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Simultaneous Quantitation and Validation of Paracetamol, Phenylpropanolamine Hydrochloride and Cetirizine Hydrochloride by RP-HPLC in Bulk Drug and Formulation

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ABSTRACT

A HPLC method has been described for simultaneous determination of Paracetamol, Phenylpropanolamine hydrochloride and Cetirizine hydrochloride in formulation. This method is based on HPLC separation of the three drugs on the Thermo Hypersil Gold C_{18} column (250 mm × 4.6 mm, 5.0µ), with isocratic conditions and mobile phase containing methanol: 0.01M disodium hydrogen phosphate dihydrate buffer [pH 7, adjusted with Ortho Phosphoric Acid (OPA)] (60: 40) at a flow rate of 1 ml/min, using UV detection at 217 nm. This method has been applied to formulation without any interference of excipients of formulation. The linear regression analysis data for the calibration plots showed a good linear relationship over the concentration range of 0.4-1.4µg/ml for Paracetamol, 7-12µg/ml for Phenylpropanolamine hydrochloride and 5-10µg/ml for Cetirizine hydrochloride respectively. The mean values of the correlation coefficient, slope and intercept were 0.9993, 51489 and 5844.4 for Paracetamol, 0.9991, 23235 and 70540 for Phenylpropanolamine hydrochloride and 0.9990, 40416 and 93404 for Cetirizine hydrochloride respectively. The method was validated as per the ICH guidelines. The limit of detection (LOD) and limit of quantitation (LOQ) was 0.2µg/ml and 0.4µg/ml for Paracetamol, 5µg/ml and 7µg/ml for Phenylpropanolamine hydrochloride and 4µg/ml and 5µg/ml for Cetirizine hydrochloride, respectively. Statistical analysis showed that the method is repeatable and selective for the estimation of Paracetamol, Phenylpropanolamine hydrochloride and Cetirizine hydrochloride.

Keywords: Paracetamol, Phenylpropanolamine hydrochloride, Cetirizine hydrochloride, HPLC, Validation.

INTRODUCTION

Paracetamol (Fig. 1) is chemically N-(4-hydroxyphenyl) acetamide. It is a widely used over-the-counter analgesic (pain reliever) and antipyretic (fever reducer). It is commonly used for the relief of headaches, other minor aches and pains, and is a major ingredient in numerous cold and flu remedies. The onset of analgesia is approximately 11 minutes after oral administration of paracetamol. It is the active metabolite of phenacetin, once popular as an analgesic and antipyretic in its own right, but unlike phenacetin and its combinations, paracetamol is not considered to be carcinogenic at therapeutic doses. Paracetamol is considered to be the inhibitor of cyclooxygenase (COX), and recent findings suggest that it is highly selective for COX-2. While it has analgesic and antipyretic properties comparable to those of aspirin or other NSAIDs, its peripheral anti-inflammatory activity is usually limited by several factors, one of which is high level of peroxides present in inflammatory lesions. [1]

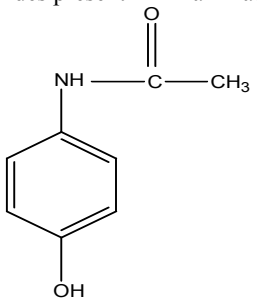

Fig. 1: Structure of Paracetamol

Phenylpropanolamine hydrochloride (Fig. 2) is chemically (1S, 2R)-2-amino-1-phenylpropan-1-ol. It is a psychoactive drug of the phenethylamine and amphetamine chemical classes which is used as a stimulant, decongestant, and anorectic agent. It is commonly used in prescription and over-the-counter cough and cold preparations. Phenylpropanolamine acts as a potent and selective releasing

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agent of norepinephrine and epinephrine, or as a norepinephrine releasing agent (NRA). It also acts as a dopamine releasing agent (DRA) to a lesser extent. It works by mimicking the effects of endogenous catecholamines such as epinephrine and norepinephrine and to a lesser degree dopamine. [2]

