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# Method Development and Validation of Simultaneous Estimation of Pseudoephedrine Ambroxol Desloratadine in Tablet Dosage Form and Degradation Studies By Rp-Hplc Method

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**Abstract**

A simple, Accurate, precise method was developed for the simultaneous estimation of the Pseudoephedrine, Ambroxol and Desloratadine in Tablet dosage form. Chromatogram was run through ODS 250mm x 4.6 mm, 5µ. Mobile phase containing Buffer, Acetonitrie and Methanol in the ratio of 50:20A:30M was pumped through column at a flow rate of 1ml/min. Buffer used in this method was 0.01N $KH_2PO_4$ buffer at pH 3.2. Temperature was maintained at 30°C. Optimized wavelength for Pseudoephedrine and Ambroxol was 225nm. Retention time of Pseudoephedrine, Ambroxol and Desloratadine were found to be 2.417min, 4.518min and 7.464min respectively. %RSD of the Pseudoephedrine, Ambroxol and Desloratadine were and found to be 1.03 0.64 and 1.15respectively. %Recover was Obtained as 99.92%, 100.21% and 99.92% for Pseudoephedrine, Ambroxol and Desloratadine respectively. LOD, LOQ values are obtained from regression equations of Pseudoephedrine(0.01ppm, 0.02ppm), Ambroxol(0.15ppm, 1.44ppm) and Desloratadine(0.06ppm, 0.17ppm). Regression equation of Pseudoephedrine is y = 16947x + 319.2 Ambroxol is y = 19401x + 3236.and Desloratidine y = 38993x +2051.

**Key Words:** Pseudoephedrine, Ambroxol and Desloratadine RP-HPLC

## 1. Introduction

**Pharmaceutical Analysis** is that core branch of pharmacy education and research, which is advancing very fast. It can be categorized as synthesis of new drugs molecules and pharmaceutical analysis. Analytical chemistry is the science of making quantitative and qualitative measurements. In practice, quantifying an analyte in a complex sample becomes an exercise in problem solving. To be efficient and effective, an analytical chemist must know the tools that are available to tackle a wide variety of problems. Analytical chemistry is divided into two branches qualitative and quantitative. A qualitative method provides information about the identity of atomic or molecular species or functional groups in sample. A quantitative

method provides numerical information as to the relative amount of one or more of the components. Varieties of analytical methods are used for the analysis of drugs in bulk, formulations and biological samples. In pharmaceutical industry, spectrophotometric and chromatographic methods have gained the significance in recent years. Spectrophotometric methods3,4 It is defined as a method of analysis that embraces the measurement of absorption by chemical species of radiant energy at definite and narrow wavelength approximating monochromatic radiation. The electromagnetic spectrum extends from 100-780 nm. Traditionally, analytical chemistry has been split into two main types,

**Qualitative And Quantitative:** Qualitative Inorganic Analysis seeks to establish the presence of a given element or inorganic compound in a sample. Qualitative Organic Analysis seeks to establish the presence of a given functional group or organic compound in a sample. Quantitative analysis seeks to establish the amount of a given element or compound in a sample. There are various techniques used for analysis of mixtures. Spectroscopy measures the interaction of the molecules with electromagnetic radiation. Chromatography is the collective term for a family of laboratory techniques for the separation of mixtures. It involves passing a mixture dissolved in a "mobile phase" through a stationary phase, which separates the analyte to be measured from other molecules in the mixture and allows it to be isolated. Analytical Chromatography is used to determine the existence and possibly also the concentration of analyte(s) in a sample6. Analytical chemistry has played critical roles in the understanding of basic science to a variety of practical applications, such as biomedical applications, environmental monitoring, quality control of industrial manufacturing, forensic science and so on.

Various methods have been reported in literature to detect and quantify the individual drugs pseudoephedrine, ambroxal and desloratadine. But there is no method has been reported for the simultaneous estimation of pseudoephedrine, ambroxal and desloratadine. Hence, it felt that, there is a need of new stability and analytical method development with a simple, accurate and precise RP-HPLC method for the simultaneous determination of the three drugs in formulation.

Present work is aimed to develop and validate the proposed method for the intended analytical application.
To apply the proposed methods for analysis of these drugs in their dosage form.

