Simultaneous Determination of Ambroxol Hydrochloride, Cetirizine Hydrochloride, Methylparaben and Propylparaben in Liquid Pharmaceutical Formulation

Overview

The present chapter deals with the simultaneous determination of ambroxol hydrochloride, cetirizine hydrochloride, methylparaben, and propylparaben in liquid pharmaceutical formulation using the developed and validated, stability indicating, RP-UPLC method.

3.1 LITERATURE REVIEW

Liquid preparations are particularly susceptible to microbial growth because of the nature of their ingredients. Such preparations are protected by the addition of preservatives that prevent the alteration and degradation of the product formulation [1]. The finished product release specifications should include an identification test and a content determination test with acceptance criteria and limits for each antimicrobial preservative present in the formulation [2]. The finished product self-life specification should also include an identification test and limits for the antimicrobial preservatives present [2]. Hence their (MP and PP) antimicrobial and antifungal properties make them an integral part of the product formulation. This encourages the development of new stability indicating method for simultaneous estimation of all compounds (AMB, CTZ, MP and PP) to provide driving force in today’s pharmaceutical industry.

UPLC is a new category of separation technique based upon well-established principles of liquid chromatography, which utilizes sub-2 µm particles for stationary phase. These particles operate at
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Elevated mobile phase linear velocities affect dramatic increase in resolution, sensitivity and speed of analysis. Owing to its speed and sensitivity, this technique is gaining considerable attention in recent years for pharmaceuticals and biomedical analysis. In the present work, this technology has been applied to the method development and validation study of assay determination (AMB, CTZ, MP and PP) in liquid pharmaceutical formulation.

Several spectrophotometric methods have been reported for the qualitative and quantitative determination of AMB from pharmaceuticals formulations [2-6]. Various HPLC [7-10], GLC [11, 12], sequential injection technique coupled with monolithic column [13] LC-MS [14], capillary electrophoretic [15] and by capillary electrophoresis and fluorescence detection [16] are also reported for its determination from biological fluids.

Literature survey revealed that several spectrophotometric [17-19] methods, HPLC methods [20-23], HPLC coupled to tandem mass spectroscopy [24], capillary electrophoretic [25, 26] have been also reported for determination of CTZ from Pharmaceutical formulations and biological fluids. Detailed literature survey for MP and PP revealed that many existing analytical procedures are available in literature for the determination of present preservatives studied, either alone or in combination with other drugs by HPLC and other techniques [10, 27-35].

A detailed literature survey for AMB + CTZ revealed that few analytical methods are available using spectrophotometric and HPLC where; Neela M. Bhatia et al. [36], describe RP-HPLC and spectrophotometric estimation of AMB and CTZ in combined dosage form; Mukesh Maithani et al. [37], simultaneous estimation of AMB and CTZ in tablet dosage form by RP-HPLC method; Trivedi Aditya et al. [38], development of modified spectrophotometric and HPLC method for simultaneous estimation of AMB and CTZ in tablet dosage forms; A. S. Birajdar et al. [39], simultaneous analysis of AMB with CTZ and of AMB with levo-Cetirizine dihydrochloride in solid dosage forms by RP-HPLC; NM Gowekar et al. [40], spectrophotometric estimation of AMB and CTZ from tablet dosage form. HPTLC method is also reported by S.B. Bagade et al. [41].
Ambroxol Impurity-A, B, C, D and E are official in British Pharmacopoeia [42]. Cetirizine specified impurities A, B, C, D, E and F are also official in British Pharmacopoeia [43]. Cetirizine CDH1 (impurity G as per British Pharmacopoeia) impurity is completely characterized in house (Dr. Reddy’s Laboratory) by using IR, Mass and NMR.

3.2 THE SCOPE AND OBJECTIVES OF PRESENT STUDY

The combination of AMB and CTZ is not official in any pharmacopoeia. So far, no RP-UPLC stability indicating method has been reported for the rapid simultaneous determination of AMB, CTZ, MP and PP in liquid pharmaceutical formulation yet. Therefore, the research is undertaken to develop a new rapid and stability-indicating method for simultaneous determination of four compounds (AMB, CTZ, MP and PP) in liquid pharmaceutical formulation. The developed method is able to separate AMB, CTZ, MP and PP with each other and from its all twelve (AMB impurities A, B, C, D, E and CTZ impurities A, B, C, D, E, F, CDH1) known impurities/ degradation products and one unknown degradation product within 3.5 min. Thereafter, this method is validated according to the ICH guidelines [44] and successfully applied for separation and quantification of all compounds of interest in the liquid and solid pharmaceutical formulation.

The objectives of the present work are as follow:

- Development of rapid, stability indicating RP-UPLC method for simultaneous determination of AMB, CTZ, MP, and PP in liquid pharmaceutical formulation.

- Forced degradation study.

- To separates AMB, CTZ, MP and PP with each other and from its all twelve (AMB impurities A, B, C, D, E and CTZ impurities A, B, C, D, E, F, CDH1) known impurities/ degradation products and any unknown degradation product generated during forced degradation study.

- Perform analytical method validation for the proposed method as per ICH guideline.

- Application of developed and validated method on various pharmaceutical dosage forms (various marketed products).
3.3 AMBROXOL HYDROCHLORIDE

Ambroxol hydrochloride (AMB) is a semi-synthetic derivative of vasicine obtained from Indian shrub *Adhatoda vasica*. It is a metabolic product of bromhexine. AMB is a clinically proven systemically active mucolytic agent. When administered orally, onset of action occurs after about 30 minutes. The breakdown of acid mucopolysaccharide fibers makes the sputum thinner and less viscous and therefore more easily removed by coughing. Although sputum volume eventually decreases, its viscosity remains low for as long as treatment is maintained [45]. AMB is chemically \((1s,4s)-4-((2\text{-amino-3,5-dibromocyclohexyl})\text{methylamino})\text{cyclohexanol hydrochloride} [\text{Figure 3.1}]. Its molecular weight is 414.6 g/mole with molecular formula \(\text{C}_{13}\text{H}_{18}\text{Br}_{2}\text{N}_{2}\text{O}.\text{HCl}\). Its dissociation constant (pKa) 8.2 is reported. Its melting point is 235°C to 240°C.

![Chemical structure of Ambroxol hydrochloride (AMB)](image)

3.3.1 Indications

All forms of tracheobronchitis, emphysema with bronchitis pneumoconiosis, chronic inflammatory pulmonary conditions, bronchiectasis, bronchitis with bronchospasm asthma. During acute exacerbations of bronchitis it should be given with the appropriate antibiotic [45].