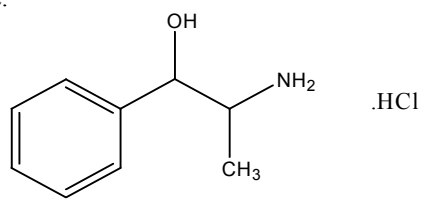

Fig. 2: Structure of Phenylpropanolamine hydrochloride

Cetirizine hydrochloride (Fig. 3) is chemically 2-(2-{4-[(4-Chlorophenyl) (phenyl)-methyl] piperazino} ethoxy) acetic acid hydrochloride. It is a second-generation antihistamine, is a major metabolite of hydroxyzine, and a racemic selective H_1 receptor inverse agonist used in the treatment of allergies, hay fever, angioedema, and urticaria. Cetirizine crosses the blood-brain barrier only slightly, reducing the sedative side-effect common with older antihistamines. It has also been shown to inhibit eosinophil chemotaxis and LTB4 release. At a dosage of 20 mg it was found that it inhibited the expression of VCAM-1 in patients with atopic dermatitis. The levorotary enantiomer of Cetirizine, known as levocetirizine, is the more active form. [1]

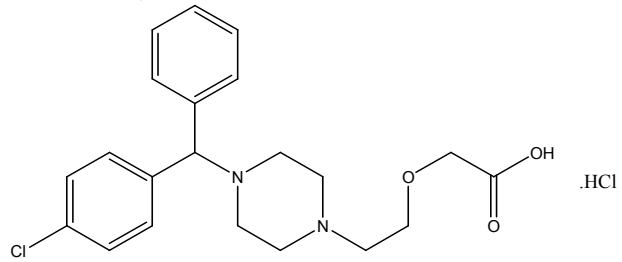

Fig. 3: Structure of Cetirizine hydrochloride

Literature review reveals that methods have been reported for analysis of Paracetamol, Phenylpropanolamine hydrochloride and Cetirizine hydrochloride either alone or in combination with other drugs. HPLC method, [3-9] stability indicating HPLC method, [10-11] HPLC assay [12-13] and some bioanalytical work by capillary electrophoresis [14] and HPLC [15] in combination with other drugs are reported for Paracetamol. Spectrophotometric and HPLC method for Ambroxol hydrochloride and Cetirizine hydrochloride [16-17] and stability indicating method for Cetirizine hydrochloride by HPLC [18] have been reported. Similarly methods have been reported for stability indicating HPLC method for Phenylpropanolamine hydrochloride, [19] spectrophotometric method [20] and HPLC method for estimation of Phenylpropanolamine hydrochloride alone [21] and in combination with other drugs. [22-25] Capillary electrophoretic method has also been reported for simultaneous determination of Cetirizine dihydrochloride, Paracetamol and Phenylpropanolamine in tablets. [26]

To date, there have been no published reports about the simultaneous quantitation of Paracetamol, Phenylpropanolamine hydrochloride, and Cetirizine hydrochloride by chromatographic method in bulk drug and in tablet dosage form. This present study reports for the first time simultaneous quantitation of the same drugs by RP-HPLC in bulk drug and in tablet dosage form. The proposed method is validated as per ICH guidelines. [27]

MATERIALS AND METHODS

Working standards of pharmaceutical grade Paracetamol (Batch no. 260738), Phenylpropanolamine hydrochloride (Batch no. 16043/01) and Cetirizine hydrochloride (Batch no. 102/4321) were obtained as generous gifts from AGIO Pharma Limited, MIDC, (Pune, Maharashtra, India). They were used without further purification and certified to contain 99.21%, 99.62% and 99.79% on dry weight basis for Paracetamol, Phenylpropanolamine hydrochloride and Cetirizine hydrochloride, respectively. Fixed dose combination tablet CHESTON COLD (CIPLA Limited) Batch no. DUO423 (Exp date Dec 2012), containing 500 mg Paracetamol, 25 mg Phenylpropanolamine hydrochloride and 5 mg Cetirizine hydrochloride was purchased from local market, Pune, Maharashtra, India. All the chemicals were of HPLC grade, purchased from Merck Chemicals, India. Water used was double distilled and filtered through 0.45µm filter.

