ORIGINAL RESEARCH PAPER

Pharmacy

KEY WORDS: UPLC, validation,stabilitystudies,Method development

ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY FOR SIMULTANEOUS ESTIMATION OF ACETAMINOPHEN, DEXTROMETHORPHAN, LEVOCETIRIZINE AND PHENYL PROPONOLAMINE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A novel, rapid and stability indicatinganalytical method is developed and validated for simultaneous quantification of levocetirizen, dextromethorphan, paracetamol, and phenylpropanolaminein bulk and its Pharmaceutical formulations by UPLC a Kromosil C18 (250 x 4.6 mm, 5m).column with a solvent mixture of sodium dihydrogen phosphate :methanol(60:40 %V/V) 0.1N hydrochloric acid is used to adjusted the pH : 7.5 as mobilephasewith a flow rate of 1 ml/min. Isocratic mode was used for the separation of Levocetirizen, dextromethorphan, paracetamol, and phenylpropanolamine.The retention times of Levocetirizen, dextromethorphan, paracetamol, andphenylpropanolamine were found to be around 2.5 min,5.2min,3.9,5.9minrespectively From the linearity studies the specified range for Levocetirizen was determined to be 1.25-7.5 g/ml and for dextromethorphan was determined to be 3.75-22. g/ml, and for paracetamol was determined to be 125-750 g/ml and for phenylpropanolamine was determined to be 6.25-37.5 g/ml. The proposed method was successful in simultaneous quantification of Levocetirizen, dextromethorphan, paracetamol, and phenylpropanolamine.

INTRODUCTION

Levocetirizine, chemically is [2-[4- [(r)-(4-chlorophenyl) phenylmethyl]-1- piperazinyl] ethoxy] (fig. 1).Levocetirizine works by blocking histamine receptors.[1] Montelukast sodium and an antihistamine levocetirizine, shows significantly better symptom relief compared with the modest improvement of rhinitis symptomatically with each of the treatments alone.[2]Levocetirizine is rapidly and extensively absorbed, minimally metabolized and has a volume of distribution which is lower than other compounds from the same group[3]. Cetirizine dihydrochloride is a piperazine derivative and metabolite of hydroxyzine. It is used for the symptomatic relief of allergic conditions including rhinitis and chronic urticaria.[4].Levocetirizine dihydrochloride falls under the BCS Class III, highly soluble and poorly permeable drug.[5] . Combination of acetaminophen(fig. 2), and caffeine is an analgesic-antipyretic formulation with known therapeutic efficacy .[6] Acetaminophen, phenylephrine and chlorpheniramine are frequently associated in pharmaceutical formulations against the common cold.[7]Paracetamol is a popular analgesic and antipyretic agent, with the following Molecular formula: C8H9NO2[8]. Paracetamol is available in different dosage forms: tablet, capsules, drops, elixirs, suspensions and suppositories.[9]paracetamol is an acetanilide derivative chemically 4-hydroxy acetanilide having analgesic, antipyretic and also administered in the management of more severe pains in advanced cancers.[10].

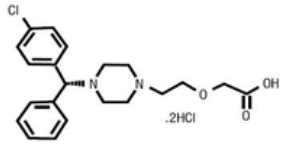

Fig. 1: Chemical Structure of Levocitrizen

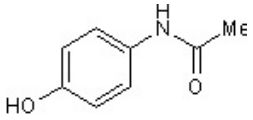

Fig. 2: Chemical Structure of Acetaminophen

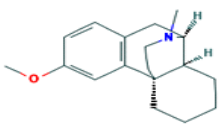

Fig. 3: Chemical Structure of Dextromethorphan

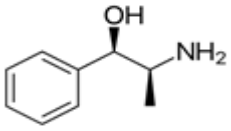

Fig. 4: Chemical Structure of phenylpropanol amine

MATERIAL AND INSTRUMENTS:

Analysis was performed on a chromatographic system of Waters Alliance, with photodiode array detector.A chromatographic separation was achieved on Discovery C18(250 x 4.6 mm, 5 µ). analytical column. Data acquisition was made with Empower 2 software.

REAGENTS AND CHEMICALS:

Levocetrizine, dextromethorphan, paracetamol, phenylpropanolamine working standard working standard supplied by spectrum pharma laboratories Hyderabad. Bioder plus (Sample) Cetirizine -5mg ,Dextromethorphan–10 mg,Paracetamol-500mg,Phenylpropanol amine-12.5mg,purchasedfrom Biochemix Health care pvt.ltd. Potassium dihydrogenphosphate, Acetonitrile, orthophosphoric Acid,Distilled water.

selection of mode of separation and stationary phase

UPLC method was preferred as mode of separation. Column utilized in the study was Discovery C18(250 x 4.6 mm, 5 µ).

selection of detector wavelength

The wave length selection was made at 220 nm in UV spectrum.

