient(r^2)	0.999µg/ml	1

Table 7. Observation of Precision

S.No	**Sample Weight**	**Sample Area-Trifluoperazine**	**Sample Area-Isopropamide**	**%Assay of Trifluoperazine**	**% Assay**
1	2408.70	4031892	6533221	99.34	99.68
2	2408.70	4027885	6535078	99.24	99.71
3	2408.70	4022263	6539830	99.10	99.78
4	2408.70	4023117	6537569	99.12	99.75
5	2408.70	4017716	6538743	98.99	99.77
6	2408.70	4018316	6533591	99.00	99.69
Avarage Assay:				99.13	99.73
STD				0.14	0.04
%RSD				0.14	0.04

Table 8. LOD and LOQ results

Drug	**LOD ((µg/mL))**	**LOQ ((µg/mL)**
Trifluoperazine	2.963	9.877
Isopropamide	2.9851	9.9502

Table 9. Flow Rate Observation of Trifluoperazine

Flow Rate (ml/min)		System Suitability Results		
		USP Plate Count	USP Tailing	Retention time(min)
Low	0.6	4938	1.48	1.924
Actual*	0.8	5194	1.57	2.400
High	0.10	4568	1.57	3.191

*****Results for actual flow rate have been considered from Assay standard.

Table 10. Flow rate observation for Isoprpamide

Flow Rate (ml/min)		System Suitability Results		
		USP Plate Count	USP Tailing	Retention time(min)
Low	0.6	6255	1.38	2.897
Actual*	0.8	6738	1.40	7.60
High	0.10	6129	1.39	4.816

Table 11. Temperature Observation of Trifluoperazine

Change in column temperature	System Suitability Results		
	USP Plate Count	USP Tailing	Retention time(min)
20^{o}c	5021	1.52	2.403
Actual*	5194	1.57	2.400
30^{o}c	4962	1.54	2.399

Table 12. Temperature Observation of Isopropamide Sodium

Change in column temperature	System Suitability Results		
	USP Plate Count	USP Tailing	Retention time(min)
20^{o}c	6169	1.37	7.32
Actual*	6738	1.40	3.623
30^{o}c	6302	1.39	7.39

Table 13. Summary for RP-HPLC Method

S. No	Parameter	Acceptance Criteria	Results obtained
1	System suitability	Theoretical Plates-NLT 2000	Trifluoperazine-5194 Isopropamide-6738
		Tailingfactor-NMT2	Trifluoperazine-1.57 Isopropamide-1.40
		Resolution-NLT2	Trifluoperazine- Isopropamide-7.60
		Retention time	Trifluoperazine-2.400 Isopropamide-3.623
2	Precision	% RSD of FOS- NLT2 % RSD of HCTZ- NLT2	Trifluoperazine-0.14 Isopropamide-0.04
3	Linearity	Correlation coefficient NLT 0.999	Trifluoperazine-0.999 Isopropamide-1
4	Accuracy	Percentage Recovery 98-102%	Trifluoperazine-100.15 Isopropamide-100.023
5	Limit of detection	-	Trifluoperazine-2.963 Isopropamide-2.9851
6	Limit of quantitation	-	Trifluoperazine-9.877 Isopropamide-9.9502