## 2.Drug Profile

### Pseudoephedrine:

Pseudoephedrine is a decongestant that shrinks blood vessels in the nasal passages. Dilated blood vessels can cause nasal congestion (stuffy nose).Pseudoephedrine is used to treat nasal and sinus congestion, or congestion of the tubes that drain fluid from your inner ears, called the eustachian (yoo-STAY-shun) tubes.Pseudoephedrine may also be used for purposes not listed in this medication.

**Description:**

An alpha- and beta-adrenergic agonist that may also enhance neither release of nor epinephrine. It has been used in the treatment of several disorders including asthma, heart failure, rhinitis, and urinary incontinence, and for its central nervous system stimulatory effects in the treatment of narcolepsy and depression. It has become less extensively used with the advent of more selective agonists.

**Pharmacodynamics:** Pseudoephedrine is a sympathomimetic agent, structurally similar to ephedrine, used to relieve nasal and sinus congestion and reduce air-travel-related otalgia in adults. The salts pseudoephedrine hydrochloride and pseudoephedrine sulfate are found in many over-the-counter preparations either as single-ingredient preparations, or more commonly in combination with antihistamines and/or paracetamol/ibuprofen. Unlike antihistamines, which modify the systemic histamine-mediated allergic response, pseudoephedrine only serves to relieve nasal congestion commonly associated with colds or allergies. The advantage of oral pseudoephedrine over topical nasal preparations, such as oxymetazoline, is that it does not cause rebound congestion (rhinitis medicamentosa).

**Mechanism of Action**:

Pseudoephedrine acts directly on both alpha- and, to a lesser degree, beta-adrenergic receptors. Through direct action on alpha-adrenergic receptors in the mucosa of the respiratory tract, pseudoephedrine produces vasoconstriction. Pseudoephedrine relaxes bronchial smooth muscle by stimulating beta2-adrenergic receptors. Like ephedrine, pseudoephedrine releasing nor-epinephrine from its storage sites, an indirect effect. This is its main and direct mechanism of action. The displaced nor-adrenaline is released into the neuronal synapse where it is free to activate the postsynaptic adrenergic receptors.

**Side Effects**

Get emergency medical help if you have any of these signs of an allergic reaction to pseudoephedrine: hives; difficulty breathing; swelling of your face, lips, tongue, or throat. Stop using pseudoephedrine and call your doctor at once if you have a serious side effect such as:fast, pounding, or uneven heartbeat;severe dizziness or anxiety;easy bruising or bleeding, unusual weakness, fever, chills, body aches, flu symptoms; ordangerously high blood pressure (severe headache, blurred vision, ringing in your ears, anxiety, confusion, chest pain, trouble breathing, uneven heart rate, seizure).

**Less serious pseudoephedrine side effects may include:**loss of appetite;warmth, tingling, or redness under your skin;feeling restless or excited (especially in children);sleep problems (insomnia); orskin rash or itchingng. **AMBROXOL**

Ambroxol is a secretolytic agent used in the treatment of respiratory diseases associated with viscid or excessivemucus. It is the active ingredient of Mucosolvan, Mucobrox, Mucol, Lasolvan, Mucoangin, Surbronc, Ambolar, and Lysopain. The substance is a mucoactive drug with several properties including secretolytic and secretomotoric actions that restore the physiological clearance mechanisms of

the respiratory tract, which play an important role in the body's natural defence mechanisms. It stimulates synthesis and release of surfactant by type II pneumocytes. Surfactant acts as an anti-glue factor by reducing the adhesion of mucus to the bronchial wall, in improving its transport and in providing protection against infection and irritating agents

**Description**

Ambroxol hydrochloride is a small molecule expectorant and mucolytic agent with antioxidant and anti inflammatory effects. Ambroxol stimulates the secretion of surfactant which decreases mucus adhesion to the bronchial lining and produces expectorant effects. The antioxidant effects of Ambroxol are well demonstrated, where lipid oxidation initiated by t-butyl hydroperoxide or doxorubicin is suppressed in the presence Ambroxol, and scavenging of hydroxyl radicals and cellular superoxide radical anions is also observed. Ambroxol produces anti inflammatory effects by blocking the expression of pro inflammatory messengers, inhibiting the release of histamine from mast cells and the generation of cytokines and interleukins. Ambroxol hydrochloride is an inhibitor of Sodium Channel Protein.