3.3.2 Pharmacokinetics

Alteplase initiates local fibrinolysis and dissolution of clots by binding to fibrin in a thrombus and the fibrin-bound plasminogen is converted to plasmin.
3.3.3 **Ambroxol dosage** [45]

- Adults: daily dose of 30 mg (one ambroxol tablets) to 120 mg (4 ambroxol tablets) taken in 2 to 3 divided dose.
- Children up to 2 years: half a teaspoonful ambroxol syrup twice daily.
- Children 2-5 years: half a teaspoonful ambroxol syrup 3 times daily.
- Children over 5 years: one teaspoonful ambroxol syrup 2-3 times daily.

3.4 **CETIRIZINE HYDROCHLORIDE (CETIRIZINE DIHYDROCHLORIDE)**

Cetirizine hydrochloride (CTZ) is an orally active and selective H1-receptor antagonist. It is piperazine derivative and metabolite of hydroxyzine. The drug substance, cetirizine dihydrochloride, is commonly referred to as cetirizine hydrochloride or cetirizine HCl. The chemical name is (±) - [2-[4-[4-(4-chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]acetic acid, dihydrochloride. Cetirizine hydrochloride is a racemic compound with an empirical formula of C\textsubscript{21}H\textsubscript{25}ClN\textsubscript{2}O\textsubscript{3}•2HCl. The molecular weight is 461.82 and the chemical structure is shown below [Figure 3.2]. Cetirizine HCl has three ionizable moieties resulting in pKa values of 2.2, 2.9 and 8.0. At physiological pH, it predominantly exists as a zwitterion or an anion. Cetirizine HCl is a white or almost white powder that is freely soluble in water, practically insoluble in acetone and in methylene chloride. Its melting point is 110°C to 115°C.

![Chemical structure of Cetirizine dihydrochloride (CTZ)]
3.4.1 Indications

For the relief of symptoms associated with seasonal allergic rhinitis, perennial allergic rhinitis and the treatment of the uncomplicated skin manifestations of chronic idiopathic urticaria. [46]

3.4.2 Pharmacokinetics

Cetirizine undergoes rapid absorption where the maximum plasma concentration is reached at about 1 hour following oral administration of tablets, chewable tablets, and syrup. Comparable bioavailability is found between the tablet and syrup dosage forms. No accumulation is observed following multiple dosing of cetirizine HCl (10 mg tablets once daily for 10 days) in healthy subjects. Cetirizine pharmacokinetics is linear for oral doses ranging from 5 to 60 mg. The mean plasma protein binding of cetirizine hydrochloride is 93%, independent of concentration in the range of 25 to 1000 ng/mL, which includes the therapeutic plasma concentrations. The mean elimination half-life is 8.3 hours and the apparent total body clearance for cetirizine is 53 mL/min following oral administration of cetirizine in healthy subjects. [47]

3.4.3 Ambroxol dosage

Cetirizine can be taken without regard to food consumption. Cetirizine is available as 5 mg and 10 mg tablets, 1 mg/mL syrup, and 5 mg and 10 mg chewable tablets which can be taken with or without water [48].

- Adults and children 12 years and older: The recommended initial dose of cetirizine is 5 mg or 10 mg per day in adults and children 12 years and older, depending on symptom severity.
- Children 6 to 11 years: The recommended initial dose of cetirizine in children aged 6 to 11 years is 5 mg or 10 mg once daily depending on symptom severity.
- Children 2 to 5 years: The recommended initial dose of cetirizine in children aged 2 to 5 years is 2.5 mg (half teaspoon) syrup once daily. The dosage in this age group can be increased to a maximum dose of 5 mg per day given as 1 teaspoon syrup once a day or one ½ teaspoon syrup given every 12 hours, or one 5 mg chewable tablet once a day.
• Children 6 months to <2 years: The recommended dose of cetirizine syrup in children 6 months to 23 months of age is 2.5 mg (half teaspoon) once daily. The dose in children 12 to 23 months of age can be increased to a maximum dose of 5 mg per day, given as half teaspoon (2.5 mg) every 12 hours. Syrup is recommended for children under the age of 2 years.

3.5 PARABENS (METHYLPARABEN AND PROPYLENPARABEN)

Parabens are a class of chemicals widely used as preservatives by cosmetic and pharmaceutical industries. Parabens are effective preservatives in many types of formulas. These compounds, and their salts, are used primarily for their bactericidal and fungicidal properties. They can be found in shampoos, commercial moisturizers, shaving gels, personal lubricants, topical/parenteral pharmaceuticals, spray tanning solution, makeup, and toothpaste [49]. They are also used as food additives.

Their efficacy as preservatives, in combination with their low cost, the long history of their use, and the inefficacy of some natural alternatives like grapefruit seed extract (GSE) [50], probably explains why parabens are so commonplace. They are becoming increasingly controversial, however, because they have been found in extremely low concentrations in breast cancer tumors (an average of 20 nanograms/g of tissue) [51]. Parabens have also displayed the ability to slightly mimic estrogen (a hormone known to play a role in the development of breast cancer) [51]. No effective direct links between parabens and cancer have been established, however [52]. Another concern is that the estrogen-mimic aspect of parabens may be a factor in the increasing prevalence of early puberty in girls [53].

3.5.1 Chemistry

Parabens are esters of para-hydroxybenzoic acid, from which the name is derived. Common parabens include methylparaben (E number E218), ethylparaben (E214), propylparaben (E216) and
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butylparaben. Less common parabens include isobutylparaben, isopropylparaben, benzylparaben and their sodium salts. The general chemical structure of a paraben is shown in Figure 3.3, where R symbolizes an alkyl group such as methyl, ethyl, propyl or butyl.

![Chemical structure of Paraben]

**Figure 3.3 Chemical structure of Paraben**

### 3.5.2 Occurrence

Some parabens are found naturally in plant sources. For example, methylparaben is found in blueberries [54-56], where it acts as an antimicrobial agent.

### 3.5.3 Synthesis

All commercially used parabens are synthetically produced, although some are identical to those found in nature. They are produced by the esterification of *para*-hydroxybenzoic acid with the appropriate alcohol. *para*-Hydroxybenzoic acid is in turn produced industrially from a modification of the Kolbe-Schmitt reaction, using potassium phenoxide and carbon dioxide.

### 3.5.4 Toxicology

Studies on the acute, subchronic, and chronic effects in rodents indicate that parabens are practically non-toxic [57, 58]. Parabens are rapidly absorbed, metabolized, and excreted [57]. The major metabolites of parabens are *p*-hydroxybenzoic acid (pHBA), *p*-hydroxyhippuric acid (M1), *p*-hydroxybenzoyl glucuronide (M3), and *p*-carboxyphenylsulfate (M4) [59].

### 3.5.5 Regulation

The European Scientific Committee on Consumer Products (SCCP) stated in 2006 that the available data on parabens do not enable a decisive response to the question of whether propyl,
butyl and isobutyl paraben can be safely used in cosmetic products at individual concentrations up to 0.4%, which is the allowed limit in the EU [60].

