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Reverse Phase High Performance Liquid Chromatographic Method for Simultaneous Determination of Paracetamol, Cetirizine and Dextromethorphan in Pure and Synthetic Mixture.

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ABSTRACT

A simple, precise and sensitive method was developed for simultaneous estimation of paracetamol (PAR), cetirizine (CET) and dextromethorphan (DTM) using reverse phase high performance liquid chromatography. A good separation was achieved using a RP C-18 chromatographic column (Phenomenex, 150 mm length × 4.6 mm i.d) and a mobile phase consisting of methanol and phosphate buffer pH 5.5 in the ratio 60:40 v/v at a flow rate of 1 ml/min and the detection wavelength at 210 nm. The retention time of PAR, CET and DTM was found to be at 2.22, 7.83 and 4.50 min respectively. The linearity of the proposed method was found to lie in the range of 100 – 500 µg/ml, 3 – 15 µg/ml and 10 -50 µg/ml for PAR, CET and DTM respectively. The limit of detection (LOD) was 0.0022, 0.1022 and 0.0106 for PAR, CET and DTM respectively. The Limit of Quantification (LOQ) was 0.0068, 0.3098 and 0.0322 for PAR, CET and DTM respectively. The relative standard deviation of six replicate analysis was found to be less than 2%. This method could be used successfully for simultaneous quantitative analysis of PAR, CET and DTM from pharmaceutical formulations.

Keywords: Paracetamol, Cetirizine, Dextromethorphan, RPHPLC

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INTRODUCTION

Paracetamol (PAR) is N-(4-hydroxy phenyl) acetamide. It is a popular analgesic and antipyretic drug used for the relief of fever, head ache and other pains. Cetirizine hydrochloride (CET), chemically [2-[4-[(4- chlorophenyl) phenylmethyl]-1-piperazinyl] ethoxy] acetic acid], belongs to the group of second generations antagonists of H1-receptors, inhibits the allergic reaction mediated by histamine. It is a non-sedative antihistamine, used in the treatment of seasonal rhinitis, hay fever, running nose, control sneezing of allergic origin. Both PAR and CET are official in IP [1] and BP [2]. Dextromethorphan hydrobromide (DTM) is antitussive (cough suppressant) drug used for the pain relief and in psychological conditions. It acts on cough centre to elevate the threshold for coughing. Chemically, it is morphinan, 3-methoxy-17-meth (9, 13, 14)-, hydrobromide [2].

A literature survey revealed that PAR has been analyzed separately and in combination with other drugs by HPLC [3,4], HPTLC [5] and UV spectrophotometry [6-9]. Literature reveals that many analytical methods are specified for the determination of CTZ as individual and combined dosage form with other combination of drugs and also in biofluid viz., UV-visible spectophotometry [10-12], HPLC [13-18] and HPTLC [19-21] methods. Spectrophotometry [22], RP-HPLC [23, 24] and capillary electrophoresis [25], methods have been reported for the estimation of dextromethorphan hydrobromide in pharmaceutical formulations.

Experimental

Materials and Reagents

Pure drug of PAR, CET and DTM were obtained as gift sample from Madras Pharmaceuticals Ltd, Chennai. Methanol HPLC grade, Potassium dihydrogen phosphate AR grade, disodium hydrogen phosphate AR grade, Lactose, starch, magnesium state and talc were obtained from Rankem Laboratories (A division of Ranbaxy). HPLC grade water was prepared in the laboratory using Milli Pore – Milli Q system.

HPLC Instrumentation and Conditions

The HPLC system consisted of a dual pump Shimadzu – LC – 10AT VP and a LC-20 AD UV detector. The chromatographic separation was achieved on a Phenomenex 5 µm, 150 mm × 4.6 mm i.d column using a mobile phase consisting of methanol and phosphate buffer pH 5.5 (60:40 v/v) with isocratic elution. The mobile phase was filtered through 0.45 µ membrane filter. The eluent was monitored using UV detector at a wavelength of 210 nm. The column was maintained at room temperature and the injection volume of 20 µl was used.

Preparation of Stock and Standard Solutions

Stock solution of PAR, CET and DTM (equivalent to 1000 µg/ml) were prepared separately in methanol. Aliquots of standard stock solutions of PAR, CET and DTM were

transferred to 10 ml volumetric flasks and the volume was made up to the mark with methanol to yield the concentrations of 100 – 500 µg/ml, 3 – 15 µg/ml and 10 -50 µg/ml of PAR, CET and DTM respectively.

Preparation of sample solution for assay

The synthetic mixture was prepared by mixing 250 mg of Paracetamol, 5 mg of Cetirizine, 20 mg of Dextromethorphan, 130 mg of lactose, 40 mg of talc, 50 mg of starch and 5 mg of magnesium 185tate by geometric dilution. An amount equivalent to 250 mg of drug was weighed from the mixture and transferred to a 100 ml standard flask and diluted with 30 ml of HPLC grade water. This solution was sonicated for 15 mins and the final volume was made up to the mark with HPLC grade water. The solution was filtered through 0.45µ membrane filter and used as sample stock solution. From the stock solution aliquot dilutions were made with mobile phase and used for further analysis.

