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Research Article

SIMULTANEOUS DETERMINATION OF CHLORPHINERAMINE MALEATE, DEXTROMETHORPHAN HBR AND PHENYLEPHRIN HCL IN CODILAR SYRUP USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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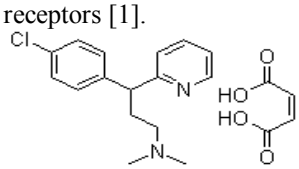
ABSTRACT
A simple, selective, sensitive and precise, simultaneous high performance liquid chromatographic analysis of syrup containing Chlorphineramine Maleate, Dextromethorphan HBr and Phenylephrin HCl was described. Good chromatographic separation was achieved using a Zorbax C18 (4.6 cm x 250 mm, 5 µm) and a mobile phase consisting of acetonitrile-phosphate buffer pH 3.5 (15:85, v/v) at a flow rate 0.9 mL/min. The ultraviolet detector was set at wavelength 280 nm. Chlorphineramine Maleate, dextromethorphan HBr and Phenylephrin HCl were measured at 2.789, 3.645 and 13.521 min, respectively. The linear ranges for chlorphineramine maleate, dextromethorphan HBr and phenylephrin HCl were 10-50, 10-50 and 5-45 µg/mL, respectively. The recoveries of chlorphineramine maleate, dextromethorphan HBr and phenylephrin HCl in pharmaceutical preparation were all greater than 98% and their relative standard deviations were less than 2.0%. The limits of detection were 2.57, 0.19 and 0.003 µg/mL for Chlorphineramine Maleate, Dextromethorphan HBr and Phenylephrin HCl, respectively.
Keywords: High performance liquid chromatography, Suppression irritant unproductive cough medicaments

INTRODUCTION

Chlorphineramine maleate, dextromethorphan HBr and phenylephrin HCl present in Codilar syrup which is used for suppression of ittitant unprodu-ctive cough with or without nasal catarrh.

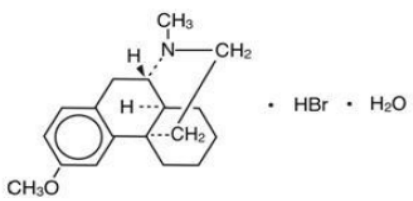
Chlorpheniramine maleate belong to the oldest first-generation classic H_1-receptor blockers drugs. Most of these drugs produce sedation by breaking in CNS. Additionally, they are producing a variety of unwanted adverse effects due to there interaction with other receptors as adrenergic receptors, muscarinic cholinergic receptors (atropine like), and serotonin receptors [1].



γ-(4-Chlorophenyl)-*N,N*-dimethyl–2– pyridinepropanamine

Chlorpheniramine maleate was determined in pharmaceutical dosage forms and plasma samples by Chromatographic [2-12], spectrophotometric [13-16] and electrochemical methods [17, 18].

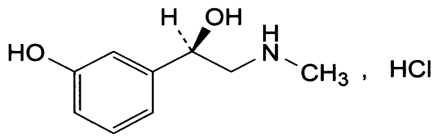
Dextromethorphan acts centrally. It elevates the threshold for coughing, without inhibiting ciliary activity. Dextromethorphan (DXM) is rapidly absorbed from the gastrointestinal tract and converted into lower active metabolite (dextrorphan). The duration of action after oral administration is approximately three to eight hours for dextromethorphan-hydrobromide [1].



3-methoxy-17-methyl-(8α, 13 α, 14 α)-morphinan hydrobromide

Several methods have been reported for the analysis of the cited drug either in bulk powder, different dosage forms or in biological fluids. These methods include spectrophotometric [19], Chromatographic [20-25] and voltametric [26] methods.

Phenylephrine HCl used as a decongestant. Oral phenylephrine is extensively metabolised by MAO enzyme in the GIT and liver. So compared to orally-taken pseudoephedrine, it has a reduced and variable bioavailability of only up to 38 %. It is a direct selective α-adrenergic receptor agonist, it does not cause release of endogenous noradrenaline, as pseudoephedrine does. So phenylephrine has low side-effects like CNS stimulation, irritability, insomnia, anxiety and restlessness [1].



