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Simultaneous Estimation of Cetrizine Hydrochloride and Phenylpropanolamine Hydrochloride in Tablet Dosage Form using Reverse Phase HPLC

Gautam R. Chauhan[1*] • Kamlesh K. Tiwari[1] • Jaime A. Teixeira da Silva[2] • Pinak Patel[3] • Falgun Mehta[4]

[1] Sophisticated Instrumentation Centre for Applied Research and Testing (SICART), Motabazar, Vallabh Vidyanagar-388 120, Anand, Gujarat, India
[2] Department of Horticultural Science, Faculty of Agriculture and Graduate School of Agriculture, Kagawa University, Miki-cho, Ikenobe 2393, Kagawa-ken 761-0795, Japan
[3] Dharmaj Degree Pharmacy College and Research Centre, Dharmaj-388 430, Gujarat, India
[4] Indukak Ipcowala College of Pharmacy, New, Vallabh Vidyanagar-388 120, Anand, Gujarat, India

Corresponding author: * **gautamsicart@gmail.com**

ABSTRACT

An accurate, sensitive and specific reverse phase high-performance liquid chromatographic (RP-HPLC) method for the estimation of cetrizine (CET) and phenylpropanolamine (PPA) in combined dosage form have been developed and validated. The molecular mass of CET and PPA conformed with molecular masses derived from a mass spectrometer (MS) in an electro-spray ionization method. Liquid chromatography was carried out on an ODS AQ Prontosil C_{18} column by binary elution with a mobile phase (acetonitrile: methanol: phosphate buffer (50: 20: 30, v/v/v) with pH adjusted with orthophosphoric acid to 3. The mobile phase was pumped at a flow rate of 1.0 ml/min and the response to UV was monitored at 244 nm. The average retention time for CET and PPA was 4.02 and 5.13 min, respectively. Method calibration showed linearity in the range of 5-65 µg/ml for CET and 150-325 µg/ml for PPA. The method is accurate and precise with recoveries of CET and PPA in the range of 100.37 ± 0.62 and 99.99 ± 0.74, respectively. The recovery values (> 99.4%) were high, indicating reliability and suitability of the method.

Keywords: accuracy, mass spectrometry, reproducibility, simultaneous equation, validation
Abbreviations: CET, cetrizine; **MS**, mass spectrometry; **PPA**, phenylpropanolamine; **RP-HPLC**, reversed phase high performance liquid chromatography

INTRODUCTION

Cetrizine (CET) hydrochloride, an 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 (http://www.prescriptiondrug-info.com/topics/cetirizine). Histamines cause symptoms of allergy when released by allergic reactions in the body and CET competes with histamine for binding at H1-receptor sites on the effector cell surface, as an H_1 receptor blocker, resulting in suppression of histaminic edema, flare, and purities (James *et al.* 1998; Sharma 2001). The low incidence of sedation can be attributed to reduced penetration of CET into the central nervous system as a result of fewer lipophilic carboxyl groups on the ethylamine side chain (Windholz and Bundavari 1976; Hobart *et al.* 2001).

PPA, also known as norephedrine and oxyamphetamine, is a psychoactive drug of the phenethylamine and amphetamine chemical classes and is used as a stimulant, decongestant, and anorectic agent (Windholz and Bundavari 1988). It is commonly used in prescription and over-the-counter cough and cold preparations. In veterinary medicine, it is used to control urinary incontinence in dogs under trade names such as Propalin and Proin (Christian 2003; Beringer *et al*. 2005; Silverstain *et al*. 2006).