RESULT AND DISCUSSION

HPLC method development and Optimization

Phenomenex RP 5 µm, 150 mm × 4.6 mm id, column maintained at ambient temperature was used for the separation and the method was validated for the determination of PAR, CET and DTM in synthetic mixture. The composition, pH and flow rate of the mobile phase were changed to optimize the separation conditions using main substances of the three compounds of interest. A mobile phase consisting of methanol and phosphate buffer (pH 5.5) in the ratio 60:40 v/v with isocratic elution was selected for quantification after several preliminary investigatory chromatographic runs. Under the optimized experimental conditions all peaks were well defined and free from tailing. The elution orders were PAR (2.22 min), DTM (4.50 min) and CET (7.83 min) at a flow rate of 1 ml/min. The optimum wavelength for detection was 210 nm at which better response for the three drugs were obtained.

Validation of the method

Linearity

Linearity was established by least square linear regression analysis of the calibration curve. The constructed calibration curves were linear over the concentration range of 100 – 500 µg/ml, 3 – 15 µg/ml and 10 -50 µg/ml for PAR, CET and DTM respectively. Peak areas of PAR, CET and DTM were plotted versus their respective concentrations and linear regression analysis was performed on the resultant curves. The regression equations were y = 22.192x +14.843 (r = 0.999), y = 7.8576x + 0.491 (r = 0.9996), y = 4.7457x + 20.683 (r = 0.9989) for PAR, CET and DTM respectively.

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LOD and LOQ

LOD and LOQ were performed on samples containing analytes based on calibration curve method. Standard solution of PAR, CET and DTM were injected in six replicates. Average peak area of analytes was plotted against concentration. LOD and LOQ were calculated using following equations

$$LOD = (3.3 \times \sigma)/S \quad LOQ = (10.0 \times \sigma)/S$$

Where σ = the standard deviation of y-intercepts of regression lines of the calibration curve,
S = the slope of the calibration curve.

The LOD and LOQ were found to be 0.0022µg/ml, 0.1022µg/ml, 0.0106µg/ml and 0.0068µg/ml, 0.3098µg/ml, 0.0322µg/ml for PAR, CET and DTM respectively (Table 1).

Table 1: Statistical Data of standard curve of PAR, CET and DTM

Parameters	PAR	CET	DTM
Linearity range (µg/ml)	100 – 500	3.0 – 15	10 – 50
Slope	22.192	7.857	4.745
Intercept	14.843	0.490	20.682
Linear Equation	Y = 22.192x +14.843	Y = 7.857 + 0.490	Y = 4.745 + 20.682
LOD (µg/ml)	0.0022	0.1022	0.0106
LOQ (µg/ml)	0.0068	0.3098	0.0322
R^2 Value	0.9999	0.9996	0.9989

LOD – Limit of Detection; LOQ – Limit of Quantification

Precision

The intra and inter day variability were assessed by using standard solutions prepared at three different concentration levels. Intra-day precision was investigated by injecting three replicate samples of each of the samples of three different concentrations. Inter-day precision was assessed by injecting the same three samples over three consecutive days. Repeatability was investigated by injecting six replicate samples of each of the samples of five different concentrations. The % RSD values were found to be less than 2% (Table 2) and the method was found to be precise.

Table 2: Summary of validation parameter

Parameters	PAR	CET	DTM
Recovery (%)	99.4 – 101.9	99.8 – 101.8	98.1 – 101.5
Repeatability (RSD, n = 6)	0.54 – 1.23	0.63 – 1.33	0.67 – 0.98
Intra-day precision (n = 3)	0.653	0.546	0.632
Inter-day precision (n = 3)	1.232	1.342	1.332
Specificity	Specific	Specific	Specific

RSD – Relative Standard Deviation

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Recovery

A known amount of standard drug at various levels were added to synthetic mixture, which was then mixed, extracted and subsequently diluted to yield a starting concentration of 125µg/ml, 2.5µg/ml and 10µg/ml for PAR, CET and DTM respectively. The observed percentage recovery of PAR, CET and DTM were ranging from 99.42-101.91%, 99.80-101.80% and 98.81-101.51% respectively.

Assay

The validated method was applied to the determination of PAR, CET and DTM in laboratory prepared synthetic mixture. The estimated amount was found to be close to 100% proving the accuracy of the method (Table 3).