Instrumentation

The HPLC system consisted of Intelligent HPLC pump model (Jasco PU 2080 Plus) with sampler programmed at 20µl capacity per injection. The detector consisted of a UV/ VIS (Jasco UV 2075 Plus). Data was integrated using Jasco Borwin version 1.5, LC-Net II/ADC system. The column used was, Thermo Hypersil Gold C_{18} column (250 mm × 4.6 mm, 5.0µ), with isocratic conditions. Mobile phase consisted of a mixture of methanol: 0.01M disodium hydrogen phosphate dihydrate buffer (pH 7 adjusted with OPA) (60: 40) at a flow rate of 1 ml/min using UV detection at 217 nm. The mobile phase was filtered through a 0.45 micron membrane filter and degassed. The injection volume was 20µl and analysis was performed at ambient temperature.

Preparation of Standard Stock Solutions

Standard stock solutions of concentration 1000µg/ml of Paracetamol, 1000µg/ml of Phenylpropanolamine hydrochloride and 1000µg/ml of Cetirizine hydrochloride were prepared separately using water. The stock solution was stored at 2-8°C. From the standard stock solution, the working standard solutions were prepared using mobile phase to get 10µg/ml of each drug. The stock solutions were stored at 2-8°C.

Optimization of HPLC Method

All drugs were subjected to chromatographic analysis using mobile phases of differing pH, flow rate using the under mentioned chromatographic conditions. The changes in the retention time of all drugs were noted as a function of changing mobile phase, pH, flow rate, strength and selectivity. Initially methanol: water in the ratio of 70: 30 was tried but splitting of paracetamol peak was observed. Then acetonitrile: water in the ratio of (70: 30) was tried but all the three peaks of drug got merged into each other. Later methanol: 0.01 M disodium hydrogen phosphate dihydrate buffer (pH 7 adjusted with OPA) in various ratios were tried. It was found that methanol: 0.01 M disodium hydrogen phosphate dihydrate buffer (pH 7 adjusted with OPA) in the ratio of (60: 40) at flow rate of 1 ml/min gave acceptable retention time of 3.10, 4.00 and 13.39 with number of plates (N) 5471, 5031 and 6665 and good resolution (2.71 between Paracetamol and Phenylpropanolamine hydrochloride, 19.77 between Phenylpropanolamine hydrochloride and Cetirizine Hydrochloride) for Paracetamol, Phenylpropanolamine