### 3.5.6 Methylparaben

Methylparaben (MP) is effective preservative in many types of pharmaceutical formulations. It is the Methyl ester of p-hydroxybenzoic acid. Methylparaben is chemically Methyl 4-hydroxybenzoate and its chemical structure is shown in Figure 3.4.

![Chemical structure of Methylparaben (MP)](image)

Its molecular weight is 152.15 g/mole with molecular formula C₈H₈O₃. MP is a white crystalline powder that is easily soluble in diethyl ether, acetone and slightly soluble in cold water, hot water. Its melting point is 126°C to 128°C.

### 3.5.7 Propylparaben

Propylparaben (PP) is effective preservative in many types of pharmaceutical formulations. It is the Propyl ester of p-hydroxybenzoic acid. Propylparaben is chemically Propyl 4-hydroxybenzoate and its chemical structure is shown in Figure 3.5.

![Chemical structure of Propylparaben (PP)](image)
Its molecular weight is 180.2 g/mole with molecular formula C\textsubscript{10}H\textsubscript{12}O\textsubscript{3}. PP is a white crystalline powder that is easily soluble in methanol, diethyl ether, acetone and slightly soluble in cold water, hot water. Its melting point is 96°C to 99°C.

3.6 EXPERIMENTAL

3.6.1 Materials and reagents

Drug product, placebo solution, working standards and reference standards are provided by Dr. Reddy’s laboratories Ltd., Hyderabad, India. HPLC grade acetonitrile and methanol are obtained from J.T.Baker (NJ., USA). GR grade potassium dihydrogen phosphate, GR grade orthophosphoric acid and GR grade triethylamine are obtained from Merck Ltd. (Mumbai, India). 0.22 µm nylon membrane filter and nylon syringe filters are purchased from Pall life science limited (India). 0.22 µm PVDF syringe filter is purchased from Millipore (India). High purity water is generated by using Milli-Q Plus water purification system (Millipore\textsuperscript{®}, Milford, MA, USA).

3.6.2 Equipments

Acquity UPLC\textsuperscript{TM} system (Waters, Milford, USA), consisting of a binary solvent manager, sample manager and PDA (photo diode array) detector. System control, data collection and data processing are accomplished using Waters Empower\textsuperscript{TM}-2 chromatography data software. Cintex digital water bath is used for specificity study. Photo stability studies are carried out in a photo-stability chamber (SUNTEST XLS+, ATLAS, Germany). Thermal stability studies are performed in a dry air oven (Cintex, Mumbai, India).

3.6.3 Preparation of mobile phase and its gradient program

Mobile Phase-A (MP-A): Mixture of 0.01M phosphate buffer (KH\textsubscript{2}PO\textsubscript{4}) in 0.1% triethylamine.

Mobile phase-B (MP-B): Acetonitrile.

MP-A and MP-B is filtered through 0.22 µm nylon membrane filter and degassed under vacuum prior to use.
Table 3.1  Gradients program for elution

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Flow rate (mL/min)</th>
<th>% MP-A</th>
<th>% MP-B</th>
<th>Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>0.5</td>
<td>70</td>
<td>30</td>
<td>Isocratic</td>
</tr>
<tr>
<td>0.2</td>
<td>0.5</td>
<td>70</td>
<td>30</td>
<td>Isocratic</td>
</tr>
<tr>
<td>3.0</td>
<td>0.5</td>
<td>5</td>
<td>95</td>
<td>Linear</td>
</tr>
<tr>
<td>3.1</td>
<td>0.5</td>
<td>70</td>
<td>30</td>
<td>Isocratic</td>
</tr>
<tr>
<td>3.5</td>
<td>0.5</td>
<td>70</td>
<td>30</td>
<td>Equilibration</td>
</tr>
</tbody>
</table>

3.6.4  Diluent preparation

Mixture of water and methanol in the ratio of 50:50 (v/v) respectively.

3.6.5  Chromatographic conditions

The chromatographic condition is optimized using Agilent Eclipse Plus C18, RRHD 1.8 µm (50 mm x 2.1 mm) column. The finally selected and optimized conditions are as follows: injection volume 4 µL, gradient elution [Table 3.1], at a flow rate of 0.5 mL/min at 50°C (column oven) temperature, detection wavelength 237 nm. Under these conditions, the backpressure in the system is about 6,000 psi. The stress degraded samples and the solution stability samples are analyzed using a PDA detector covering the range of 200-400nm.

3.6.6  Standard solution preparation

Standard solution is prepared by dissolving standard substances in diluent to obtain solution containing 120 µg/mL of Ambroxol hydrochloride, 20 µg/mL of Cetirizine hydrochloride, 40 µg/mL of Methylparaben and 4 µg/mL of Propylparaben.

3.6.7  Sample solution preparation

An accurately weighed 2 g of sample solution is taken into the 100 mL volumetric flask. About 70 mL of diluent is added to this volumetric flask and sonicated in an ultrasonic bath for 5 min. This
solution is then diluted up to the mark with diluent and mixed well. It is then filtered through 0.22 µm PVDF syringe filter and the filtrate is collected after discarding first few milliliters.

3.6.8 Placebo solution preparation

An accurately weighed 2 g of placebo solution is taken into the 100 mL volumetric flask. About 70 mL of diluent is added to this volumetric flask and sonicated in an ultrasonic bath for 5 min. This solution is then diluted up to the mark with diluent and mixed well. It is then filtered through 0.22 µm PVDF syringe filter and the filtrate is collected after discarding first few milliliters.

3.6.9 Market product sample solution preparation (for oral solution)

An accurately weighed X g of sample solution is taken into 100 mL volumetric flask (where X= 4 gm for Xyzal® [UCB, India Pvt. Ltd.; B.No.-VO 10001], 2 gm of ZyrCold® [UCB, India Pvt. Ltd.; B.No.-LI10035] and 2 g of Relent® [Dr. Reddy’s Lab. Ltd. India; B.No.-L 0590]). About 70 mL of diluent is added to this volumetric flask and sonicated in an ultrasonic bath for 3 min. This solution is then diluted up to the mark with diluent and mixed well. It is then filtered through 0.22 µm PVDF syringe filter and the filtrate is collected after discarding first few milliliters.

3.6.10 Market product sample solution preparation (for oral tablet)

Twenty tablets are crushed to fine powder. An accurately weighed portion of the powder equivalent to 5 mg of CTZ is taken into 100 mL volumetric flask (Cetzine® Tablets [GSK Pharmaceuticals Ltd.; B.No.-L473], Dio-1® Tablets [Unison pharmaceuticals; B.No.-2005] and ZyrCold® Tablets [UCB, India Pvt. Ltd.; B.No.-5829]). About 70 mL of diluent is added to this volumetric flask and sonicated in an ultrasonic bath for 15 min. This solution is then diluted up to the mark with diluent and mixed well. It is then filtered through 0.22 µm PVDF syringe filter and the filtrate is collected after discarding first few milliliters.

