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Original Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF CLOPAMIDE, DIHYDROERGOCRISTINE MESYLATE AND RESERPINE IN TABLETS

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ABSTRACT

Objective: To develop a new, rapid, precise and sensitive RP-HPLC method for the simultaneous determination of Clopamide (CPM), Dihydroergocristine mesylate (DHECM) and Reserpine (RSP) in tablets.

Methods: Caffeine (CAFF) was used as internal standard, a BDS Hypersil column C8 (4.6 x 150 mm) with mobile phase containing a mixture of inorganic phase (ammonium acetate solution in water) and organic phase (acetonitrile and methanol), adjusted with phosphoric acid (10% v/v) to a pH-3.5, the flow rate was 2.0 ml/min, UV detection was performed at a wavelength of 220 nm and injection volume was 20 µl. The validation of this method was done as per ICH guidelines.

Results: Retention times were observed as 1.277, 2.397, 3.799 and 5.202 min for Caffeine, Clopamide, Dihydroergocristine mesylate and Reserpine, respectively. Linearity ranges were observed to be: 1.50-300.00, 2.50-500.00 and 1.00-200.00 µg/ml for Clopamide, Dihydroergocristine mesylate and Reserpine respectively. Relative Standard Deviation did not exceed 2.00%. Detection limits were observed to be 0.08, 0.07 and 0.05 µg/ml for Clopamide, Dihydroergocristine mesylate and Reserpine, respectively.

Conclusion: This method was applied, the first time as simple, rapid, precise and sensitive method for determination of Clopamide, Dihydroergocristine mesylate and Reserpine either alone or in combination, in pure form and in pharmaceutical preparations without interference with any excipient.

Keywords: RP-HPLC, Method development, Validation, Clopamide, Dihydroergocristine mesylate, Reserpine.

INTRODUCTION

Clopamide is described chemically as 4-Chloro-N-[(2RS, 6SR)-2,6-dimethylpiperidin-1-yl]-3-sulfamoylbenzamide.

The empirical formula is $C_{14}H_{20}ClN_3O_3S$. The structural formula is shown in fig. 1.

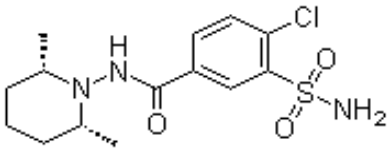

Fig. 1: Structure of clopamide

Clopamide is a white or almost white, hygroscopic, crystalline powder. It is slightly soluble in water and in anhydrous ethanol, sparingly soluble in methanol. It shows polymorphism. It contains not less than 99.0% and not more than 101.0% of C14H20ClN3O3S in bulk powder. Its melting point ranges between 244.0 and 246.0 °C, its loss on drying is not more than 2.5 per cent at 105.0 °C, its molecular weight is 345.84 g.

Clopamide is a piperidine diuretic, prescribed for fluid retention, it reduces the action of adrenaline and nor adrenaline.

Clopamide was identified by many methods as titration with perchloric acid in a medium of anhydrous acetic acid [1], GC-MS [2], derivative spectra [3], chromatographic–densitometric method [4] and RP-HPLC [5].

Dihydroergocristine mesylate is described chemically as (6aR,9R,10aR)-N-[(2R,5S,10aS,10bS)-5-Benzyl-10b-hydroxy-2-(1-methylethyl)-3,6-dioxo-octahydro-8H-oxazolo[3,2-a]pyrrolo[2,1-c] pyrazin-2-yl]-7-methyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-fg]quinoline-9-carboxamide methanesulphonate.

The empirical formula is $C_{36}H_{45}N_5O_8S$. The structural formula is shown in fig. 2.