CET and PPA are available in tablet dosage form in the ratio of 1: 5. Chemically, CET is (±) - [2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl] ethoxy]acetic acid, dihydrochloride and PPA is (1*R*,2*S*)-2-amino-1-phenyl-propan-1-ol. CET is official in the Indian and US pharmacopoeia while PPA is official in the US (Patel and Mehta 2011). Several analystical methods such as UV spectrophotometry (Panda *et al.* 2002; Ramesh *et al.* 2002; Bhatia *et al.* 2008), RP-HPLC from pharmaceutical preparations (Zarapakar *et al*. 1998; Khanolkar *et al.* 1999; Kaddoumi *et al*. 2004; Likar *et al.* 2005; Bajerski *et al.* 2005; Indian Pharmacopoeia 2007) and for HPTLC (Patel *et al*. 2011).

However, no analytical methods have been reported for the estimation of both the drugs from a combined dosage form. This paper presents a simple rapid, reproducible and economic method for simultaneous analysis of CET and PPA from its combined dosage form.

High performance liquid chromatography methods are useful in the determination of drugs in pharmaceutical formulations, especially those containing more than one active component. The aim of this work was to develop a relatively simple HPLC method for simultaneous quantification of CET and PPA in their combined dosage form (Marketed formulation: ALERID-D, Cipla).

The reverse phase HPLC method was found to be simple and convenient for the simultaneous determination of the two drugs and results indicate high accuracy and precision (Shethi *et al.* 1983; Shethi 2001). Over the last decade, electrospray ionization and atmospheric pressure chemical ionization have become the dominant techniques superseding thermospray, etc. and are likely to remain so for the foreseeable future being inherently the most suitable for analytes in solution presented to the Mass spectrometry-electrospray ionization was originally proposed by Dole *et al.* (1968) who suggested using charged droplets as a source of ions for MS (Whitehouse *et al.* 1985) pioneered its development as an ionization source for MS, leading to the first commercially available instrument in 1989.

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Table 1 Recovery studies for developed reversed phase chromatographic method.

Amount of sample in mixture in (µg/ml)		Amount added in mixture in (µg/ml)		Amount recovered in (µg/ml)		%R (Recovery)	
CET	PPA	CET	PPA	CET	PPA	CET	PPA
30	150	-	-	-	-	-	-
30	150	15	75	45.5	224.25	101.3	99.6
30	150	24	120	54	269.7	100	99.8
30	150	30	150	60.10	298.25	100.1	99.4

Abbreviations: CET, cetrizine; **PPA**, phenylpropanolamine; Zero level spiking.

MATERIALS AND METHODS

Sample determination for HPLC

Acetonitrile (HPLC grade) and methanol (HPLC grade) were purchased from Ranchem (Mumbai, India) and Milli-Q water (HPLC grade) was prepared in institute. Standard gift samples of CET and PPA were procured from Vital Formulations, Vallabh Vidyanagar, Gujarat. Combined CET and PPA tablets were purchased from local market (Vallabh Vidyanagar). Perkin Elmer Series 200, Totalchrom software solvent delivery gradient system (Pump), Rheodyne 7125 injector with a 20-µL loop, analytical column (ODS AQ Prontosil C18, 5 µ particle size, 25 cm × 4.6 mm id) was photo diode array detector series 200 were employer in this investigation. All the chemicals, reagents and solvents used were of HPLC grade.

Chromatographic method (RP-HPLC method)

The HPLC system used consisted of the 'Perkin Elmer' Series 200, photo diode array detector series 200 (Perkin Elmer, USA). The data station was computer controlled using Total Chrome software (Perkin Elmer, USA), which includes an integrator and a recorder. The injector device was "Rheodyne 7125" (Perkin Elmer, USA) with a 20 µL fixed volume loop. The flow rate was kept at 1.0 ml/min with an average operating pressure of 2500 PSI and the UV response was monitored at 244 nm. The mobile phase was filtered through a 0.45 µm nylon 66 membrane (Merck, Mumbai). The mobile phase automated degassed by system (Perkin Elmer series 200, Perkin Elmer, USA). The analytical column used was ODS AQ prontosil C18, 5 µm particle size, 25 cm × 4.6 mm id.