3.6.11 Method Validation

The method described herein has been validated for simultaneous, assay determination by UPLC.
3.6.11.1 Specificity

Forced degradation studies are performed to demonstrate selectivity and stability-indicating the capability of the proposed method. The sample is exposed to acid hydrolysis [0.1N HCl (3 mL), 60°C, 1h], base hydrolysis [0.1N NaOH (3 mL), 60°C, 1h], oxidative [6% w/v H₂O₂ (3 mL), 60°C, 1h], thermal [60°C, 1h] and photolytic degradation [1.2 million Lux hours]. All exposed samples are then analysed by the developed method.

3.6.11.2 System suitability

System suitability parameters are measured so as to verify the system performance. System precision is determined on six replicate injections of standard preparation. All important characteristics including % RSD, resolution (between CTZ and PP), tailing factor and theoretical plate number are measured.

3.6.11.3 Precision

The precision of the system is determined using the sample preparation procedure described above for six real samples of liquid formulation and analysis using the same proposed method. Intermediate precision is studied by other scientist, using different columns, different UPLC, and is performed on different days.

3.6.11.4 Accuracy

To confirm the accuracy of the proposed method, recovery experiments are carried out by the standard addition technique. Three levels (50 %, 100 % and 150 %) of standards are added to pre-analyzed placebo samples in triplicate. The percentage recoveries of AMB, CTZ, MP and PP at each level and each replicate are determined. The mean of percentage recoveries (n = 9) and the relative standard deviation are also calculated.

3.6.11.5 Linearity

Linearity is demonstrated from 1.5 to 150 % of standard concentration using a minimum of seven calibration levels (25 %, 50 %, 75 %, 100 %, 125 %, 150 % and 200 %) for AMB, CTZ, MP and
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PP. The method of linear regression is used for data evaluation. The peak areas of the standard compounds are plotted against the respective AMB, CTZ, MP and PP concentrations. Linearity is described by the linearity equation, correlation coefficient and Y-intercept bias is also determined.

3.6.11.6 Robustness

The robustness is a measure of the capacity of a method to remain unaffected by small but deliberate changes in flow rate (± 0.05 mL/min) and change in column oven temperature (± 5 °C). The theoretical plates, tailing factor and retention behaviour of all interested compounds (AMB, CTZ, MP and PP) and the resolution (between CTZ and PP) are evaluated.

3.6.11.7 Solution stability

The stability of the sample solution is established by storage of the sample solution at ambient temperature for 24h. The sample solution is re-analyzed after 12 and 24h, and the results of the analysis are compared with the results of the fresh sample. The stability of standard solution is established by the storage of the standard solution at ambient temperature for 24h. The standard solution is re-injected after 12 and 24h, and % RSD is calculated.

3.6.11.8 Filter compatibility

Filter compatibility is performed for nylon 0.22 μm syringe filter (Pall Life sciences) and PVDF 0.22 μm syringe filter (Millipore). To confirm the filter compatibility in proposed analytical method, filtration recovery experiment is carried out by sample filtration technique. Sample is filtered through both syringe filters and percentage assay is determined and compared against centrifuged sample.

3.6.2 Application of the Method to Dosage Forms

The present method is applied for the estimation of drugs and preservatives in the commercially available various dosage forms.
3.7 RESULTS AND DISCUSSION

3.7.1 Method Development and Optimization

The main objective of the RP-UPLC method development is to rapid and simultaneous determination of AMB, CTZ, MP and PP in liquid pharmaceutical formulation are: the method should be able to determine assay of four compounds in single run and should be accurate, reproducible, robust, stability indicating, filter compatible, linear, free of interference from blank / placebo / impurities / degradation products and straightforward enough for routine use in quality control laboratory.

The spiked solution of AMB (120 μg/mL), CTZ (20 μg/mL), MP (40 μg/mL) and PP (4 μg/mL) is subjected to separation by RP-UPLC. Label claim of compounds and its working concentration is presented in Table 3.2.

Table 3.2 Formulation label claim with its working concentration

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formulation label claim per 5 mL</th>
<th>Working concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg/mL</td>
</tr>
<tr>
<td>AMB</td>
<td>Ambroxol hydrochloride 30 mg</td>
<td>0.12</td>
</tr>
<tr>
<td>CTZ</td>
<td>Cetirizine hydrochloride 5 mg</td>
<td>0.02</td>
</tr>
<tr>
<td>MP</td>
<td>Methylparaben 10 mg</td>
<td>0.04</td>
</tr>
<tr>
<td>PP</td>
<td>Propylparaben 1 mg</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Initially the separation of all compounds is studied using water as a MP-A and acetonitrile as a MP-B on UPLC column (Eclipse Plus C18, RRHD, 50 x 2.1 mm; 1.8 μm) and Waters (UPLC) system with the linear gradient program. The flow rate of 0.5 mL/min is selected with regards to the backpressure and analysis time as well. During this study column oven temperature is capped at 50°C. When study performed with above condition we observed broad peak of all the compounds.
Various types of MP-A and B are studied to optimize the method, which are summarized in Table 3.3 with the observation. Based on above solvent selection study optimized UPLC parameters are; flow rate 0.5 mL/min; column oven temperature 50°C; gradient solvent program as per Table 3.1; 0.01M phosphate buffer in 0.1% triethylamine as a MP-A and acetonitrile as a MP-B.

### Table 3.3 Summary of solvent used to optimize the method

<table>
<thead>
<tr>
<th>MP-A</th>
<th>MP-B</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Retention time (t_R)</td>
<td>USP tailing</td>
</tr>
<tr>
<td>Water</td>
<td>Acetonitrile</td>
<td>AMB=0.947; MP=1.373</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CTZ=2.016; PP=2.748</td>
</tr>
<tr>
<td>0.1M KH₂PO₄</td>
<td>Acetonitrile</td>
<td>AMB=1.101; MP=1.451</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CTZ=2.234; PP=2.851</td>
</tr>
<tr>
<td>0.1M KH₂PO₄ buffer (pH 3.0 with H₃PO₄)</td>
<td>Acetonitrile</td>
<td>AMB=1.223; MP=1.477</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CTZ=2.019; PP=2.835</td>
</tr>
<tr>
<td>0.01M KH₂PO₄ + 0.1% triethylamine</td>
<td>Acetonitrile</td>
<td>AMB=2.185; MP=0.605</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CTZ=1.217; PP=1.389</td>
</tr>
</tbody>
</table>