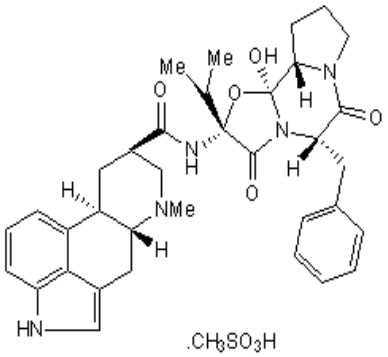

Fig. 2: Structure of Dihydroergocristine mesylate

Dihydroergocristine mesylate is a white or almost white, fine crystalline powder. It is slightly soluble in water, soluble in methanol. It contains not less than 98.0% and not more than 102.0% of C36H45N5O8S in bulk powder. Its maximum loss on drying is 3.0 per cent at 80.0 °C, pH for its hydrous solution is in the range between 4.00-5.00, molecular weight 707.84 g.

Dihydroergocristine mesylate is used to treat dementia and age-related cognitive impairment (such as in Alzheimer disease), as well as to aid in recovery after stroke.

DHECM was identified by titration with tetrabutyl ammonium hydroxide 0.1M in medium of pyridine using nitrogen [1], HPLC with fluorescence detection [6] and LC-MS [7, 8].

Reserpine is described chemically as Methyl 11,17α-dimethoxy-18β-[(3,4,5-trimethoxybenzoyl)oxy]-3β,20α-yohimban-16β-carboxylate.

The empirical formula is $C_{33}H_{40}N_2O_9$. The structural formula is shown in fig. 3.

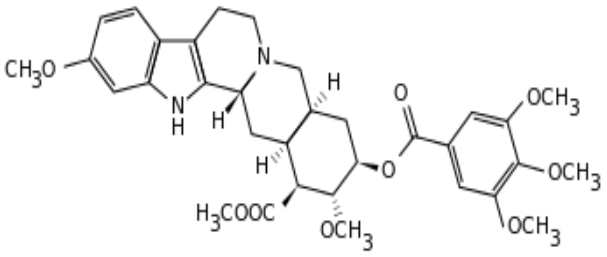

Fig. 3: Structure of Reserpine

Reserpine is a white or slightly yellow, small crystals or crystalline powder, darkening slowly on exposure to light. It is practically insoluble in water, very slightly soluble in ethanol (96 per cent). It contains not less than 97.0% and not more than 101.0% of $C_{33}H_{40}N_2O_9$ in bulk powder, its melting point is 264.5 °C, its molecular weight is 608.68 g.

Reserpine is used with or without other medications to treat high blood pressure (hypertension). Lowering high blood pressure helps prevent strokes, heart attacks and kidney problems, it works by decreasing certain substances in the body (such as norepinephrine), this causes the blood vessels to relax so that blood can flow more easily and also slows the heart rate, these effects help to lower blood pressure.

A spectro fluorimetric method was established for determination of RSP [10]. RSP was identified with rescinnamine by GC [11], in two steps by HPLC [12], in rauwolfia serpentine by HPTLC [13], in equine plasma by LC-MS [14] and spectrophotometry [15].

The significance of this study due to the simultaneous determination of three drug substances (Clopamide, Dihydroergocristine mesylate and Reserpine) in one drug product used for heart diseases which should be very precise. The objective of this study is to develop a new, rapid, precise and sensitive RP-HPLC method for the simultaneous determination of Clopamide, Dihydroergocristine mesylate and Reserpine in pharmaceutical dosage forms as per ICH guidelines [16]. The validation procedure followed the guidelines of USP 34 [9].

MATERIALS AND METHODS

Chemicals and reagents

All reagents and chemicals were highly-pure, phosphoric acid, HPLC grade acetonitrile and HPLC grade methanol were obtained by SCHARLAU company (Spain), ammonium acetate was obtained by RIEDEL-DE HAEN AG SEELLE company (Germany), Clopamide produced by INTERPHARMA PRAHA company (Czech Republic), Dihydroergocristine mesylate produced by MAYALS ROAD company (China), Reserpine produced by VITAL LABORATORIES company (China), Caffeine produced by CHEMPI FINE CHEMICAL company (India) and Brindine tablets manufactured by SHIFA (Syria). Water was deionized and passed through Millie Q system, Millie pore (USA).