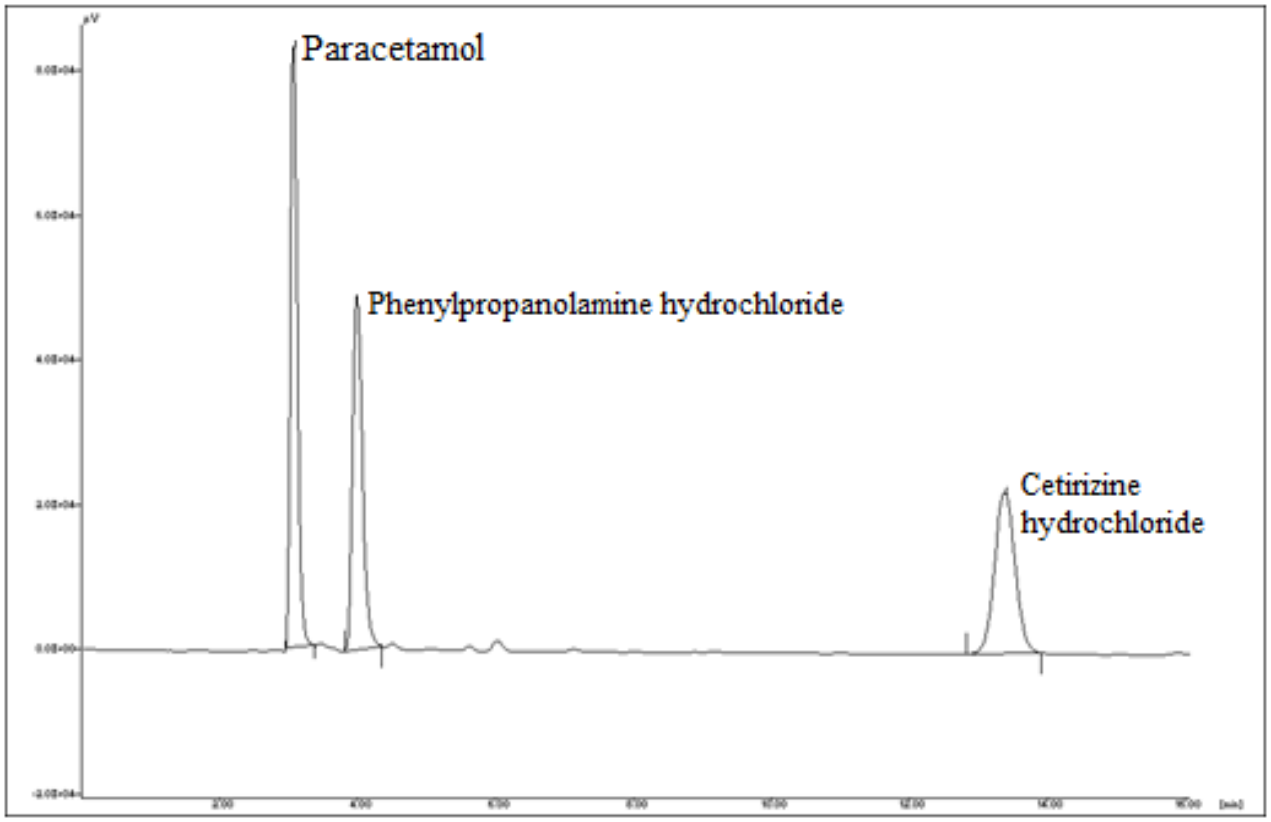

Fig. 4: Chromatogram of Paracetamol R_t (3.108), Phenylpropanolamine hydrochloride R_t (4.008) and Cetirizine hydrochloride R_t (13.392)

hydrochloride and Cetirizine hydrochloride, respectively (Fig. 4).

Validation of the method

Validation of the optimized HPLC method was carried out with respect to the following parameters.

Linearity and range

Linearity of the method was studied by injecting six concentrations of the drug prepared in the mobile phase in the range of 0.4-1.4µg/ml for Paracetamol, 7–12µg/ml for Phenylpropanolamine hydrochloride and 5–10µg/ml for Cetirizine hydrochloride; in triplicate into the HPLC system keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

Precision

The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations 0.4, 0.8, 1.2µg/ml for Paracetamol, 7, 9, 11µg/ml for Phenylpropanolamine hydrochloride and 5, 7, 9µg/ml for Cetirizine hydrochloride six times on the same day. The intermediate precision of the method was checked by repeating studies on three different days.

Limit of detection and limit of quantitation

Limits of detection (LOD) and quantification (LOQ) represent the concentration of the analyte that would yield signal-to-noise ratios of 3 for LOD and 10 for LOQ, respectively. To determine the LOD and LOQ, Serial dilutions of mixed standard solution of these three drugs were made from the standard stock solution in the range of 0.1–1µg/ml for Paracetamol, 1–10µg/ml for Phenylpropanolamine hydrochloride and Cetirizine hydrochloride.

Robustness of the method

To evaluate robustness of the HPLC method, few parameters were deliberately varied. The parameters included variation of flow rate, percentage of methanol in the mobile phase and solvents from different lot. Robustness of the method was checked at three different concentration levels 0.4, 0.8, 1.2µg/ml, 7, 9, 11µg/ml and 5, 7, 9µg/ml for Paracetamol, Phenylpropanolamine hydrochloride and Cetirizine hydrochloride, respectively.

Specificity

The specificity of method was accessed from the chromatogram where complete separation of Paracetamol, Phenylpropanolamine hydrochloride and Cetirizine hydrochloride was achieved. The peaks obtained were sharp and well separated at the baseline. Also excipients from formulation were not interfering with assay.

Accuracy

Accuracy of the method was carried out by applying the method to preanalyzed drug sample (Paracetamol, Phenylpropanolamine hydrochloride and Cetirizine hydrochloride combination tablet) to which known amount of Paracetamol, Phenylpropanolamine hydrochloride and Cetirizine hydrochloride standard powder corresponding to 80, 100 and 120 % of label claim had been added (Standard addition method), mixed and the powder was extracted and analyzed by running chromatogram in optimized mobile phase.