USP = United state pharmacopoeia

In order to achieve symmetrical peak of all substances and more resolution between CTZ and PP different stationary phases are explored. Peak merging (CTZ and PP) is observed with Acquity BEH C8 (50 x 2.1 mm, 1.7µm) column. Poor resolution (Rₛ=2.3 between CTZ and PP) is observed with Acquity BEH C18 (50 x 2.1 mm, 1.7µm) column. Finally desired separation with symmetrical peaks is obtained using Eclipse Plus C18, RRHD (50 x 2.1mm, 1.8µm) column. Column oven temperature is also studied (at low temperature and 50°C) and found that 50°C is more appropriate with respect to separation and peak shape. Based on compounds UV spectrums 237nm is found more appropriate for the simultaneous determination. UV spectra and IUPAC name of AMB, CTZ, MP and PP are presented in Figure 3.6.
Ambroxol hydrochloride (AMB)
(1s,4s)-4-((2-amino-3,5-dibromocyclohexyl)methylamino)cyclohexanol hydrochloride

Cetirizine hydrochloride (CTZ); Cetirizine dihydrochloride
2-(2-(4-(4-chlorophenyl)(phenyl)methyl)piperazine-1-yl)ethoxy)acetic acid

Methylparaben (MP): Methyl 4-hydroxybenzoate
Propylparaben (PP): Propyl 4-hydroxybenzoate

[Figure 3.6  UV spectra and IUPAC name of AMB, CTZ, MP and PP]

AMB, CTZ, MP and PP are well resolved with each other and also well resolved with all twelve known impurities/ degradation products in reasonable time of 3.5 minutes which is presented in Figure 3.7. There is no chromatography interference due to blank (diluent) and excipients (placebo) at the retention time of AMB, CTZ, MP and PP which is presented in Figure 3.8.
3.7.2 Analytical Parameters and Validation

After satisfactory development of RP-UPLC method it is subjected to method validation as per ICH guidelines [44]. The method is validated to demonstrate that it is suitable for its intended purpose by the standard procedure to evaluate adequate validation characteristics (system suitability, accuracy, precision, linearity, robustness, solution stability, filter compatibility and stability indicating capability).

3.7.2.1 Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities [44]. There is no any interferences at the RT (retention time) of AMB, CTZ, MP and PP due to blank, placebo, impurities and degradation products [Figure 3.7 and 3.8]. Peak purity plot of AMB, CTZ, MP and PP are presented in Figure 3.9.
Simultaneous Determination of AMB, CTZ, MP and PP in Liquid Pharmaceutical Formulation

**Figure 3.9 Spectral purity plot of AMB, CTZ, MP and PP**

Chemical name of all impurities with its purity plot are presented in Figure 3.10.

**AMB IMP-A**
(2-amino-3,5-dibromophenyl)methanol

**AMB IMP-B**
trans-4-(6,8-dibromo-1,4-dihydroquinazolin-3(2H)-yl)cyclohexanol

**AMB IMP-C**
trans-4-[(E)-2-amino-3,5-dibromobenzyliden] amino]cyclohexanol
**Chapter 3**

**AMB IMP-D**

cis-4-[(2-amino-3,5-dibromobenzyl)amino] cyclohexanol

![Structure of AMB IMP-D](image1.png)

**AMB IMP-E**

2-amino-3,5-dibromobenzaldehyde

![Structure of AMB IMP-E](image2.png)

**CTZ IMP-A**

R1 = R2 = H, R3 = Cl:
(RS)-1-[(4-chlorophenyl)phenylmethyl] piperazine

![Structure of CTZ IMP-A](image3.png)

**CTZ IMP-B**

R1 = CH$_2$-CO$_2$H, R2 = H, R3 = Cl:
(RS)-2-[4-[(4-chlorophenyl)phenylmethyl] piperazin-1-yl]acetic acid

![Structure of CTZ IMP-B](image4.png)
Simultaneous Determination of AMB, CTZ, MP and PP in Liquid Pharmaceutical Formulation

CTZ IMP-C
R1=CH₂-CH₂-O-CH₂-CO₂H, R2=Cl, R3=H: (RS)-2-[(2-[4-[(2-chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy)acetic acid

CTZ IMP-D
1,4-bis[(4-chlorophenyl)phenylmethyl]piperazine

CTZ IMP-E
R1=CH₂-[CH₂-O-CH₂]₂-CO₂H, R2=H, R3=Cl: (RS)-2-[(2-[(4-chlorophenyl)phenylmethyl][piperazin-1-yl]ethoxy]ethoxy]acetic acid (ethoxycetirizine)

CTZ IMP-F
R1 = CH₂-CH₂-O-CH₂-CO₂H, R2 = R3 = H: [2-[(4-(diphenylmethyl)piperazin-1-yl]ethoxy]acetic acid
Degradation are observed when the drug product is subjected to acid hydrolysis (0.1N HCl, 60°C, 1h, Figure 3.11), base hydrolysis (0.1N NaOH, 60°C, 1h, Figure 3.12), oxidative (6% H₂O₂, 60°C, 1h, Figure 3.13), thermal (60°C, 1h, Figure 3.14) and photolytic degradation (1.2 million Lux hours, Figure 3.15). Significant degradation is observed when the drug product is subjected to base hydrolysis leading to the formation of unknown impurity after the peak of MP, CTZ impurity-D, E and AMB impurity-B (Figure 3.12). Peaks due to AMB, CTZ, MP and PP are investigated for spectral purity in the chromatogram of all exposed samples and found spectrally pure [Table 3.4].
Simultaneous Determination of AMB, CTZ, MP and PP in Liquid Pharmaceutical Formulation

Figure 3.12 Overlaid chromatograms of base hydrolysis study

Figure 3.13 Overlaid chromatograms of peroxide degradation study

Figure 3.14 Overlaid chromatograms of heat degradation study
Table 3.4  Peak purity data obtained from forced degradation study

<table>
<thead>
<tr>
<th>Stress conditions</th>
<th>AMB</th>
<th>CTZ</th>
<th>MP</th>
<th>PP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unstressed sample</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>0.1N HCl at 60°C for 1h</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>0.1N NaOH at 60°C for 1h</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>6 % H₂O₂ at 60°C for 1h</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Heat at 60°C for 1h</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Photolytic (1.2 million Lux hours)</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

$=$For waters UPLC system,

Purity flag: No, which indicates that purity angle is less than purity threshold and
Purity flag: Yes, which indicates that purity angle is more than purity threshold

3.7.2.2  System suitability

System suitability results from precision and intermediate precision study are summarized in Table 3.5 with its proposed acceptance criteria. The percentage RSD of area counts of six replicate injections is below 1.0 %, which indicates that the system is precise. The parameters all complied with the acceptance criteria and system suitability is established. Overlaid chromatograms of six replicate standards are presented in Figure 3.16.
Table 3.5  System suitability results (precision and intermediate precision study)

