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Short communication

Determination of cisapride in pharmaceutical dosage forms by reversed-phase liquid chromatography

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1. Introduction

Cisapride (CISA) is chemically (*RS*)-*cis*-4-amino-5-chloro-*N*-{1-[3-(4-fluorophenoxy)propyl]-3-methoxy-4-piperidyl}-2-methoxybenzamide monohydrate. It stimulates gastrointestinal motility and is used in management of gastro-oesophageal reflux, non-ulcer dyspepsia [1]. It is official in BP [2]. Various methods such as high-performance thin-layer chromatography [3] and spectrophotometry [4,5] for its determination in tablets and fluorometry [6] and liquid chromatography (LC) [7,8,9] for its determination in plasma have been reported in the literature. However, there is no reported LC method for its determination in pharmaceutical dosage forms. We report a new reversed-phase (RP)–LC method for the determination of CISA in pharmaceutical dosage forms.

2. Experimental

2.1. Instrumentation

A liquid-chromatographic system comprising Rheodyne injector, ternary gradient pump (9012) and UV detector (9050) connected to star chromatography workstation software (version 4.51) for processing the data generated and photodiode array (PDA) detector (9065) for determining the peak purity were used. This system was supplied by Varian, USA.

2.2. Solvents and chemicals

Standard of cisapride was obtained from Centaur Chemicals (P) Ltd, India. Purity of this was checked as per BP 93 addendum 1996 and found to be 99.28%. Acetonitrile used was of HPLC grade whereas methanol, triethylamine and orthophosphoric acid were of AR grade, which were supplied by S.D. Fine Chemicals, Thane, India. Tablets and suspension formulations were procured from the market.

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2.3. Mobile phase and stationary phase

A mixture of water–acetonitrile–triethylamine (65: 35: 0.25, v/v), adjusted to pH 3.5 with orthophosphoric acid was used as a mobile phase. A Microsorb C18 column, (250 mm × 4 mm i.d., 5 μm particle size) was used as a stationary phase.

2.4. Standard stock solution

Standard stock solution was prepared by dissolving accurately weighed 20 mg of CISA in 100 ml of methanol (0.2 mg/ml).

2.5. Working standard solution

A 5-ml volume of standard stock solution was diluted to 50 ml with mobile phase.This solution was used as working standard for assay analysis.

2.6. Sample solutions

2.6.1. Tablets

Twenty tablets were weighed and crushed to a fine powder. An accurately weighed portion of this powder equivalent to 25 mg of CISA was taken in a 50-ml volumetric flask, about 30 ml of methanol was added to it and the flask was kept in an ultrasonic bath for 10 min. The solution was then diluted to 50 ml with methanol. This solution was then centrifuged, 2 ml of the supernatent solution was diluted to 50 ml with mobile phase and used for the analysis.

2.6.2. Suspension

Suspension equivalent to 5 mg of CISA (5 ml) was taken in a 50-ml volumetric flask, about 30 ml of methanol was added and the flask was kept in an ultrasonic bath for 10 min, then the solution was diluted to the mark with methanol; 2 ml of this solution was further diluted to 10 ml with mobile phase and used for the analysis.

2.7. Calibration

Aliquots of standard stock solution of CISA were taken in different standard volumetric flasks and diluted with mobile phase to obtain the final concentrations of CISA in the range 0.5–200 μg/ml; 20 μl of each solution were injected into the chromatograph. The evaluation of CISA was performed with UV detector at 275 nm. Peak areas were recorded for all the chromatograms. Calibration curve was constructed by plotting peak areas (Y axis) against the amount of drug in μg/ml (X axis) and the linear relationship was evaluated by calculation of regression line by the method of least squares.

2.8. Assay

Each working standard and sample solution were injected into the chromatograph and the peak areas were recorded as described in the calibration procedure. From the peak area of CISA in standard and sample solutions the amount of CISA was computed by external standard quantification.