Equipments

HPLC model 503V by JASCO (Japan), equipped with a degasser and column oven, and an auto sampler with injection volume ranges of 1.0-100.0 µl, UV detection at 190-900 nm, pump range of flow rate 0.0-10.0 ml/min, separation was achieved on a BDS HYPERSIL column C_8 (4.6 x 150 mm) produced by THERMO company (USA), pH apparatus model 320 equipped with electrode produced by ORION company (France), sensitive analytical balance: 220 g, d=0.1 mg produced by KERN company (Germany), ultrasonic produced by DAIHAN company (Korea) and laboratory glasses produced by ISOLAB company (Germany).

Chromatographic conditions

The mobile phase consists of a mixture of ammonium acetate (prepared by dissolving 462.0 mg of ammonium acetate in 340 ml of water) (34 volumes), acetonitrile (16 volumes) and methanol (10 volumes), adjusted using phosphoric acid (10% v/v) to a pH-3.5 and filtered through 0.45 µm nylon membrane filter before use. The injection volume was 20 µl with a flow rate 2.0 ml/min and detection wavelength 220 nm having ambient condition.

Standard preparation

Stock solutions were prepared by accurately weighing 40.0, 37.5, 250.0 and 50.0 mg of Caffeine, Clopamide, Dihydroergocristine mesylate and Reserpine respectively and transferring to a 100 ml volumetric and dissolving in mobile phase. Volume was made up to the mark with mobile phase, which gave 400.00, 375.00, 2500.00 and 500.00 µg/ml.

Samples' preparation

Twenty tablets were weighed and the coats were removed. An accurately weighed amount of the finely powdered Brindine® tablets equivalent to 5.00 mg Clopamide, 0.50 mg Dihydroergocristine mesylate and 0.10 mg Reserpine was transformed to 20 ml volumetric flask and dissolved using mobile phase, sonicated for 15 min and filtered, the sample was prepared with Caffeine 20.00 µg/ml as internal standard.

Method development

An analytical procedure is developed to test a defined characteristic of the drug substance or drug product against established acceptance criteria for that characteristic. Early in the development of a new analytical procedure, the choice of analytical instrumentation and methodology should be selected based on the intended purpose and scope of the analytical method.

The solutions of Caffeine (internal standard, 20.00 µg/ml), Clopamide, Dihydroergocristine mesylate and Reserpine (50.00 µg/ml for each component) were chromatographed at the same chromatographic conditions described above.

Retention times were as follows: 1.277, 2.397, 3.799 and 5.202 min for Caffeine, Clopamide, Dihydroergocristine mesylate and Reserpine respectively. These results are shown in fig. 4-7 respectively as follows:

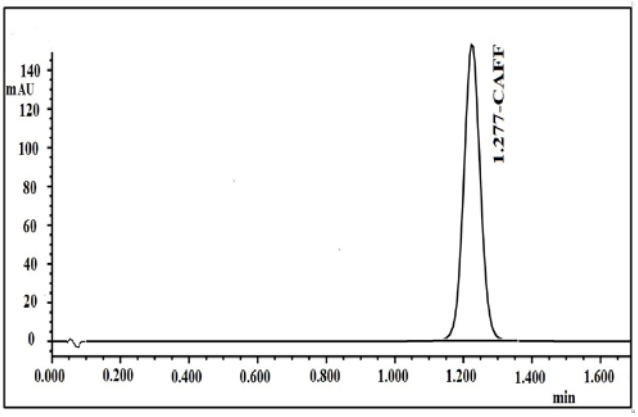

Fig. 4: Typical chromatogram of CAFF (internal standard) 20.00 µg/ml (mobile phase 34/16/10 v/v/v, ammonium acetate/acetonitrile/methanol, column hypersil C8 (4.6 x 150 mm), λ=220 nm and flow rate 2.0 ml/min)