<table>
<thead>
<tr>
<th>Test</th>
<th>Parameters</th>
<th>MP</th>
<th>CTZ</th>
<th>PP</th>
<th>AMB</th>
<th>Proposed criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precision (n=6)</td>
<td>USP resolution</td>
<td>--</td>
<td>--</td>
<td>4.34</td>
<td>--</td>
<td>NLT 3.5</td>
</tr>
<tr>
<td></td>
<td>USP tailing</td>
<td>1.3</td>
<td>1.0</td>
<td>1.0</td>
<td>0.9</td>
<td>NMT 1.5</td>
</tr>
<tr>
<td></td>
<td>USP plate count</td>
<td>4549</td>
<td>24009</td>
<td>18630</td>
<td>33643</td>
<td>NLT 3000</td>
</tr>
<tr>
<td></td>
<td>Area % RSD</td>
<td>0.2</td>
<td>0.2</td>
<td>0.4</td>
<td>0.1</td>
<td>NMT 2.0%</td>
</tr>
<tr>
<td>Intermediate precision (n=6)</td>
<td>USP resolution</td>
<td>--</td>
<td>--</td>
<td>4.35</td>
<td>--</td>
<td>NLT 3.5</td>
</tr>
<tr>
<td></td>
<td>USP tailing</td>
<td>1.3</td>
<td>1.0</td>
<td>1.0</td>
<td>0.9</td>
<td>NMT 1.5</td>
</tr>
<tr>
<td></td>
<td>USP plate count</td>
<td>4656</td>
<td>23951</td>
<td>18444</td>
<td>33537</td>
<td>NLT 3000</td>
</tr>
<tr>
<td></td>
<td>Area % RSD</td>
<td>0.3</td>
<td>0.2</td>
<td>0.5</td>
<td>0.2</td>
<td>NMT 2.0%</td>
</tr>
</tbody>
</table>

NLT= Not less than; NMT= Not more than; USP=United State Pharmacopeia

[Figure 3.16  Overlaid chromatograms of six replicate standard injections]

3.7.2.3  Precision

The precision of the assay method is evaluated by carrying out six independent determination of AMB, CTZ, MP and PP (120 µg/mL of AMB, 20 µg/mL of CTZ, 40 µg/mL of MP and 4 µg/mL of PP) test samples against qualified working standard. The method precision study shows the repeatability of the results obtained by the testing method. The % RSD (n=6) is 0.3 % for AMB, 0.5
% for CTZ, 0.4 % for MP and 0.7 % for PP, which are well within the acceptable limit of 2.0%. It is confirmed from results that the method is precise for the intended purpose [Table 3.6].

The purpose of this study is to demonstrate the reliability of the test results with variations. The reproducibility is checked by analyzing the samples by different analyst using different chromatographic system and column on different day. The analysis is conducted in the same manner as the method precision and the % RSD of all six sets of sample preparations is determined [Table 3.6]. The % RSD is 0.4 % for AMB, 0.6 % for CTZ, 0.7 % for MP and 0.9 % for PP, which are well within the acceptance criteria of 2.0%, so this study proves that the method to be rugged enough for day to day use. Overlaid chromatograms of precision and intermediate precision study are presented in Figure 3.17 and Figure 3.18 respectively.

### Table 3.6  Precision (n=6) and Intermediate precision (n=6) results

<table>
<thead>
<tr>
<th>Substance</th>
<th>Precision at 100%</th>
<th>Intermediate precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean % assay</td>
<td>% RSD</td>
</tr>
<tr>
<td>AMB</td>
<td>101.1</td>
<td>0.3</td>
</tr>
<tr>
<td>CTZ</td>
<td>99.3</td>
<td>0.5</td>
</tr>
<tr>
<td>MP</td>
<td>98.1</td>
<td>0.4</td>
</tr>
<tr>
<td>PP</td>
<td>97.5</td>
<td>0.7</td>
</tr>
</tbody>
</table>

[Figure 3.17  Overlaid chromatograms of precision study]
3.7.2.4 Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method compared with the true values. To confirm the accuracy of the proposed method, recovery experiments are carried out by standard addition technique. The accuracy of the method is carried out by adding known amounts of each drug corresponding to three concentration levels; 50, 100, and 150% of the label claim [Table 3.2] along with the excipients in triplicate. The samples are given the same treatment as described in sample preparation. The percentage recoveries of AMB, CTZ, MP and PP at each level and each replicate are determined. The mean of percentage recoveries (n=3) and the relative standard deviation is calculated. The amount recovered is within ±1.0 % of amount added, which indicates that there is no interference due to excipients present in liquid oral formulation. It is confirmed from results that the method is highly accurate [Table 3.7]. Accuracy study chromatograms are presented in Figure 3.19.
Table 3.7  Accuracy results

<table>
<thead>
<tr>
<th>Substance</th>
<th>At 50% (n=3)</th>
<th></th>
<th>At 100% (n=3)</th>
<th></th>
<th>At 150% (n=3)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Recovery</td>
<td>% RSD</td>
<td>% Recovery</td>
<td>% RSD</td>
<td>% Recovery</td>
<td>% RSD</td>
</tr>
<tr>
<td>AMB</td>
<td>100.1</td>
<td>0.3</td>
<td>99.8</td>
<td>0.2</td>
<td>99.8</td>
<td>0.2</td>
</tr>
<tr>
<td>CTZ</td>
<td>99.7</td>
<td>0.4</td>
<td>99.9</td>
<td>0.3</td>
<td>100.2</td>
<td>0.2</td>
</tr>
<tr>
<td>MP</td>
<td>100.2</td>
<td>0.3</td>
<td>99.8</td>
<td>0.2</td>
<td>99.7</td>
<td>0.2</td>
</tr>
<tr>
<td>PP</td>
<td>100.6</td>
<td>0.5</td>
<td>100.4</td>
<td>0.4</td>
<td>99.6</td>
<td>0.6</td>
</tr>
</tbody>
</table>

[Figure 3.19  Overlaid chromatograms of accuracy study]

3.7.2.5  Linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in sample within a given range. The nominal concentrations of standard and test solutions for AMB, CTZ, MP and PP are 120, 20, 40 and 4 μg/mL, respectively. The response function is determined by preparing standard solutions at seven different concentration levels ranging from 30.04-240.32 μg/mL for AMB, 5.01-40.08 μg/mL for CTZ, 9.97-79.76 μg/mL for MP and 1.005-8.04 μg/mL for PP (25 to 200% of analyte concentration). The response is found linear from 25% to 200% of standard concentration. For all compounds the correlation coefficient is greater than 0.999. The regression statistics are shown in Table 3.8. Overlaid specimen chromatograms of linearity study are presented in Figure 3.20. Linearity plot are also presented in Figure 3.21-3.24.
Table 3.8  Regression statistics