Mass spectrometer (MS) condition

The condition used in MS was: Sheth gas flow: 25 arb; I spray voltage: 5.00 Kv; capillary voltage: 1.00 V; tube lens: 40.00 V.

Reagents and solvents

Acetonitrile HPLC grade (Ranchem), methanol (Ranchem), potassium dihydrogen phosphate buffer (pH 3.0), ortho-phosphoric acid HPLC grade (1% v/v in water), acetic acid, water (HPLC grade from Milli-Q system).

Acetonitrile: methanol: phosphate buffer (50: 20: 30, v/v/v), pH adjusted to 3.0 was used as the mobile phase.

Preparation of the standard solution

10 mg of CET and 50 mg of PPA was weighed accurately and transferred to a 10 m1 and 50 ml standard volumetric flask and dissolved using methanol up the volume up to the mark. Aliquots of these solutions were transferred to 10 ml volumetric flask and volume was made up to mark with mobile phase to give 6 mixtures (5 µg/ml of CET and 150 µg/ml of PPA up to 65 µg/ml of CET and 325 µg/ml of PPA). These standard mixtures were chromatogramed and used to prepare the calibration curve.

Preparation of sample solution

Twenty tablets were weighed accurately and powdered. A quantity equivalent to 5 mg of CET and 25 mg of PPA was taken and diluted to 50 ml with methanol. Aliquot of above solution was transferred to 10 ml volumetric flask and volume was made up to mark with mobile phase (Alerid–D tablet, 5 mg CET and 25 mg PPA-Cipla).

Preparation of solution for MS analysis

10 mg CET and PPA mix was dissolved in 10 ml of 0.1% acetic acid in methanol. Sample was filtered through hydrophobic filter and filtrate was sonicated for 10 min. Then it was infused directly on ion trap MS (Thermo Scientific LCQ Fleet, Thermo Fischer, USA) system. Analysis was done in ESI mode.

Procedure of HPLC analysis

The chromatographic system was set at the conditions mentioned earlier and a steady baseline was recorded, after stabilization of the baseline, the standard solution containing 5 µg/ml CET and 150 µg/ml PPA was injected. The above procedure was repeated in the same way with the remaining 5 standard concentrations. The chromatograms were obtained for all the concentrations. This procedure was done in triplicate to ensure that repeatability was obtained. The retention time of CET and PPA was found to be 4.02 and 5.13, respectively. The chromatograms are shown in **Fig. 1**. The calibration curve of concentration vs. peak area of CET and PPA was plotted from the results.

The sample solution was prepared as mentioned above and then injected and the procedure as described for the standard solution was followed and the chromatogram was recorded and integrated (**Fig. 1**). The concentration of CET and PPA was determined from the regression equation of calibration curve.

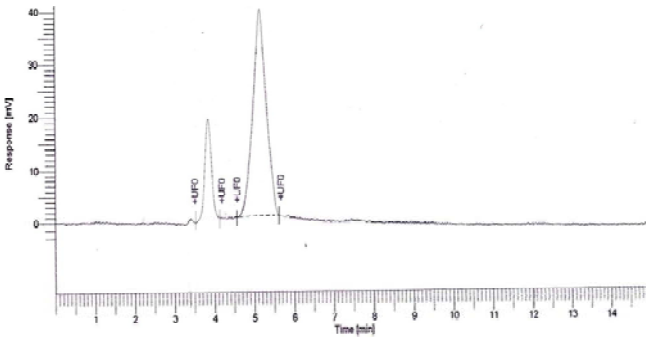

Fig. 1 Chramatogram of cetrizine and phenylpropanolamine by HPLC.