Table 3: Assay Results of Synthetic Mixture

Formulation	Amount Present (mg)			Amount Found* (mg)		
	PAR	CET	DTM	PAR	CET	DTM
Synthetic Mixture	125	2.5	10	125.05 ± 0.54	2.49 ± 0.62	9.96 ± 0.55

* - Mean of three estimations; Mean ± SD; SD – Standard Deviation

Robustness

Robustness of the method was performed by intentionally modifying the chromatographic conditions such as composition and flow rate of the mobile phase and the detection wavelength. The chromatographic parameters of each analyte such as retention time, tailing factor, and number of theoretical plates were measured at each changed conditions (Table 4).

Table 4: Robustness Study of selected drugs

Factor	Level	Retention time (min)			Tailing Factor			Theoretical Plates		
		PAR	CET	DTM	PAR	CET	DTM	PAR	CET	DTM
Flow rate (ml/min)										
1.2	+1	1.83	6.90	3.49	1.3	1.21	1.11	35672	4668	2879
1.0	1	2.22	7.83	4.50	1.1	1.1	1.01	35189	4338	2981
0.8	-1	2.62	10.83	5.99	1.3	1.31	1.32	35762	4588	2787
Detection Wavelength (nm)										
215	+5	2.21	7.82	4.50	1.1	1.1	1.1	33245	4237	2764
210	0	2.22	7.83	4.50	1.1	1.1	1.0	35189	4338	2981
205	-5	2.22	7.83	4.50	1.1	1.1	1.1	31009	4119	2233
Mobile Phase Ratio (ml) – Methanol : Phosphate buffer pH 5.5										
62:38	+2	2.21	7.81	4.48	1.1	1.1	1.1	34652	4334	2932
60:40	0	2.22	7.83	4.50	1.1	1.1	1.0	35189	4338	2981
58:42	-2	2.21	7.84	4.48	1.1	1.1	1.1	34883	4324	2876

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Paracetamol Dextromethorphan etrizine

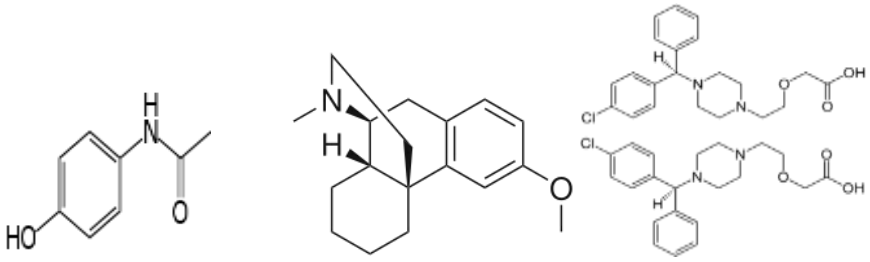

Figure 1: Structure of selected drugs

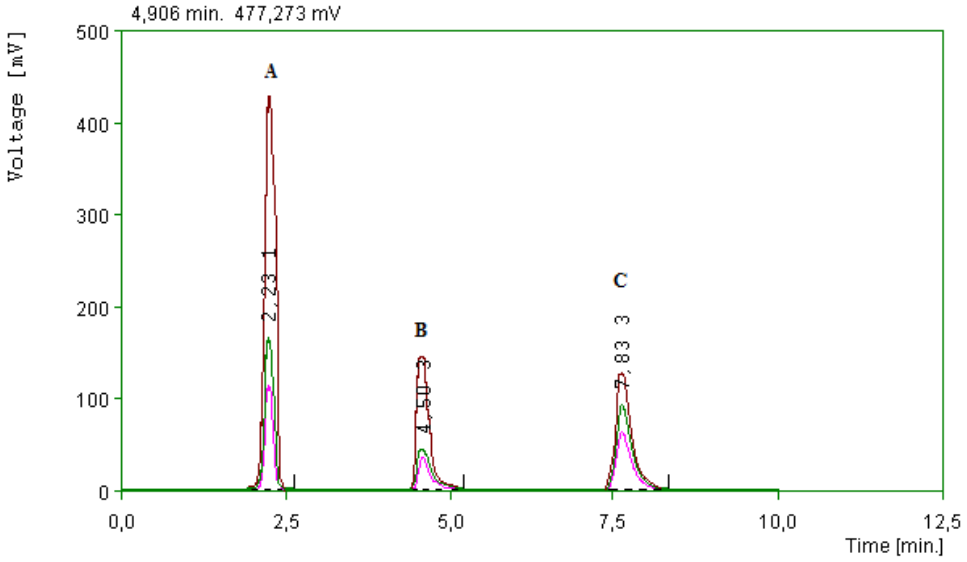

Figure 2: HPLC Chromatogram showing retention time of PAR, CET and DTM

CONCLUSION

A simple and accurate reverse phase HPLC method has been developed for the simultaneous determination of paracetamol, cetirizine and dextromethorphan. The method was validated by testing its linearity, accuracy, precision, limits of detection and quantitation, selectivity and robustness. The run time of less than ten minutes allows its application for the routine determination of paracetamol, cetirizine and dextromethorphan. Further, the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.

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