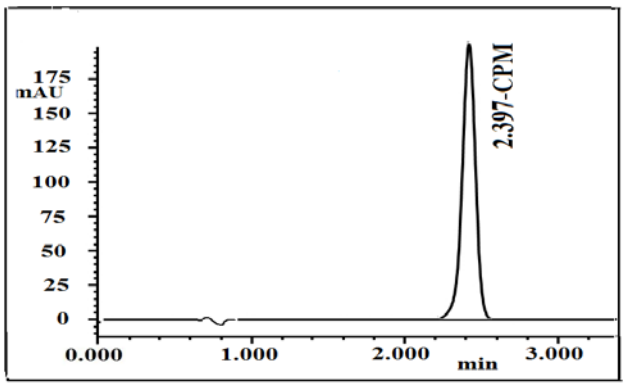

Fig. 5: Typical chromatogram of CPM 50.00 µg/ml (mobile phase 34/16/10 v/v/v, ammonium acetate/acetonitrile/methanol, column hypersil C8 (4.6 x 150 mm), λ=220 nm and flow rate 2.0 ml/min)

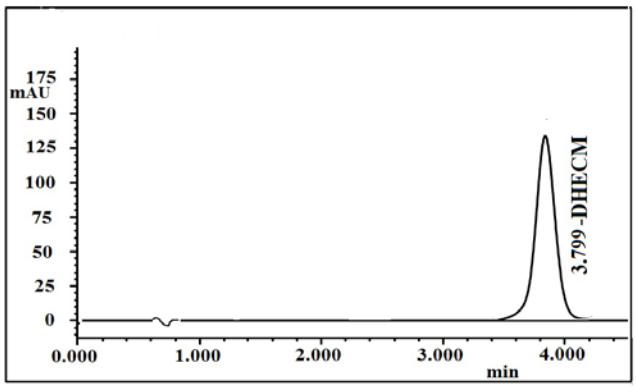

Fig. 6: Typical chromatogram of DHECM 50.00 µg/ml(mobile phase 34/16/10 v/v/v, ammonium acetate/acetonitrile/methanol, column hypersil C8 (4.6 x 150 mm), λ=220 nm and flow rate 2.0 ml/min)

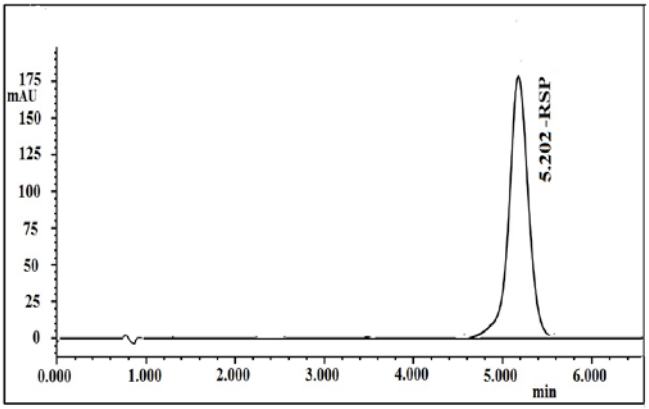

Fig. 7: Typical chromatogram of RSP 50.00 µg/ml (mobile phase 34/16/10 v/v/v, ammonium acetate/acetonitrile/methanol, column hypersil C8 (4.6 x 150 mm), λ=220 nm and flow rate 2.0 ml/min)

The solution containing the four components: Caffeine (20.00 µg/ml), Clopamide, Dihydroergocristine mesylate and Reserpine (50.00 µg/ml for each) was chromatographed in the same conditions. It was found that their peaks are well separated (good resolution), see fig. 8.

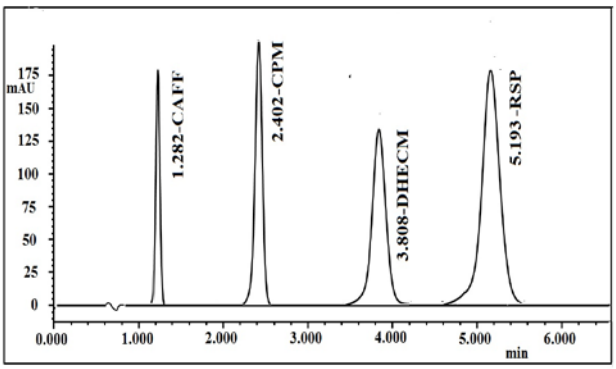

Fig. 8: Typical chromatograms of CAFF 20.00 µg/ml, CPM, DHECM and RSP 50.00 µg/ml for each (mobile phase 34/16/10 v/v/v, ammonium acetate/acetonitrile/methanol, column hypersil C8 (4.6 x 150 mm), λ =220 nm and flow rate 2.0 ml/min)

Method validation

Validation of an analytical method is the process that establishes, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications. The validation of this suggested method is according to ICH guidelines. Method validation is the process of demonstrating that analytical procedures are suitable for their intended use, therefore the objective of this analytical procedure validation is to demonstrate that it is suitable for its intended purpose.