(R)- 3[1-m-hydroxy-2-(methylamino)methyl] benzy alcohol hydrochloride

Several Spectrophotometeric [27-29] and Chromatographic [30-32] methods have been reported for the analysis of Phenylephrine hydrochloride in bulk powder, different dosage or in biological fluids.

Experimental

Equipments

Agilent 1200 series, vacuum degasser, thermos tatted column compartment G1316A/G1316B, diode array and multiple wavelength detector SL, quaternary pump (Germany).

Chemicals

Chlorphineramine Maleate, dextromethorphan HBr and phenylephrin HCl were purchased from Merck (Germany).

Pharmaceutical preparation

Codilar® syrup; B.N. 079001 (labeled to contain 10 mg dextromethorphan HBr, 2 mg phenylephrin HCl and 1 mg chlorphineramine Maleate per 10 mL) were kindly supplied from El—Nile Pharmaceutical Co., Egypt.

HPLC procedure

Chromatographic conditions

The analytical column was a Zorbax C18 (4.6 cm x 250 mm, 5 µm) and a mobile phase consisting of acetonitrile and phosphate buffer pH 3.5 (15:85, v/v) at a flow rate of 0.9 mL/min and at room temperature. The ultraviolet detector was set a wavelength of 280 nm. Solutions and mobile phase were freshly prepared at the time of use.

Standard solution preparation

Stock solutions of chlorphineramine Maleate, dextromethorphan HBr and phenylephrin HCl were prepared daily by dissolving the appropriate amount of drug standards in mobile phase to yield a final concentration of 5.0, 2.0 and 1.0 mg/mL, respectively. Separate stock solutions were prepared for the calibration standards and quality control samples. Further, solutions were obtained by serial dilutions of stock solutions with mobile phase.

Preparation of pharmaceutical dosage sample

The contents of five bottles Codilar were mixed well and transferred to 100.mL volumetric flasks. Each 10-mL equivalent to 10 mg dextromethorphan HBr, 2 mg phenylephrin HCl and 1 mg chlorphineramine Maleate. Working solutions were prepared individually by diluting the stock solutions with distilled water to obtain concentration range of 10-50 ug ml^{-1} for chlorphineramine Maleate, 10-50 $ugml^{-1}$ for dextromethorphan HBr and 5-45 $ugml^{-1}$ for phenylephrin HCl.

RESULTS AND DISCUSSION

Chromatograms of samples

The aim of this research was to develop a new, simple, accurate, reproducible, sensitive HPLC method for the simultaneous determination of chlorphineramine Maleate, dextromethorphan HBr and phenylephrin HCl. A satisfactory separation of each drug from pharmaceutical excipients was obtained. To optimize the appropriate HPLC conditions for separation of the examined drugs, various reversed-phase columns, isocratic and gradient mobile phase systems were tried. The optimum wavelength for detection was 280 nm at which much better detector responses for the three drugs were obtained. The mobile phase was found to be suitable to improve the sharpness and thinness of the chlorphineramine Maleate, dextromethorphan HBr and phenylephrin HCl. The retention times for the investigated drugs were found to be 2.869 min, 3.752 and 13.689 min, respectively. No pharmaceutical excipients eluted at the retention times of the peaks of interest.

Calibration and linearity

Calibration curves were constructed in the ranges of 10-50, 10-50 and 5-45 µg/mL for chlorphineramine Maleate, dextromethorphan HBr and phenylephrin HCl, respectively. The slope, intercept and regression coefficient for each compound were estimated.

Accuracy

Absolute recoveries of six different authentic concentrations of chlorphineramine Maleate, dextromethorphan HBr and phenylephrin HCl (Table 1) and the studied drugs in syrup (Table 2) were determined by assaying the samples as described above. Mean recoveries, standard deviations and the relative standard deviations were calculated by standard method (Tables 1 and 2) .

Table 1. Statistical analysis of the results of authentic chlorphineramine maleate, dextromethorphan HBr and phenylephrin HCl compared with official methods.