Preparation of mobile phase

Sodium dihydrogen orthophosphate buffer was prepared by dissolving 0.22gm of sodium dihydrogen phosphate into a 1000 ml beaker with HPLC water. 0.1NHydrochloric acid was added to maintain the PH level at 7.5

Preparationof Standard Solution of Levocetrizine,dextromethorphan,paracetamol, phenylpropanolamine

Working standard was prepared by taking 1 ml and pipetted out into a 10 ml volumetric flask to get a concentration of 5 µg/ml of Levocitrizine, 10µg/ml of Dextromethorphan,500µg/ml of paracetamol,12.5µg/ml

Phenylpropanolamine then make up to the final volume with diluents.The optimised chromatogram is shown in figure no 5.

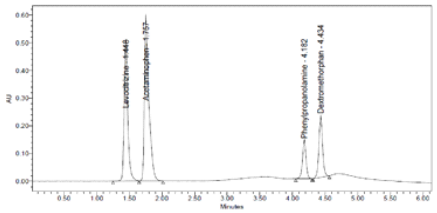

Fig. no 5: optimised Chromatogramfor Levocetrizine,dextromethorphan,paracetamol, phenylpropanolamine

Assay of Levocetrizine,dextromethorphan,paracetamol, phenylpropanolamine

10 µl of the mixed standard solution containing Levocetrizine, dextromethorphan, paracetamol, phenylpropanolamine was injected into the UPLC.The obtained data of Quantitative determination for Levocetirizen, dextromethorphan, paracetamol, phenylpropanolamine are reported in table no1.

Analytical method validation for simultaneous estimation of levocetirizen, dextromethorphan,paracetamol,and phenylpropanolamine for developed method by uplc

Specificity

Preparation of Blank for Specificity

The mobile phase used is prepared by mixing phosphate buffer and methanol in the ratio of 60:40 % v/v respectively.

Preparation of Placebo for Specificity

100 mg of placebo was placed in 50 ml volumetric flask, about 15 ml of diluent was added.

Preparation of Standard solution of Levocetirizen, dextromethorphan, paracetamol, and phenylpropanolamine and placebo for specificity

5mg of Levocitrizine, 10mg Dextromethorphan ,500mg of paracetamol, and 12.5mg of Phenylpropanolamine were placed in 100 ml graduated flask..From the above stock solutions, 1ml was pipetted out in to a 10ml volumetric flask and then make final volume with diluent.

System Suitability Test

Mixed standard solution 5 µg/ml of Levocitrizine,10µg/ml of Dextromethorphan,500µg/ml of paracetamol,12.5µg/ml Phenylpropanolamine was prepared by using a diluent as per test method and 10 µl of this solution was injected six times into the UPLC.

System Precision

Mixed standard solution containing 5 µg/ml of Levocitrizine,10µg/ml of Dextromethorphan,500µg/ml of paracetamol,12.5µg/ml Phenylpropanolamine was prepared by using a diluent as per test method and 10 µl of this solution was injected six replicate times into the UPLC

Method Precision

Sample powder was accurately weighed and working standard was prepared by taking 1 ml and pipetted out into a 10 ml volumetric flask to get a concentration of 5 µg/ml of Levocitrizine,10µg/mlofDextromethorphan,500µg/mlofpara cetamol,12.5µg/ml Phenylpropanolamine then make up to the final volume with diluent.

Linearity & Range

Working standard at concentration levels from 1.25- 7.5 g/ml of Levocetirizen, 2.5-15 g/ml ofDextromethorphan,125-750 g/mlparacetamol and3.125-18.75 g/ml phenylpropanolamine respectively.

Accuracy

Working standards were spiked with Placebo and made up with diluent to get the targeted concentrations of 50 %, 100 % and 150 % for Levocetirizen, dextromethorphan, paracetamol, and phenylpropanolamine.10 1of placebo was injected and standard solutions of Accuracy - 50 %, Accuracy - 100 % and Accuracy - 150 % solutions into UPLC.

Robustness

Flow rate variation

Mixedstandard solution of 5 µg/ml of Levocitrizine, 10µg/ml of Dextromethorphan,500µg/ml of paracetamol,12.5µg/ml Phenylpropanolamine was prepared by using a diluent and 10 µl of this solution was injected six times for varied flow rates (0.8 ml, 1.2 ml), detection wavelength (215 nm, 225nm), and the obtained data compared with flow rate (1.0 ml),method detection wavelength (220 nm).

Ruggedness

Analyst to Analyst variation

MixedStandard solution of 5 µg/ml of Levocitrizine, 10µg/ml ofDextromethorphan,500µg/ml of paracetamol,12.5µg/ml Phenylpropanolamine was prepared separately by Analyst – I and Analyst – II.

