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Determination of *cis*- and *trans*-centchroman in its dosage forms by high-performance liquid chromatography☆

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Abstract

A high-performance liquid chromatographic (HPLC) method was developed and validated for the determination of *cis* and *trans*-centchroman, an oral contraceptive, in bulk drug samples and its dosage forms. After extraction with methanol, separation was accomplished by reversed-phase HPLC on a C_{18} column with acetonitrile–water (80:20) containing 0.4% of tetramethylammonium hydroxide [10% (v/v) aqueous solution] as the mobile phase. The pH was adjusted to 7.6 with 0.1 *M* orthophosphoric acid. The recoveries of *cis*- and *trans*-centchroman were always greater than 95%. The calibration graphs were linear over the range 0.11–4.0 μg for *cis*-centchroman and 0.18–4.0 μg for *trans*-centchroman.

1. Introduction

Centchroman [1], *trans* - 7 - methoxy - 2,2 - dimethyl - 3 - phenyl - 4 - [4 - (2 - pyrrolidinoethoxy)-phenyl]chroman, is a non-steroidal, once-a-week [2] oral contraceptive for females [3,4]. It is also effective against breast cancer [5]. Several methods have been reported for the determination of centchroman in its dosage forms [6] and in human serum [7], but they were unsuccessful in the separation of *cis* and *trans* isomers of centchroman. In this study, an HPLC method was developed to determine the *cis* and *trans* isomers of centchroman in bulk drug samples and in its dosage forms.

2. Experimental

2.1. Reagents and solvents

Pure *cis*- and *trans*-centchroman (**I** and **II**, respectively; Fig. 1) were obtained from Dr. S. Ray, Medicinal Chemistry Division, CDRI. Tetramethylammonium hydroxide (analytical-reagent grade) and acetonitrile (HPLC grade) were procured from Merck (Bombay, India). Triply distilled water from an all-glass apparatus was used as a solvent. All glassware was washed with detergent, rinsed thoroughly with triply distilled water and dried prior to use.

2.2. HPLC apparatus and chromatographic conditions

The HPLC instrument consisted of Micromeritics (Norcross, GA, USA) Model 750

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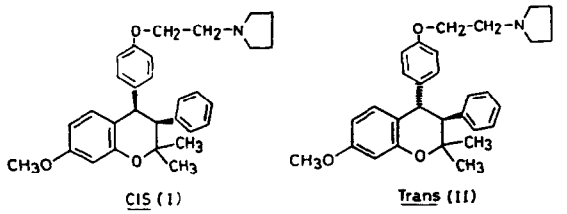

Fig. 1. Structures of *cis*- and *trans*-centchroman.

solvent-delivery system, a Rheodyne (Cotati, CA, USA) Model 7125 injector with a 20-μl loop and a Perkin-Elmer Model 235 diode array detector. Separation was carried out on an ODS Phenomenex (5 μm particle size) column (250 mm × 4.6 mm I.D.). The column effluent was monitored at 280 nm. Chromatograms were recorded on a G.P. 100 printer–plotter. The mobile phase was acetonitrile–water–tetramethylammonium hydroxide [10% (v/v) aqueous solution] (80:20:0.4), adjusted to pH 7.6 with 0.1 *M* orthophosphoric acid. It was filtered and degassed before use. Chromatography was performed at 27 ± 3°C at a flow-rate of 1.5 ml/min.

2.3. Preparation of stock and working standard solutions

Stock standard solutions containing 200 μg/ml of **I** and **II** as hydrochlorides were prepared in the mobile phase. Working standard solutions were prepared in the mobile phase in the range 9–200 μg/ml for **I** and 5.5–200 μg/ml for **II**.

2.4. Extraction

Twenty tablets were weighed and finely powdered. Powder equivalent to 10 mg of centchroman hydrochloride was extracted three times with 5-ml volumes of methanol, the combined extracts were centrifuged and the volume was made up to 25 ml with methanol. A 250-μl volume of this solution was further diluted to 25 ml with mobile phase.

2.5. Accuracy and precision

Known amounts of **I** and **II** were added to the mixed contents of tablets and *cis*- and *trans*-centchroman were determined by interpolation on the corresponding calibration graphs. The accuracy of the method was calculated on the basis of the difference in the mean calculated and added concentrations and the precision was obtained by calculating the inter-day relative standard deviations (R.S.D.s).

3. Results and discussion

3.1. Chromatography

A C_{18} column was used to separate *cis*- and *trans*-centchroman. pH plays an important role in the separation. A decrease in pH results in peak sharpening but with a decrease in the retention time difference between the *trans* and *cis* isomers, leading to merging of the peaks (Fig. 2a and b). Similarly, an increase in pH leads to a better separation of the *cis* and *trans* isomers but with peak broadening (Fig. 2c). Accordingly, pH 7.6 was chosen as higher pH may decrease the column lifetime. Acetonitrile–water–tetramethylammonium hydroxide [10% (v/v) aqueous solution] (80:20:0.4), adjusted up pH 7.6 with 0.1 *M* orthophosphoric acid was found to be the optimum mobile phase for the effective resolution of *cis*- and *trans*-centchroman in bulk drug samples and in formulations.