3. Results and discussion

3.1. Chromatography

Initial experiments were carried out using mobile phases of water and acetonitrile in different proportions and adjusting the pH to 3.5. A lot of tailing was observed for the peak of CISA. Since these mobile phases did not give proper peak shapes and retention, it was decided to add some modifiers to the mobile phase. Triethylamine (TEA) was selected as the reagent because of its organic nature and ability to reduce tailing drastically. TEA in the proportion of 0.05%, 0.1%, 0.15%, 0.2% and 0.25% was used with a mixture of water and acetonitrile in the ratio of 65:35 and adjusting the pH to 3.5, and retention time, theoretical plates and tailing factor of CISA were studied as a function of TEA concentration. These results are tabulated in Table 1. In view of these results it was decided to use the concentration of 0.25% of triethlyamine to get proper retention, good efficiency and acceptable tailing factor for the CISA peak. A typical chromatogram of CISA is shown in Fig. 1.

Table 1
System suitability parameters as a function of TEA concentration

% TEA	R_t of CISA	Theoretical plates	Tailing factor
0.05	18.91	5858	2.23
0.1	15.57	7487	1.89
0.15	9.72	9590	1.78
0.2	9.69	9987	1.62
0.25	9.37	10402	1.59

The wavelength of 275 nm was selected for the UV detection because at this wavelength CISA exhibits a maximum.

3.2. System suitability

To ascertain effectiveness of system suitability test, seven replicate injections of freshly prepared standard stock solution of CISA (20 μg/ml) were injected into the chromatograph and the relative standard deviation (R.S.D.) of peak areas was calculated. Observed R.S.D. was 0.29% (USP limit is not more than 2%). The peak purity of the peak due to CISA was tested using PDA detector and was found to be pure.

3.3. Linearity, limit of detection and limit of quantification

The plot of the peak area versus the respective concentration of CISA was found to be linear in the range of 0.5–200 μg/ml. The calibration curve could be represented by the following linear regression equation:

$$Y_{CISA} = 19361X + 6029.60 \qquad (r = 0.99999)$$

where Y is area, X is concentration in μg/ml and r is correlation coefficient.

The limit of detection (LOD) and the limit of quantification (LOQ) of CISA was calculated by using equations given in the International Conference on Harmonisation (ICH) guideline [10]:

$$\text{LOD} = \frac{3.3\sigma}{S}$$

$$\text{LOQ} = \frac{10\sigma}{S}$$

where σ is the noise estimate and is the S.D. of the blank responses (11 injections), S is the slope of the calibration curve of the drug. The limit of detection and the limit of quantification for CISA were found to be 0.02 μg/ml and 0.06 μg/ml, respectively.

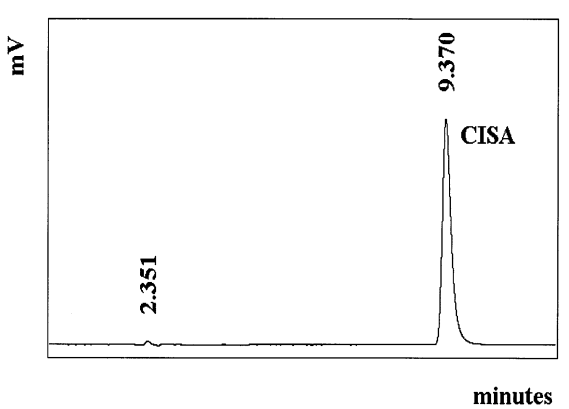

Fig. 1. Typical chromatogram of CISA.

3.4. Assay

The contents of CISA in three commercial brands of tablets and a brand of suspension found by the proposed method are shown in Table 2. The low values of R.S.D. indicate that the method is precise and accurate.

3.5. Accuracy and precision

To study the accuracy and the precision of the proposed method, recovery experiments were carried out by standard addition technique. Three different levels of standards were added to preanalysed tablet/suspension samples and each level was repeated three times. The percentage recoveries were calculated and the results obtained are shown in Table 3. The percentage recovery were in the range of 97.80–101.71%. These results indicate that the method is accurate and precise, and also there is no interference due to the excipients present in these formulations.