<table>
<thead>
<tr>
<th>Compound</th>
<th>Linearity range (µg/mL)</th>
<th>Correlation coefficient ($r^2$)</th>
<th>Linearity (Equation)</th>
<th>Y- intercept bias in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMB</td>
<td>30.04 to 240.32</td>
<td>0.9996</td>
<td>$y = 9020.7497(x) + 6130.5328$</td>
<td>0.557</td>
</tr>
<tr>
<td>CTZ</td>
<td>5.01 to 40.08</td>
<td>0.9998</td>
<td>$y = 11111.220(x) - 263.1311$</td>
<td>-0.117</td>
</tr>
<tr>
<td>MP</td>
<td>9.97 to 79.76</td>
<td>0.9997</td>
<td>$y = 18542.8499(x) + 5084.6885$</td>
<td>0.676</td>
</tr>
<tr>
<td>PP</td>
<td>1.005 to 8.04</td>
<td>0.9997</td>
<td>$y = 16185.3682(x) - 118.5082$</td>
<td>-0.181</td>
</tr>
</tbody>
</table>

[Figure 3.20  Overlaid chromatograms of linearity study]

$y = 9020.7497x + 6130.5328$
$R^2 = 0.9996$

[Figure 3.21  Linearity of Ambroxol hydrochloride]
[Figure 3.22  Linearity of Cetirizine hydrochloride]

\[ y = 11111.1220x - 263.1311 \]
\[ R^2 = 0.9998 \]

[Figure 3.23  Linearity of Methylparaben]

\[ y = 18,542.8499x + 5,084.6885 \]
\[ R^2 = 0.9997 \]

[Figure 3.24  Linearity of Propylparaben]

\[ y = 16185.3682x - 118.5082 \]
\[ R^2 = 0.9997 \]
3.7.2.6 Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness parameters are selected based on critical method attribute. The effect of change in flow rate (± 0.05 mL/min) and column oven temperature (± 5°C) on the retention time, resolution (between CTZ and PP), theoretical plates and tailing factor are studied. During study other chromatographic conditions are kept same as per the experimental section. It is conformed from results that the method is robust with respect to variability in above conditions [Table 3.9]. Robustness study chromatograms are presented in Figure 3.25 and 3.26.

Table 3.9 Robustness study results

<table>
<thead>
<tr>
<th>Condition</th>
<th>Parameters</th>
<th>MP</th>
<th>CTZ</th>
<th>PP</th>
<th>AMB</th>
<th>Proposed criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal methodology</td>
<td>USP resolution</td>
<td>--</td>
<td>--</td>
<td>4.34</td>
<td>--</td>
<td>NLT 3.5</td>
</tr>
<tr>
<td></td>
<td>USP tailing</td>
<td>1.3</td>
<td>1.0</td>
<td>1.0</td>
<td>0.9</td>
<td>NMT 1.5</td>
</tr>
<tr>
<td></td>
<td>USP plate count</td>
<td>4549</td>
<td>24009</td>
<td>18630</td>
<td>33643</td>
<td>NLT 3000</td>
</tr>
<tr>
<td></td>
<td>Retention time in min</td>
<td>0.603</td>
<td>1.224</td>
<td>1.385</td>
<td>2.183</td>
<td>--</td>
</tr>
<tr>
<td>At flow rate 0.45 mL/min</td>
<td>USP resolution</td>
<td>--</td>
<td>--</td>
<td>4.79</td>
<td>--</td>
<td>NLT 3.5</td>
</tr>
<tr>
<td></td>
<td>USP tailing</td>
<td>1.3</td>
<td>1.0</td>
<td>1.0</td>
<td>0.9</td>
<td>NMT 1.5</td>
</tr>
<tr>
<td></td>
<td>USP plate count</td>
<td>4657</td>
<td>24838</td>
<td>18993</td>
<td>34634</td>
<td>NLT 3000</td>
</tr>
<tr>
<td></td>
<td>Retention time in min</td>
<td>0.667</td>
<td>1.309</td>
<td>1.498</td>
<td>2.319</td>
<td>--</td>
</tr>
<tr>
<td>At flow rate 0.55 mL/min</td>
<td>USP resolution</td>
<td>--</td>
<td>--</td>
<td>3.88</td>
<td>--</td>
<td>NLT 3.5</td>
</tr>
<tr>
<td></td>
<td>USP tailing</td>
<td>1.3</td>
<td>1.0</td>
<td>1.0</td>
<td>0.9</td>
<td>NMT 1.5</td>
</tr>
<tr>
<td></td>
<td>USP plate count</td>
<td>4790</td>
<td>22386</td>
<td>17442</td>
<td>32067</td>
<td>NLT 3000</td>
</tr>
<tr>
<td></td>
<td>Retention time in min</td>
<td>0.549</td>
<td>1.160</td>
<td>1.300</td>
<td>2.081</td>
<td>--</td>
</tr>
<tr>
<td>At 45°C column oven temp.</td>
<td>USP resolution</td>
<td>--</td>
<td>--</td>
<td>5.3</td>
<td>--</td>
<td>NLT 3.5</td>
</tr>
<tr>
<td></td>
<td>USP tailing</td>
<td>1.3</td>
<td>1.0</td>
<td>1.0</td>
<td>0.9</td>
<td>NMT 1.5</td>
</tr>
<tr>
<td></td>
<td>USP plate count</td>
<td>5310</td>
<td>23455</td>
<td>19314</td>
<td>32483</td>
<td>NLT 3000</td>
</tr>
<tr>
<td></td>
<td>Retention time in min</td>
<td>0.628</td>
<td>1.224</td>
<td>1.434</td>
<td>2.186</td>
<td>--</td>
</tr>
<tr>
<td>At 55°C column oven temp.</td>
<td>USP resolution</td>
<td>--</td>
<td>--</td>
<td>3.6</td>
<td>--</td>
<td>NLT 3.5</td>
</tr>
<tr>
<td></td>
<td>USP tailing</td>
<td>1.3</td>
<td>1.0</td>
<td>1.0</td>
<td>0.9</td>
<td>NMT 1.5</td>
</tr>
<tr>
<td></td>
<td>USP plate count</td>
<td>4481</td>
<td>22939</td>
<td>16790</td>
<td>32440</td>
<td>NLT 3000</td>
</tr>
<tr>
<td></td>
<td>Retention time in min</td>
<td>0.580</td>
<td>1.210</td>
<td>1.337</td>
<td>2.172</td>
<td>--</td>
</tr>
</tbody>
</table>
[Figure 3.25  Chromatograms of column oven temperature study]

[Figure 3.26  Chromatograms of flow rate study]
3.7.2.7 Solution stability

Drug stability in pharmaceutical formulations is a function of storage conditions and chemical properties of the drug, preservative and its impurities. Condition used in stability experiments should reflect situations likely to be encountered during actual sample handling and analysis. Stability data is required to show that the concentration and purity of analyte in the sample at the time of analysis corresponds to the concentration and purity of analyte at the time of sampling.