3.2. Selectivity and specificity

The retention times of **II** and **I** were *ca.* 24.0 and 34.0 min, respectively. No interfering peaks were detected. Based on a signal-to-noise ratio of 3, the detection limits were 0.1 and 0.05 μg for **II** and **I**, respectively. However, the limits of determination were set at 0.18 and 0.11 μg, respectively. The method provided adequate sensitivity for the determination of **I** and **II** in bulk drug substance and dosage forms.

3.3. Linearity and reproducibility

External standardization by peak area was used for the determination of *cis*- and *trans*-centchroman. The calibration graphs were linear

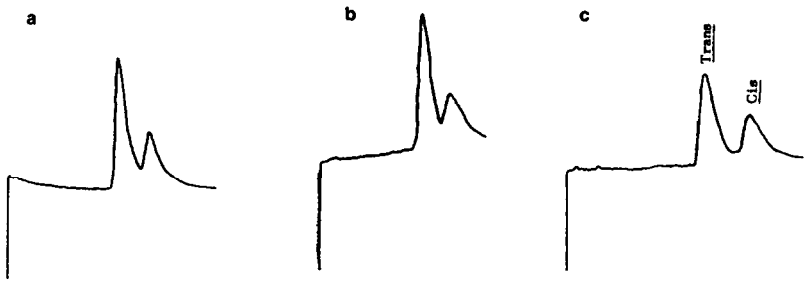

Fig. 2. Separation of *cis*- and *trans*-centchroman at pH (a) 7.4, (b) 7.2 and (c) 7.6.

over the ranges 0.18–4.0 μg for *trans*- and 0.11–4.0 μg for *cis*-centchroman with correlation coefficients (r) of 0.999 and 0.9949, respectively. The calibration equations for **I** and **II** are as follows:

unknown concentration of **I**
$= 1.0053$ (peak area) $- 0.0143$

unknown concentration of **II**
$= 0.817$ (peak area) $- 0.0025$

The reproducibility and accuracy of the method were determined by intra- and inter-assay variations (Tables 1 and 2).

3.4. Application of method in pharmaceutical analysis

The method was applied to the analysis of bulk drug samples and tablet formulations of centchroman (Table 3). The method is simple and is in use for the analysis of different bulk drug samples and tablet formulations in different manufacturing units and test laboratories. This method has also been proposed for incorporation in the Indian Pharmacopoeia for the determination of centchroman.

Table 1
Precision and accuracy for **II**

Amount taken (μg)	Mean peak area (cm^2)	Bias (%)	R.S.D. (%)
Within-day (n = 3)			
0.16	0.18	0.35	3.2
0.41	0.45	0.58	2.2
0.82	0.66	0	0
1.22	0.96	0	0
1.63	1.40	2.31	2.86
3.3	2.73	0.58	0.37
Day-to-day (n = 3)			
0.16	0.18	0.35	3.2
0.41	0.46	1.15	4.3
0.82	0.66	0	0
1.22	0.93	2.31	4.3
1.63	1.41	2.31	3.0
3.3	2.82	8.7	5.5

Table 2
Precision and accuracy for I

Amount taken (μg)	Mean peak area (cm^2)	Bias (%)	R.S.D. (%)
Within-day (n = 3)			
0.11	0.117	0.34	4.93
0.22	0.255	0.87	5.88
0.54	0.563	0.58	1.81
1.08	1.121	3.41	5.29
1.61	1.463	0.75	0.85
2.15	2.20	2.89	2.27
Day-to-day (n = 3)			
0.11	0.123	0.34	4.69
0.22	0.275	0.88	5.94
0.54	0.585	0.76	2.26
1.08	1.28	1.15	1.56
1.61	1.605	6.73	7.26
2.15	2.25	2.89	2.22

Table 3
Determination of centchroman in drug formulations

Sample	Tablets			Bulk drug sample	
	Centchroman content declared (mg)	*trans*-Centchroman found (%)	*cis*-Centchroman found (%)	*trans*-Centchroman found (%)	*cis*-Centchroman found (%)
PHT/ID/2/88	30	99.28	–		
PHT/ID/39/88	30	98.38	–		
PHT/ID/90/88	30	103.13	–		
PHT/ID/129/89	30	100.37	0.02		
PHT/ID/97/92				98.82	–
PHT/ID/97/92 (*cis*-centchroman)				–	98.78
PHT/ID/98/92				98.90	–
PHT/ID/99/92				97.5	0.97
PHT/ID/102/92				98.74	–

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