Table 3
Results of recovery analysis

Sample	Amount of drug from tablets/suspension (mg)	Amount of drug added (mg)	Total (theoretical) amount of drug (mg)	Amount found (mg)	R.S.D. (%)	Recovery (%)
Motilax tablets	10.0	0.0	10.0	10.04	0.30	100.40
	10.0	2.5	12.5	12.60	0.36	100.80
	10.0	5.0	15.0	15.00	0.12	100.00
	10.0	7.5	17.5	17.71	0.18	101.14
Ciza tablets	10.0	0.0	10.0	10.03	0.68	100.30
	10.0	2.5	12.5	12.49	0.15	99.92
	10.0	5.0	15.0	15.25	0.19	101.66
	10.0	7.5	17.5	17.80	0.14	101.71
Ciza-MPS tablets		0.0	10.0	10.08	0.38	100.80
	10.0	2.5	12.5	12.46	0.32	99.68
	10.0	5.0	15.0	15.02	0.10	100.13
	10.0	7.5	17.5	17.59	0.16	100.51
Motilax suspension	4.0	0.0	4.0	3.91	0.22	97.80
	4.0	1.0	5.0	4.89	0.32	97.80
	4.0	2.0	6.0	5.88	1.24	98.00
	4.0	3.0	7.0	6.95	0.71	99.28

Table 2
Assay of CISA in formulations

Brand[a](	Label claim	Amount found (mg)[b](	R.S.D. (%)
1, Motilax, tablets	Cisapride 10 mg/tablet	10.06	0.195
2, Ciza, tablets	Cisapride 10 mg/tablet	10.23	1.042
3, Ciza-MPS, tablets	Cisapride 10 mg/tablet, methylpolysiloxane 125 mg/tablet	10.05	0.66
4, Motilax, suspension	Cisapride 1 mg/ml suspension	0.98	1.59

[a] Brand 1: USV Ltd, India, batch no. CH 7007, manufactured August 1997. Brand 2: Intas Lab, India, batch no. 7320, manufactured October 1997. Brand 3: Intas Lab, India, batch no. 7266, manufactured September 1997. Brand 4: USV Ltd, India, batch no. GO 8002, manufactured January 1998.
[b] Average of six experiments.

3.6. Stability of the sample solution

Sample solution injected after 12 h did not show any appreciable change in assay value.

3.7. Stability indicating ability

The stability study is an integral part of pharmaceutical product development. The data collected during stability study decides shelf-life, storage condition and impurity profile of the product. If the assay method itself is stability indicating, it is considered to be versatile. Therefore, an attempt has been made to investigate stability indicating ability of the proposed method. The powdered samples of tablets equivalent to 25 mg of CISA were subjected to various stress conditions such as sunlight, heat (80°C temperature), acidic condition (1 ml of 0.1 N HCl) and alkaline condition (1 ml of 0.1 N NaOH) separately for 8 days. The exposed samples were then analysed by the proposed method. In the chromatogram of all exposed samples no degradation peak or change in assay value were observed. Peaks due to CISA in the chromatograms were also investigated using PDA detector for peak purity and it was found to be pure under all experimental stress conditions.

Since CISA did not show any degradation peak under any of the above stress conditions over a period of 8 days and also there was no significant change in assay value, it can be concluded that CISA is a fairly stable molecule. The assay value results are given in Table 4.

4. Conclusion

The proposed method is simple, precise, accurate and rapid for the determination of CISA from formulations. Hence it can be easily and conveniently adopted for the routine quality control analysis.

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Table 4
Results of samples exposed to stress conditions

Degradation with respect to:	[2]Amount of CISA found (mg/tablet)	
	Motilax-10 tablets[a](	Ciza tablets[a](
Initial (unexposed sample)	10.10	10.20
Heat @ 80°C for 8 days	10.00	10.00
Sunlight for 8 days	10.00	10.00
Alkaline condition (0.1 N NaOH) for 8 days	10.00	10.00
Acidic condition (0.1 N HCl) for 8 days	10.00	10.10

[a] Values are for study on one batch.

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