Analysis of a marketed formulation

To determine the content of Paracetamol, Phenylpropanolamine hydrochloride and Cetirizine hydrochloride in conventional tablet (Brand name: Cheston Cold, Label claim: 500 mg Paracetamol, 25 mg Phenylpropanolamine hydrochloride and 5 mg Cetirizine

hydrochloride per tablet), twenty tablets were weighed, their mean weight determined and finely powdered. The weight of the tablet triturate equivalent to 500 mg of Paracetamol, 25 mg of Phenylpropanolamine hydrochloride and 5 mg of Cetirizine hydrochloride was transferred into a 50 ml volumetric flask containing 30 ml water sonicated for 30 min and diluted up to 50 ml with water. The resulting solution was centrifuged at 3000 rpm for 5 min and the drug content of the supernatant was determined (10000, 500 and 100µg/ml for Paracetamol, Phenylpropanolamine hydrochloride and Cetirizine hydrochloride, respectively). Then 0.1 ml of the above solution was diluted to produce 100µg/ml concentrations for Paracetamol and from this solution 1 ml was taken and further diluted to 10 ml with water to make final concentration of 10µg/ml for Paracetamol. For Phenylpropanolamine hydrochloride 0.2 ml of from the supernatant was diluted to produce final concentration of 10µg/ml and for Cetirizine hydrochloride, 1 ml from the supernatant was diluted to produce final concentration of 10µg/ml. The dilutions were done individually due to the large differences in LOD and LOQ values as well as label claim. After the dilutions the sample solution was filtered using 0.45-micron filter (Millipore, Milford, MA). A 20µl volume of sample solution was injected into HPLC, six times, under the conditions described above. The peak areas were measured at 217 nm and concentrations in the samples were determined using multilevel calibration developed on the same HPLC system under the same conditions using linear regression equation.

RESULTS AND DISCUSSION

The results of validation studies on simultaneous estimation method developed for Paracetamol, Phenylpropanolamine hydrochloride and Cetirizine hydrochloride in the current study involving mobile phase methanol: 0.01 M disodium hydrogen phosphate dihydrate buffer (pH 7 adjusted with OPA) (60: 40) are given below-

Linearity

Paracetamol, Phenylpropanolamine hydrochloride and Cetirizine hydrochloride showed good correlation coefficient in concentration range of 0.4-1.4µg/ml (r^2 = 0.9993), 7-12µg/ml (r^2 = 0.9991) and 5-10µg/ml (r^2 = 0.9990) respectively. The mean values of the slope and intercept were 51489, 5844.4 for Paracetamol, 23235, 70540 for Phenylpropanolamine hydrochloride and 40416, 93404 for Cetirizine hydrochloride respectively. The detector response over wide range of concentrations of analyte were plotted to obtain the calibration curve.

Precision

The results of the repeatability and intermediate precision experiments are shown in Table 1. The developed method was found to be precise as the RSD values for repeatability and intermediate precision studies were < 2 %, respectively as recommended by ICH guidelines.

LOD and LOQ

Signal-to-noise ratios of 3:1 and 10:1 were obtained for the LOD and LOQ respectively. The LOD and LOQ were found to be 0.2µg/ml and 0.4µg/ml for Paracetamol, 5µg/ml and 7µg/ml for Phenylpropanolamine hydrochloride and 4µg/ml and 5µg/ml for Cetirizine hydrochloride, respectively.

Robustness of the method

Each factor selected (except columns from different manufacturers) was changed at three levels (−0.1, 0 and 0.1). One factor at a time was changed to estimate the effect. Thus, replicate injections (n = 6) of mixed standard solution at three concentration levels were performed under small changes of three chromatographic parameters (factors). Insignificant differences in peak areas and less variability in retention time were observed. The standard deviation of peak areas was calculated for each parameter and % RSD was found to be less than 2%. The data for robustness study is given in (Table 2.1, 2.2 and 2.3).