Linearity

Linearity was studied by preparing standard solution at five different concentration levels. The linearity range was found to be 1.50-300.00, 2.50-500.00 and 1.00-200.00 µg/ml for Clopamide, Dihydroergocristine mesylate and Reserpine respectively. 20 µl of each solution was injected into chromatograph. Peak areas were recorded for all the chromatogram. Calibration curve was constructed by plotting the ratio of area of studied component to area of Caffeine. The linearity curves of Clopamide, Dihydroergocristine mesylate and Reserpine are shown in fig. 9-11.

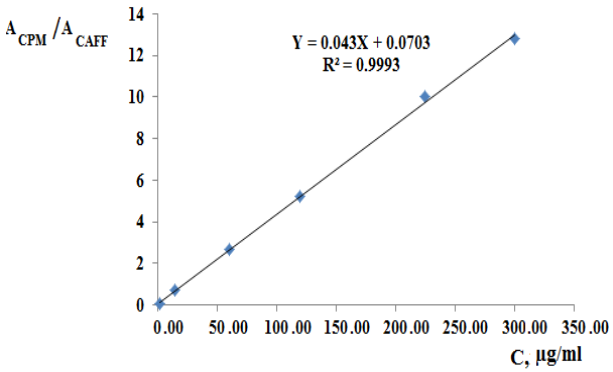

Fig. 9: Calibration curve of CPM concentrations range 1.50-300.00 µg/ml

Accuracy and precision

Accuracy and precision of the method were established by five replicate injections of the standard solution containing Clopamide, Dihydroergocristine mesylate and Reserpine in the same day. The confidence limit CL (confidence level = 95%, n-1=4 and t=2.78), recovery REC% and RSD% were calculated and presented in table 1. From the data obtained, this developed RP-HPLC method was found to be accurate and precise.