Stability of sample solution is established by storage of sample solution at ambient temperature (25°C) for 24h. Sample solution is re-analyzed after 12 and 24h time intervals and assay are determined for the compounds (AMB, CTZ, MP and PP) and compared against fresh sample. Sample solution does not show any appreciable change in assay value when stored at ambient temperature up to 24h, which are presented in Table 3.10. The results from solution stability experiments confirmed that sample solution is stable for up to 24h during assay determination.

Standard solution is re-injected after 12 and 24h time intervals and % RSD of all injected standard injections are calculated. Standard solution does not show any appreciable change in % RSD (RSD for AMB, CTZ, MP and PP are less than 1.0%) value when stored at ambient temperature up to 24h. Overlaid specimen chromatograms for standard and sample solution stability are presented in Figure 3.27 and 3.28 respectively.

![Overlaid specimen chromatograms of standard solution stability](image)
Table 3.10  Solution stability results

<table>
<thead>
<tr>
<th>Time intervals</th>
<th>AMB</th>
<th>CTZ</th>
<th>MP</th>
<th>PP</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Assay Initial</td>
<td>100.7</td>
<td>99.5</td>
<td>98.0</td>
<td>97.6</td>
</tr>
<tr>
<td>% Assay after 12h</td>
<td>100.3</td>
<td>99.6</td>
<td>98.2</td>
<td>97.7</td>
</tr>
<tr>
<td>% Assay after 24h</td>
<td>100.4</td>
<td>99.3</td>
<td>98.1</td>
<td>97.4</td>
</tr>
</tbody>
</table>

3.7.2.8  Filter compatibility

Filter compatibility is performed for nylon 0.22 μm syringe filter (Pall Life sciences) and PVDF 0.22 μm syringe filter (Millipore). To confirm the filter compatibility in proposed analytical method, filtration recovery experiment is carried out by sample filtration technique. Sample is filtered through both syringe filters and percentage assay is determined and compared against centrifuged sample. Sample solution does not show any significant changes in assay percentage with respect to centrifuged sample. Percentage assay results are presented in Table 3.11. In displayed result difference in % assay is not observed more than ±0.5, which indicates that both syringe filters having a good compatibility with sample solution. Overlaid chromatograms of filter compatibility study are presented in Figure 3.29.
Table 3.11 Filter compatibility results (Assay % w/w)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Centrifuged</th>
<th>PVDF filter 0.22µm (Millipore)</th>
<th>Nylon filter 0.22µm (Pall Life Sciences)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMB</td>
<td>100.5</td>
<td>100.3</td>
<td>100.3</td>
</tr>
<tr>
<td>CTZ</td>
<td>99.7</td>
<td>99.5</td>
<td>99.4</td>
</tr>
<tr>
<td>MP</td>
<td>98.3</td>
<td>98.3</td>
<td>98.5</td>
</tr>
<tr>
<td>PP</td>
<td>97.5</td>
<td>97.6</td>
<td>97.3</td>
</tr>
</tbody>
</table>

[Figure 3.29 Overlaid specimen chromatograms of filter compatibility study]

3.7.3 Application of the method to dosage forms

The present method is applied for the estimation of drugs and preservatives in the commercially available various dosage forms. The results obtained are as shown in Table 3.12. Based on obtained results developed method is suitable for the various marketed dosage forms. Developed method also proves the suitability for preservatives determination in various liquid dosage forms. Representative chromatograms of analysed marketed products are presented in Figure 3.30.
Table 3.12 Results of market products (mg/5 mL for syrup and mg/ tablets for tablets)

<table>
<thead>
<tr>
<th>Product Name and Labeled claim (in mg)</th>
<th>AMB</th>
<th>CTZ</th>
<th>MP</th>
<th>PP</th>
<th>LCTZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZyrCold Syrup [AMB(30); CTZ(2.5)]</td>
<td>29.8</td>
<td>2.47</td>
<td>9.53</td>
<td>0.94</td>
<td>N.A.</td>
</tr>
<tr>
<td>Relent Syrup [AMB(30); CTZ(2.5)]</td>
<td>30.1</td>
<td>2.48</td>
<td>10.10</td>
<td>1.01</td>
<td>N.A.</td>
</tr>
<tr>
<td>ZyrCold Tablets [AMB(30); CTZ(2.5)]</td>
<td>29.7</td>
<td>2.46</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>Cetzine Tablets [CTZ(10)]</td>
<td>N.A.</td>
<td>9.93</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>DOI-1 Tablets [CTZ(10)]</td>
<td>N.A.</td>
<td>9.72</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>Xyzal Syrup [LCTZ (2.5)]</td>
<td>N.A.</td>
<td>N.A.</td>
<td>10.5</td>
<td>1.07</td>
<td>2.51</td>
</tr>
</tbody>
</table>

N.A.; not applicable

[Figure 3.30 Specimen chromatograms of analysed marketed products]
3.8  CALCULATION FORMULA

3.8.1  Assay (% w/w)

Calculated the quantity, in mg, of compound (AMB, CTZ, MP and PP) in the portion of liquid pharmaceutical formulation using the following formula:

\[
\text{Assay (% w/w)} = \frac{C_{\text{std}} \times R_s \times 10,000}{C_s \times R_{\text{std}}}
\]

Where,

- \(C_{\text{std}}\) = Concentration of standard solution in mg/mL
- \(C_s\) = Concentration of sample solution in mg/mL
- \(R_s\) = Compound peak response obtained from the sample preparation
- \(R_{\text{std}}\) = Compound peak response (mean peak area) obtained from the standard preparation

3.8.2  Relative standard deviation (% RSD)

It is expressed by the following formula and calculated using Microsoft excel program in a computer.

\[
\text{Related Standard Deviation} (\%) = \frac{SD \times 100}{\bar{X}}
\]

Where,

- SD = Standard deviation of measurements
- \(\bar{X}\) = Mean value of measurements

3.8.3  Accuracy (% Recovery)

It is calculated using the following equation:

\[
\% \text{ Recovery} = \frac{\text{Amount of substance found (mg)} \times 100}{\text{Amount of substance added (mg)}}
\]
3.9 CONCLUSION

A gradient RP-UPLC method is successfully developed for the simultaneous estimation of AMB, CTZ, MP and PP in liquid pharmaceutical formulation. The developed method is selective, precise, accurate, linear, filter compatible and robust. Forced degradation data proves that the method is specific for the analytes and free from the interference of placebo / known impurities / degradation products and unknown degradation products. The run time (3.5 min) enables for rapid determination of drugs and preservatives. Moreover, it may be applied for individual and simultaneous determination of AMB, CTZ, LCTZ, MP and PP compound in pharmaceutical drug product and substance. Also it can be utilized for determination of assay, blend uniformity and content uniformity of pharmaceutical products (CTZ tablets and AMB+CTZ tablets), where sample load is higher and high throughput is essential for faster delivery of results.

Note: Intellectual Property Management (IPM) clearance number for the present research work is: PUB-00099-11.
3.10 REFERENCES


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