Figure 1. Blank Chromatogram

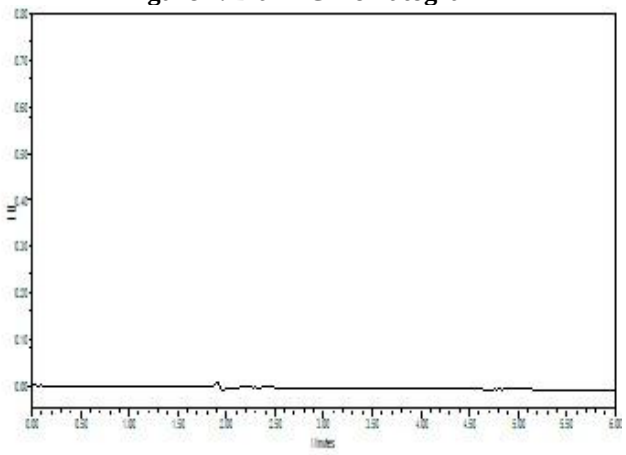


Figure 2. Standard Chromatogram

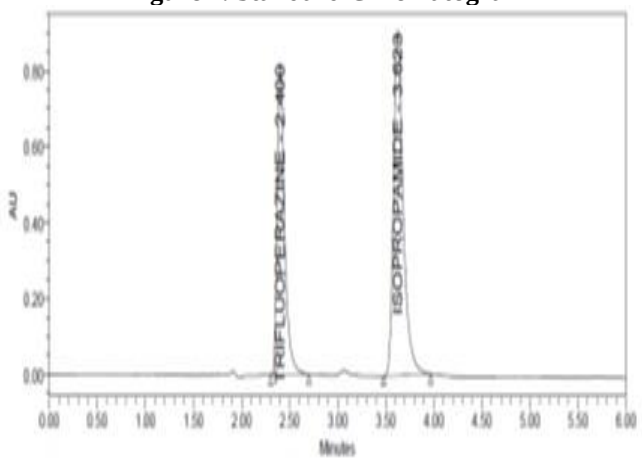


Figure 3. Sample Chromatogram -1

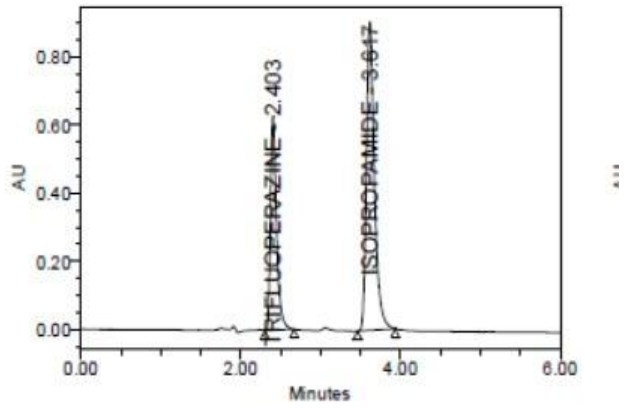


Figure 4. Calibration Curve for Trifluoperazine

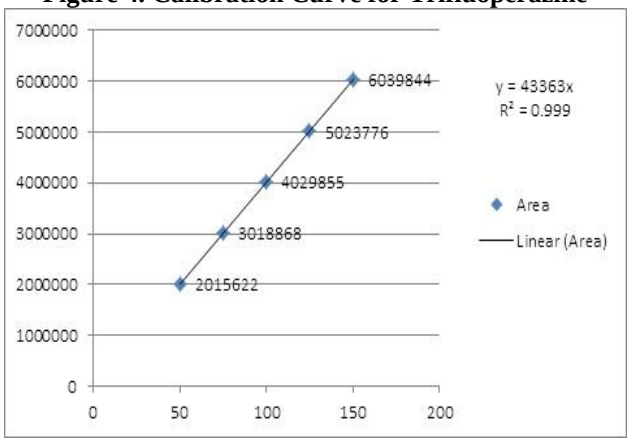
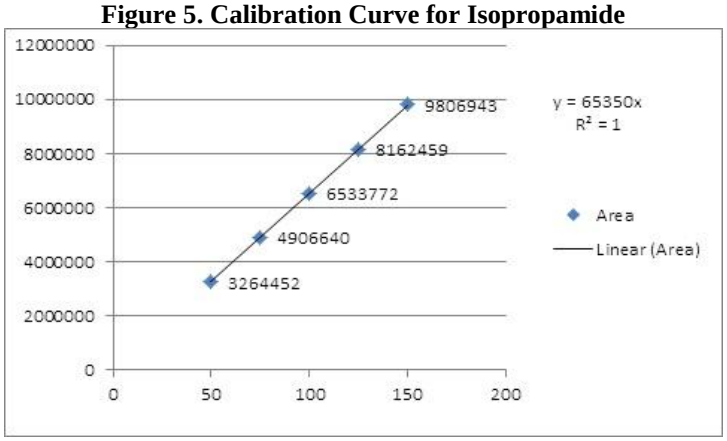


Figure 5. Calibration Curve for Isopropamide



CONCLUSION

The proposed HPLC method was found to be precise, specific, accurate, rapid and economical for simultaneous estimation of Trifluoperazine and Isopropamide in tablet dosage form. The sample recoveries in all formulations were in good agreement with their respective Label Claims and this method can be used for routine analysis. It can be applied for routine analysis in laboratories and is suitable for the quality control of the raw materials, formulations, dissolution studies and can be employed for bioequivalence studies for the same formulation.

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