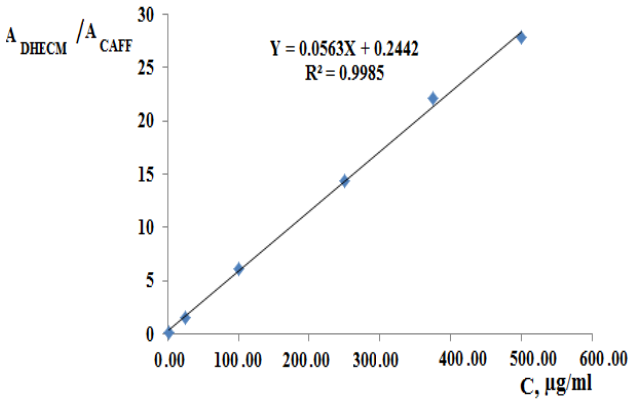

Fig. 10: Calibration curve of DHECM concentrations range 2.50-500.00 µg/ml

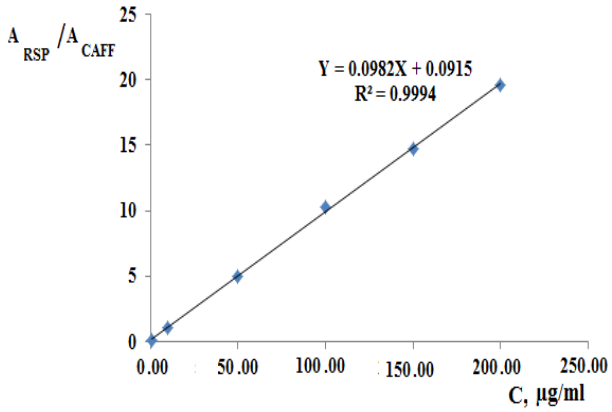

Fig. 11: Calibration curve of RSP concentrations range 1.00-200.00 µg/ml

Table 1: RP-HPLC method for simultaneous determination of CPM, DHECM and RSP

Compound	Nominal concentration, µg/ml	Mean concentration, µg/ml	RSD%	Confidence limit	REC%
CPM	10.00	10.08	1.53	10.08±0.191	100.80
	20.00	19.66	1.43	19.66±0.350	98.30
	30.00	29.64	1.09	29.64±0.399	98.80
	40.00	40.39	0.78	40.39±0.392	100.98
	50.00	49.62	0.63	49.62±0.389	99.24
DHECM	10.00	10.14	1.64	10.14±0.207	101.40
	20.00	20.20	1.44	20.20±0.362	101.00
	30.00	29.78	1.28	29.78±0.474	99.27
	40.00	39.47	1.03	39.47±0.505	98.68
	50.00	50.76	0.84	50.76±0.530	101.52
RSP	10.00	09.86	1.71	09.86±0.075	98.60
	20.00	20.33	1.53	20.33±0.387	101.65
	30.00	30.46	1.23	29.78±0.466	101.53
	40.00	40.34	0.96	39.47±0.481	100.85
	50.00	49.04	0.81	50.76±0.494	98.08

Limit of detection and limit of quantitation

Calibration lines using standard solutions for Clopamide, Dihydroergocristine mesylate and Reserpine were obtained using the same analytical conditions, linear regression analysis was used to calculate the slope, intercept and the coefficient of determination R^2 of each calibration line. The limit of detection LOD was computed as the analyte concentration giving a signal equal to the blank signal plus three standard deviation of the blank. The limit of quantitation LOQ was computed as the analyte concentration giving a signal equal to the blank signal plus ten standard deviation of the blank [17]. LOD was observed to be 0.08, 0.07 and 0.05 µg/ml, LOQ was observed to be 0.27, 0.23 and 0.17 µg/ml for Clopamide, Dihydroergocristine mesylate and Reserpine respectively. From these results, this developed RP-HPLC method was found to be sensitive.

Specificity

A solution containing a mixture of the excipients were prepared using the sample preparation procedure to evaluate possible interfering peaks. It was not seen other peaks in the chromatogram, this confirms the specificity of this applied RP-HPLC method.

Assay application in Brindine tablets (Shifa Pharmaceutical Industries)

The three studied drugs were separated and determined in the pharmaceutical form: Brindine-Shifa tablets (Shifa Pharmaceutical Industries), calibration curves were created for Clopamide, Dihydroergocristine mesylate and Reserpine using Caffeine as internal standard, the dosage of every component was calculated. It was found that the studied method was successful for simultaneous determination of Clopamide, Dihydroergocristine mesylate and Reserpine in Brindine tablets without any interference with excipients. The results of simultaneous determination for Clopamide, Dihydroergocristine mesylate and Reserpine respectively in Brindine tablets (Shifa) were shown in table 2 note that dose is for weight of one tablet (assay limits 90-110% for the three components).

Table 2: Determination results of CPM, DHECM and RSP in Brindine tablets

Compound	Dose, mg	X_1, mg	X_2, mg	X_3, mg	$\overline{X}$, mg	Assay%
CPM	5.000	5.010	4.886	4.981	4.959	99.18
DHECM	0.500	0.483	0.476	0.502	0.487	97.40
RSP	0.100	0.096	0.100	0.098	0.098	98.00

CONCLUSION

This new RP-HPLC method has been shown to be rapid, accurate, precise, sensitive and easy to perform for separation and simultaneous determination of Clopamide, Dihydroergocristine mesylate and Reserpine using Caffeine as internal standard. In addition, this proposed method was successfully applied to the quality control analysis of studied components without interference

with the additives and excipients normally used in tablet formulations, this technique can be applied for the accurate determining of dose and homogenization of individually compounded products, and this will basically provide better patient care.

CONFLICT OF INTERESTS

The authors have declared that no conflict of interests exists.

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