Table 1: Precision studies

Concentration (µg/ml)	Measured concentration ± SD, RSD (%)	
	Repeatability (n= 6)	Intermediate precision (n= 6)
	Paracetamol	
0.4	0.40 ± 0.004, 1	0.40 ± 0.001, 0.25
0.8	0.81 ± 0.1, 1.23	0.79 ± 0.012, 1.52
1.2	1.21 ± 0.006, 0.497	1.21 ± 0.016
	Phenylpropanolamine hydrochloride	
8	7.99 ± 0.15, 1.87	8.01 ± 0.13, 1.62
10	9.99 ± 0.13, 1.3	10.14 ± 0.17, 1.68
12	12.07 ± 0.21, 1.74	12.01 ± 0.18, 1.49
	Cetirizine hydrochloride	
5	5.04 ± 0.08, 1.58	4.99 ± 0.02, 0.40
7	7.01 ± 0.06, 0.85	6.99 ± 0.05, 0.73
9	8.96 ± 0.12, 1.34	9.06 ± 0.04, 0.43

Table 2.1: Robustness testing for Paracetamol (n = 6)

Factor[a]	Level	Retention time	Retention factor	Asymmetry
		A: Flow rate (mL/min)		
0.9	-1	3.112	0.2448	1.12
1.0	0	3.108	0.2432	1.10
1.1	+1	3.104	0.2416	1.09
Mean ± SD (n = 3)		3.108 ± 0.004	0.2432 ± 0.0016	1.10 ± 0.01
		B: % of methanol in the mobile phase (v/v)		
59	-1	3.110	0.244	1.11
60	0	3.108	0.2432	1.10
61	+1	3.005	0.202	1.10
Mean ± SD (n = 3)		3.074 ± 0.06	0.229 ± 0.024	1.10 ± 0.01
		C: Solvents of different lots		
First lot		3.108	0.2432	1.10
Second lot		3.111	0.2444	1.13
Mean ± SD (n = 3)		3.1095 ± 0.002	0.2438 ± 0.0008	1.11 ± 0.01

[a] Three factors were slightly changed at three levels (-0.1, 0, 0.1)

Table 2.2: Robustness testing for Phenylpropanolamine hydrochloride (n = 6)

Factor[a]	Level	Retention time	Retention factor	Asymmetry
		A: Flow rate (mL/min)		
0.9	-1	4.012	0.605	1.13
1.0	0	4.008	0.603	1.11
1.1	+1	4.005	0.602	1.07
Mean ± SD (n = 3)		4.008 ± 0.063	0.603± 0.043	1.10 ± 0.05
		B: % of methanol in the mobile phase (v/v)		
59	-1	4.013	0.605	1.14
60	0	4.008	0.603	1.11
61	+1	4.000	0.600	1.09
Mean ± SD (n = 3)		4.007 ± 0.078	0.603 ± 0.049	1.11 ± 0.05
		C: Solvents of different lots		
First lot		4.008	0.6032	1.11
Second lot		4.014	0.6056	1.10
Mean ± SD (n = 3)		4.011 ± 0.003	0.6044 ± 0.001	1.10 ± 0.01

[a] Three factors were slightly changed at three levels (-0.1, 0, 0.1)

Specificity studies

The peak purity of Paracetamol, Phenylpropanolamine hydrochloride and Cetirizine hydrochloride was assessed by comparing their respective spectra at the peak start, apex and

end positions i.e., r (S, M) = 0.9995 and r (M, E) = 0.9992. A good correlation (r = 0.9997) was also obtained between the standard and sample spectra of Paracetamol, Phenylpropanolamine hydrochloride and Cetirizine hydrochloride respectively. Also, excipients from formulation were not interfering with the assay.

Recovery

As shown from the data in Table 3 good recoveries of the Paracetamol, Phenylpropanolamine hydrochloride and Cetirizine hydrochloride in the range from 98.9 to 101.45 % were obtained at various added concentrations.

Table 2.3: Robustness testing for Cetirizine hydrochloride (n = 6)

Factor[a]	Level	Retention time	Retention factor	Asymmetry
A: Flow rate (mL/min)				
0.9	-1	13.48	4.39	1.03
1.0	0	13.39	4.36	1.15
1.1	+1	13.30	4.32	1.11
Mean ± SD (n = 3)		13.39 ± 0.09	4.36 ± 0.035	1.10 ± 0.06
B: % of methanol in the mobile phase (v/v)				
59	-1	13.45	4.38	1.19
60	0	13.39	4.36	1.17
61	+1	13.32	4.33	1.02
Mean ± SD (n = 3)		13.39 ± 0.065	4.36 ± 0.025	1.13 ± 0.09
C: Solvents of different lots				
First lot		13.39	4.36	1.06
Second lot		13.44	4.346	1.13
Mean ± SD (n = 3)		13.415 ± 0.035	4.368 ± 0.011	1.09 ± 0.05