**Pharmacodynamics:**

Ambroxol is an active N-desmethyl metabolite of mucolytic bromhexine. Preclinically, ambroxol has been shown to increase the quantity and decrease the viscosity of respiratory tract secretions. It enhances pulmonary surfactant production (surfactant activator) and stimulates ciliary motility. These actions result in improved mucus flow and transport (mucociliary clearance). Improvement of mucociliary clearance has been shown in clinical pharmacologic studies. Enhancement of fluid secretion and mucociliary clearance facilitates expectoration and eases cough. A local anaesthetic effect of ambroxol has been observed in animals (rabbit) which may be explained by the sodium channel blocking properties. It was shown in vitro that ambroxol blocks neuronal sodium channels; binding was reversible and concentration-dependent. Clinical efficacy studies for the treatment with ambroxol of upper respiratory tract symptoms have shown rapid relief of pain and pain related discomfort in the ear-nose-trachea region upon inhalation. Cytokine release from blood but also tissue-bound mononuclear and polymorphonuclear cells was found to be significantly reduced by ambroxol in vitro. Lozenges containing ambroxol have been shown to exert significant effects on pain relief in acute sore throat. Additionally redness in sore throat was significantly reduced. Following the administration of ambroxol, antibiotic concentrations (amoxicilline, cefuroxime, erythromycin) in bronchopulmonary secretions and in the sputum are increased.

**Pharmacokinetics**

**Absorption:** Absorption of all non-delayed oral forms of ambroxol hydrochloride is rapid and complete, with dose linearity in the therapeutic range. Maximum plasma levels are reached within 1 to 2.5 hours following oral administration of the immediate –release formulation and after a median of 6.5 hours of the

slow release formulation. The absolute bioavailability after a 30 mg tablet was found to be 70-80%. The slow release capsule showed a relative availability of 95% (dose-normalized) in comparison to a daily dose of 60 mg (30 mg twice daily) administered as immediate-release tablet.

**Distribution**

In the therapeutic range plasma protein binding was found to be approximately 90%. The distribution half life is 1.3 hours. Distribution of ambroxol hydrochloride from blood to tissue is rapid and pronounced, with the highest concentration of the active substance found in the lungs. The volume of distribution following oral administration was estimated to be 552L.

**Metabolism**

About 30% of an orally administered dose is eliminated via first pass metabolism. Studies in human liver microsomes have shown that CYP3A4 enzyme is responsible for the metabolism of ambroxol. Ambroxol hydrochloride is metabolized primarily in the liver by glcuronidation and some cleavage to di-bromanthranilic acid (approximately 10% of dose) aside from some minor metabolites.

**Excretion**

Ambroxol hydrochloride is eliminated with a terminal elimination half-life of approximately 10 hours. Total clearance is in the range of 660 ml/min, with renal clearance accounting for approximately 8% of the total clearance.

**Mechanism of Action and Pharmacodynamic effects**

Ambroxol is the active metabolite of bromhexine. Ambroxol causes an increase in secretion in the respiratory tract. It promotes surfactant production and stimulates ciliary activity. These effects assist the flow of mucus and its removal (mucociliary clearance). An improvement in mucociliary clearance was demonstrated in clinical pharmacological studies. The increase in secretion and mucociliary clearance facilitate expectoration and reduce the cough. In in vitro studies ambroxol showed a significant reduction in cytokine release, both in the blood and in mononuclear and polynuclear cells. The clinical relevance of these findings is unclear.

**Drug Interactions:**

Anti-tussives Concomitant administration of anti-tussives may impair the expectoration of liquefied bronchial mucus due to inhibition of the cough reflex and cause congestion of secretions (see Section

Warnings and Precautions). Antibiotics After using ambroxol the concentrations of the antibiotics amoxicillin, cefuroxime and erythromycin in bronchial secretions and sputum are increased.