Limit of Detection

It was found to be0.02 ug/ml for Levocetirizine , 0.01 µg/ml for Dextromethorphan, 0.51 µg/ml for paracetamol, 0.19 µg/ml for phenylpropanolamine respectively.

Limit of Quantification

It was found to be 0.06ug/ml for Levocetirizine , 1.53 µg/ml for Dextromethorphan, 0.03 µg/ml for paracetamol, 0.28 µg/ml for phenylpropanolamine respectively.

Degradation studies

Acidic Degradation

Sample Solutions for acid degradation studies were prepared with 10 ml of 0.01 M Hydrochloric acid. Reflux under heat at 60°C for one hour.

Basic Degradation

Sample Solutions for basic degradation studies were prepared with 10 ml of 0.1 M Sodium hydroxide. Reflux under heat at 60°C for one hour.

Neutral Degradation

Sample Solutions for neutral degradation studies were prepared with 10 ml of water. Reflux under heat at 60°C for one hour.

Oxidative Degradation

Sample Solutions for oxidative degradation studies were prepared with 10 ml of 3 % Hydrogen peroxide. Reflux under heat at 60°C for one hour.

RESULTS

Specificity

It was confirmed by injecting the placebo and placebo spiked standard and observed that there was no shift in wavelength interference due to placebo.

System Suitability:

The Tailing factor for Levocetirizen, dextromethorphan, paracetamol, and phenylpropanolamine.was found to be 1.2, 1.1, 1.1and 1.1 respectively.The Theoretical plates per unit for Levocetirizen, dextromethorphan, paracetamol, and phenylpropanolamine. was found to be 110722, 113269, 111436 and 114251respectively.The results are given in table no2.

System Precision

The % R.S.D Retention time, peak area, and tailing factor are present within the Acceptance criteria of 2 %.

Method Precision

The % R.S.D of Peak area, Retention time and Assay are present within the acceptance criteria of 2 %.

Linearity and Range

The Correlation Coefficient Levocetirizen, dextromethorphan, paracetamol, and phenylpropanolamine was found within the acceptance criteria of 0.999.

Accuracy

Recovery studies was used to evaluate Accuracy. The acceptance criteria for recovery study is 98 - 102 % w/v.

Robustness

The % R.S.D of Peak area and Retention time for flow rate variation and detection wavelength variation are present within the acceptance criteria of 2 %.

Ruggedness

The method is rugged and does not show variations in the results on slight variations of parameters.

Limit of Detection

L.O.D was found to be 0.02 ug/ml for Levocetirizine , 0.01 µg/ml for Dextromethorphan, 0.51 µg/ml for paracetamol, 0.19 µg/ml for phenylpropanolamine

Limit of Quantification

L.O.Q was found to be 0.06ug/ml for Levocetirizine , 1.53 µg/ml for Dextromethorphan, 0.03 µg/ml for paracetamol, 0.28 µg/ml for phenylpropanolamine

Degradation Studies

There was no interference of the drug peak .Thus Levocetirizen, dextromethorphan, paracetamol, and phenylpropanolamine is stable in all conditions .

Table nol :Quantitative Determination of Levocetirizen,Dextromethorphan,Paracetamol, and Phenylpropanolamine

S. No	Sample Name	Peak Area of standard	Average Peak Area of standard	Peak Area of sample	Average Peak Area of Sample	Amount found	% purity
1	Levocetirizen 5mg	184160 188700 185955	186271.7	186531 185823 187979	186777.7	4.73 mg	100.27 %w/v
2	dextromethorphan 10mg	1887437 19454 183151	186680.7	189199 186859 186964	187674	9.47mg	100.53 %w/v
3	paracetamol 500mg	3359626 3401737 3400513	3387292	3398058 3383961 3408086	3396702	499.73 mg	100.27 %w/v
4	phenylpropanolamine 12.5mg	872376 873988 871012	872458.7	876541 877552 872891	875661.3	12.14 mg	100.36 %w/v

Table no 2: System Suitability Parameters Result of Levocetirizen, dextromethorphan, paracetamol, phenylpropanolamine

	Levocetirizen	Dextromethorphan	Paracetamol	phenylpropanolamine
Tailing factor	1.2	1.1	1.1	1.1
Retention time	2.5	5.2	2.9	5.9
Theoretical plates per unit	110722	113269	111436	114251

CONCLUSION

The present paper describes the simple and accurate method for simultaneous determination of Levocetirizen, dextromethorphan, paracetamol, and phenylpropanolamine content from pharmaceutical samples using UPLC. There is no method reported for this combination of products.The developed method exhibits less retention time, faster elution and better separationcompared with other methods. Moreover, the less solvent consumption and short runtime that allows the analysis of huge number of samples with good precision andaccuracy in a short period of time, so it is affordable, convenient and economical forroutine pharmaceutical analysis.

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