[a] Three factors were slightly changed at three levels (-0.1, 0, 0.1)

Table 3: Recovery studies (n = 6)

Label claim (mg/tablet)	Amount added (mg)	Total amount (mg)	Amount Recovered (mg) ± % RSD	% Recovery
Paracetamol				
500	400 (80%)	900	907.7 ± 1.41	100.85
500	500 (100%)	1000	995.0 ± 0.89	99.50
500	600 (120%)	1100	1093.0 ± 0.90	99.36
Phenylpropanolamine hydrochloride				
25	20 (80%)	45	44.85 ± 1.32	99.67
25	25 (100%)	50	49.45 ± 1.16	98.90
25	30 (120%)	55	55.8 ± 1.40	101.45
Cetirizine hydrochloride				
5	4 (80%)	9	9.09 ± 1.52	100.94
5	5 (100%)	10	9.97 ± 0.64	99.70
5	6 (120%)	11	11.01 ± 1.56	100.07

Table 4: Analysis of commercial formulation

Drug	Lot	Drug found (mg per tablet)	
		Mean ± SD (n= 6)	Recovery (%)
Paracetamol (500 mg)	1st Lot	504.64 ± 2.31	100.93
	2nd Lot	499.5 ± 2.69	99.9
Phenylpropanolamine hydrochloride (25 mg)	1st Lot	25.07 ± 0.203	100.28
	2nd Lot	24.75 ± 0.151	99.00
Cetirizine hydrochloride (5 mg)	1st Lot	4.99 ± 0.049	99.99
	2nd Lot	4.98 ± 0.043	99.74

Cheston Cold (Paracetamol 500 mg, Phenylpropanolamine hydrochloride 25 mg and Cetirizine hydrochloride 5 mg) Batch no. DUO 423.

Analysis of a formulation

Experimental results of the amount of Paracetamol, Phenylpropanolamine hydrochloride and Cetirizine hydrochloride in tablets, expressed as a percentage of label claims were in good agreement with the label claims thereby suggesting that there is no interference from any of the excipients which are normally present. The dilutions were done individually due to the large differences in LOD and LOQ values as well as label claim. The drug content was found to be 100.41 % for Paracetamol, 99.64 % for Phenylpropanolamine hydrochloride and 99.86 % for Cetirizine hydrochloride. Two different lots of Paracetamol, Phenylpropanolamine hydrochloride and Cetirizine hydrochloride combination tablets were analyzed using the proposed procedures as shown in Table 4.

HPLC method was developed and validated as per ICH guidelines. UV detection allowed an accurate quantitation of chromophoric compounds.

The drug was analyzed by HPLC method using Thermo Hypersil Gold C_{18} column (250 mm × 4.6 mm, 5.0µ), with isocratic conditions and mobile phase containing methanol: 0.01 M disodium hydrogen phosphate dihydrate buffer (pH 7 adjusted with OPA) (60: 40) at a flow rate of 1 mL/min using UV detection at 217 nm. The procedure has been evaluated for the linearity, accuracy, precision and robustness in order to ascertain the suitability of the analytical method. The method was also applied to marketed samples. It has been proved that the method is selective and linear between concentration range 0.4-1.4µg/ml for Paracetamol, 7-12µg/ml for Phenylpropanolamine hydrochloride and 5-10µg/ml for Cetirizine hydrochloride. LOD and LOQ was found to be 0.2µg/ml and 0.4µg/ml for Paracetamol, 5µg/ml and 7µg/ml for Phenylpropanolamine hydrochloride and 4µg/ml and 5µg/ml for Cetirizine hydrochloride, respectively.

Statistical analysis proves that the method is suitable for the analysis of Paracetamol, Phenylpropanolamine hydrochloride and Cetirizine hydrochloride as bulk drug and in pharmaceutical formulation without any interference from the excipients. It may be extended to study the degradation kinetics of Paracetamol, Phenylpropanolamine hydrochloride and Cetirizine hydrochloride and also for its estimation in plasma and other biological fluids.

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