D**esloratadine:**

Desloratadine is used to relieve hay fever and allergy symptoms, including sneezing; runny nose; and red, itchy, tearing eyes. It is also used to treat hives. Desloratadine is in a class of medications called antihistamines. It works by blocking histamine, a substance in the body that causes allergic symptoms

**Description**: Desloratadine is a second generation, tricyclic antihistamine that which has a selective and peripheral H1-antagonist action. It is the active descarboethoxy metabolite of loratidine (a second generation histamine). Desloratidine has a long-lasting effect and does not cause drowsiness because it does not readily enter the central nervous system.

**Mechanism of action:** Desloratadine is a tricyclic antihistamine, which has a selective and peripheral $H_1$-antagonist action. It is an antagonist at histamine H1 receptors, and an antagonist at all subtypes of the muscarinic acetylcholine receptor. It has a long-lasting effect and in moderate and low doses, does not cause drowsiness because it does not readily enter the central nervous system.[2] Unlike other antihistamines, desloratadine is also effective in relieving nasal congestion, particularly in patients with allergic rhinitis. [3]

**Pharmacokinetics**

***Absorption***

$T_{max}$ is approximately 3 h. $C_{max}$ is approximately 4 ng/mL. AUC is approximately 56.9 ng•h/mL.

***Distribution***

82% to 87% protein bound.

***Metabolism***

Metabolized to 3-hydroxydesloratadine (active).

***Elimination***

The half-life is 27 h. approximately 87% is excreted in urine and feces.

**SIDE EFFECTS:** Most common side-effects are fatigue, dry mouth, headache, and gastro intestinal upset stomach

Dizziness sore throat dry mouth muscle pain extreme tiredness painful menstruation Some side effects can be serious. The following symptom is uncommon, but if you experience it, call your doctor immediately:

**Materials and Methods**

**Materials:**

Pseudoephedrine, Ambroxol, and Desloratadine active pharmaceutical ingredients. Pseudoephedrine, Ambroxol and Desloratadine Formulation, distilled water, acetonitrile, phosphate buffer, ammonium acetate buffer, glacial acetic acid, methanol, potassium dihydrogen phosphate buffer, tetra hydro furan, tri ethyl amine, ortho-phosphoric acid etc.

**Instrument:**

HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Pseudoephedrine and Ambroxol and Desloratadine solutions.

**Buffer: (0.01N $KH_2PO_4$)**

Accurately weighed 1.36gm of Potassium dihydrogen ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water and pH adjusted to 3.0 with dil. OPA.

**Preparation of Solutions:**

**Standard Preparation**: (100µg/ml Pseudoephrine, 100µg/ml Ambroxol and 100 µg/ml of Des loratidine)

Accurately Weighed and transferred 5mg of Pseudoephrine, 12mg of Ambroxol and 5mg of Des loratidine working Standards into a 10 ml clean dry volumetric flask, add 7ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solution, 1 ml was pipette out in to a 10ml volumetric flask and then make up to the final volume with diluents.

**Sample Preparation:**

5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 5 tablets was transferred into a 50ml volumetric flask, 30ml of diluents added and sonicated for 30 min, further the volume made up with diluents and filtered. From the filtered solution 0.2ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluents.

**Validation:**

**System suitability parameters:**

The system suitability parameters were determined by preparing standard solutions of Pseudoephedrine, Ambroxol and desloratadine and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

The % RSD for the area of six standard injections results should not be more than 2%.

**Specificity:** Checking of the interference in the optimized method. We should not found interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

**Precision:**

**Standard Preparation**:

Accurately Weighed and transferred 5mg of Pseudoephrine, 12mg of Ambroxol and 5mg of Des loratidine working Standards into a 10 ml clean dry volumetric flask, add 7ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solution, 1 ml was pipeted out in to a 10ml volumetric flask and then make up to the final volume with diluents.

**Sample Preparation:**

5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 5 tablets was transferred into a 50ml volumetric flask, 30ml of diluents added and sonicated for 30 min, further the volume made up with diluents and filtered. From the filtered solution 0.2ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluents.