Recovery studies

To confirm the suitability, reliability and accuracy of the proposed method, recovery studies were carried out. Tablet powder equivalent to 5 mg CET and 25 mg PPA was transferred to 50 ml volumetric flask and volume was made up to mark with methanol, the resulting solution was sonicated for 15 min and filtered through Whatman filter paper (Merck, USA). The filtrate thus obtained, 3 ml solution was transferred to 10 ml volumetric flask and volume was made up to mark with mobile phase (unspiked sample). Accuracy of method was confirmed by spiking 50%, 80% and 100% of standard CET and PPA and percentage (%) recovery was determined. Data for recovery studies is given in **Table 1**.

Statistical analysis

Means, standard deviation (SD), relative standard deviation (RSD), and linear regression analyses were calculated using ANOVA software.

Table 2 System suitability parameters for developed reversed phase chromatographic method.

Parameters	CET	PPA
Retention time (Rt)	4.01	5.12
Tailing factor	1.06	1.04
Peak asymmetry factor (AF)	1.01	1.14
Theoritical plates (Plates/Meter)	9687	5425
Resolution (Rs)	2.665	

Already provided by sanded chromatogram and mass spectra

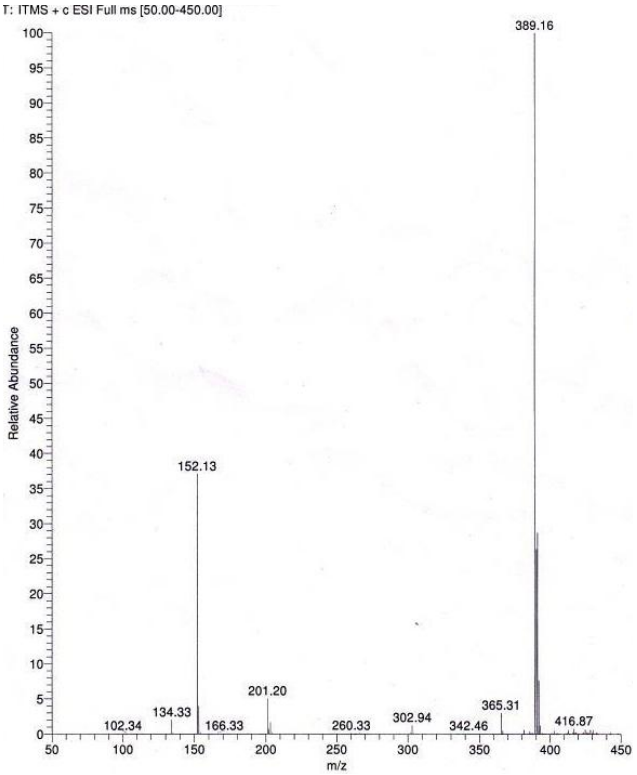

Fig. 2 Mass spectra of cetrizine and phenylpropanolamine by MS.

RESULTS AND DISCUSSION

An ESI-MS mass spectrum is shown in **Fig. 2**. The molecular weight confirmed CET [$(M + H)^+$, m/z 389] and PPA [$(M +H)^+$, m/z 152]. No other m/z impurities present in both drugs.

The calibration curve from HPLC study for both the drugs gave R^2 value as 0.995 and 0.998 for CET and PPA, respectively. The equation from calibration curve was:

Y = 10221*X + 38303 and Y = 578*X + 6306 for CET and PPA, respectively.

The method is accurate and precise with recoveries of CET and PPA in the range of 100.37 ± 0.62 and 99.99 ± 0.74.

The system suitability parameters obtained for the proposed method were found to meet the criteria as per International Conference on Harmonization ICH guidelines (**Table 2**).

CONCLUSION

Two methods were developed for determination of CET and PPA in combined dosage forms based on liquid chromatography. The method was validated and found to be simple, sensitive, accurate and precise. Statistical comparison of the assay results for determination of CET and PPA in tablet formulations using these methods indicated no significance difference, hence both the methods can be applied for routine analysis of CET and PPA in combined dosage forms. Mass spectrometer has proved to be an extremely sensitive and specific technique for the confirmation of molecular weight of drugs, pesticides and non volatile compounds. This method is less time consuming and reproducible.

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