	Chlorpheniramine Maleate	Dextromethorphan HBr	Phenylephrin HCl
	Proposed method	Proposed method	Proposed method
X	100.02	100.3	99.15
N	6	6	6
± SD	0.65	0.39	0.54
RSD%	0.65	0.39	0.54

Table 2. Determination of chlorphineramine maleate, dextromethorphan HBr and phenylephrin HCl in Codilar® Syrup

Chlorpheniramine Maleate		Dextromethorphan HBr		Phenylephrin HCl	
Taken µg/ml	Recovery %	Taken µg/ml	Recovery %	Taken µg/ml	Recovery %
10	98.8	10	101.2	5	100.2
20	99.5	20	100.8	10	99.1
30	98.8	30	100.1	15	101.5
40	100.7	40	99.8	20	99.1
50	100.1	50	100.6	25	99.7
X	100.1		100.5		100.2
± SD	0.11		0.21		0.23

Precision

Intra-day precisions were assessed injecting standard solution four to five times during a day (this solution was extracted via the same procedure as the capsules) of each analyte at two different concentrations (a low and a high concentration). The resultant standard deviations were less than 2% for all (Table

3). Inter-day precision experiments were done after treatment of the standard solution in the same method of capsules extraction, and then analyzed every day over 5 days (Table 3). All RSD% were lower than 2%.

Table 3. Reproducibility and precision

Injected amount (µg)	Intra-day (n=4-5)			Inter-day (n=5)			
	Observed amount (µg)±SD	RSD (%)	Accuracy (%)	Observed amount (µg)±SD	RSD (%)	Accuracy (%)	
Chlorpheniramine Maleate							
10	10.56±0.79	0.68	99.11	10.01	0.68	100.09	
50	49.63± 0.72	1.31	100.12	49.90	1.21	99.76	
Dextromethorphan HBr							
10	9.88±0.24	0.86	99.43	10.01±0.38	1.33	100.3	
50	49.24±.89	1.02	99.38	50.11±.0.86	0.76	100.01	
Phenylephrin HCl							
5	5.11±1.21	1.34	100.6	4.99±0.85	1.31	99.31	
45	45.52±.0.64	1.54	101.22	44.11±1.37	1.66	98.24	

Method validated

The method was validated with regard to specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy and robustness.

Peak areas of chlorphineramine Maleate, dextromethorphan HBr and phenylephrin HCl of calibration standards were proportional to the concentration in serum and dosage forms over the ranges tested 10-50, 10-50 and 5-25 µg/mL., respectively. Each concentration was tested in triplicate. The slope values for chlorphineramine Maleate, dextromethorphan HBr and phenylephrin HCl were calculated with intercept values. The standard deviations of slope were calculated and similarly standard deviations of intercept. The calibration curves were fitted by linear least-square regression and showed correlation coefficients not less than 0.9998.

The LODs and LOQs of thioctic acid, benfotiamine and cyanocobalamin were calculated on the peak area using the following equations: LOD = 3.3 x σ/S, LOQ = 10 x σ/S, where σ, is the standard deviation of the intercept of regression line of the drugs and S is the slope of the corresponding calibration curve.

Determination of authentic samples of chlorphineramine Maleate, dextromethorphan HBr and phenylephrin HCl and statistical analysis of the results obtained for the proposed method (Table 1); show that all the suggested measurements are precise and accurate for all the studied drugs (Table 1).

Application to pharmaceutical dosage form

The proposed method were successfully applied for the simultaneous determination of chlorphineramine Maleate, dextromethorphan HBr and phenylephrin HCl in Codilar syrup® without interference of the excipients present and without prior separation (Table 2). The utility of the method was also verified by applying the standard addition technique.

CONCLUSION

The chromatographic method described is adequate for quantitation of chlorphineramine maleate, dextromethorphan HBr and phenylephrin HCl in pharmaceutical dosage forms at different concentration levels. It is very simple, accurate and effective and provided no interference peaks for endogenous components and pharmaceutical excipients. Acceptable values of precision and accuracy have been obtained all levels by this method regarding the guidelines for assay validation. The separation of these drugs takes 13.68 min in one chromatogram, so a large number of samples can be analyzed in a short period of time. The method uses simple mobile phase and is very beneficial for column life. In summary, the method can be successfully applied to samples of pharmaceutical dosage form.

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