**Linearity:**

**Standard Preparation**:

Accurately Weighed and transferred 5mg of Pseudoephrine, 12mg of Ambroxol and 5mg of Des loratidine working Standards into a 10 ml clean dry volumetric flask, add 7ml of diluents, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solution, 1 ml was pipeted out in to a 10ml volumetric flask and then make up to the final volume with diluents

**25% Standard solution**: 0.25ml each from three standard stock solutions was pipette out and made up to 10ml.

**50% Standard solution:** 0.5ml each from three standard stock solutions was pipette out and made up to 10ml.

**75% Standard solution:** 0.75ml each from three standard stock solutions was pipette out and made up to 10ml.

**100% Standard solution:** 1.0ml each from three standard stock solutions was pipette out and made up to 10ml.

**125% Standard solution:** 1.25ml each from three standard stock solutions was pipette out and made up to 10ml.

**150% Standard solution:** 1.5ml each from three standard stock solutions was pipette out and made up to 10ml.

**Accuracy:**

**Standard Preparation**:

Accurately Weighed and transferred 5mg of Pseudoephrine, 12mg of Ambroxol and 5mg of Des loratidine working Standards into a 10 ml clean dry volumetric flask, add 7ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solution, 1 ml was pipette out in to a 10ml volumetric flask and then make up to the final volume with diluents

**Preparation of 50% Spiked Solution:** 500mg of drug was taken into a 50ml volumetric flask and made up with diluents followed by filtration with HPLC filters and take 0.2 ml of stock solution in 10 ml of volumetric flask and make up with diluents and labeled as Accuracy 50% Sample stock solution. 1ml from each standard stock solution was pipette out and taken into a 10ml volumetric flask to that 1ml of filtered Accuracy 100% standard stock solution was spiked and made up with diluents.

**Preparation of 100% Spiked Solution:** 1000mg of drug was taken into a 50ml volumetric flask and made up with diluents followed by filtration with HPLC filters and take 0.2 ml of stock solution in 10 ml of volumetric flask and make up with diluents and labeled as Accuracy 100% Sample stock solution. 1ml from each standard stock solution was pipette out and taken into a 10ml volumetric flask to that 1ml of filtered Accuracy 100% Standard stock solution was spiked and made up with diluents.

**Preparation of 150% Spiked Solution: 1**500 mg of drug was taken into a 50ml volumetric flask and made up with diluents followed by filtration with HPLC filters and take 0.2 ml of stock solution in 10 ml of

volumetric flask and make up with diluents and labeled as Accuracy 150% Sample stock solution. 1ml from each standard stock solution was pipette out and taken into a 10ml volumetric flask to that 1ml of filtered Accuracy 100% Standard stock solution was spiked and made up with diluents.

**Acceptance Criteria:**

The % Recovery for each level should be between 98 to 102%.

**Robustness:** Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

**LOD sample Preparation:** 0.25ml each from three standard stock solutions was pipette out and transferred to 3 separate 10ml volumetric flask and made up with diluents from the above solutions 0.3ml, 0.1ml and 0.4ml of Pseudoephrine, Ambroxol and Des loratidine solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents.

**LOQ sample Preparation:** 0.25ml each from three standard stock solutions was pipette out and transferred to 3 separate 10ml volumetric flask and made up with diluents from the above solutions 1.0ml, 0.3ml and 1.3ml of Pseudoephrine, Ambroxol and Des loratidine solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents.

**Degradation studies:**

**Oxidation:**

To 1 ml of stock solutionof Pseudoephrine, Ambroxol and Desloratidine 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at $60^0$c. For HPLC study, the resultant solution was diluted to obtain 10µg/ml, 120 µg/ml and 10µg/ml of all components and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Acid Degradation Studies:**

To 1 ml of stock s solution Phenylephrine, Ambroxol and Desloratidine, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at $60^0$c .The resultant solution was diluted to obtain 10µg/ml, 120µg/ml and 10µg/ml of all components and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Alkali Degradation Studies:**

To 1 ml of stock solution Pseudoephrine, Ambroxol and Desloratidine, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at $60^0$c. The resultant solution was diluted to obtain 10µg/ml, 120µg/ml and 10µg/ml of all components and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Dry Heat Degradation Studies:**

The standard drug solution was placed in oven at $105^0$c for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted obtain 10µg/ml, 120µg/ml and 10µg/ml of all components and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

**Photo Stability studies:**

The photochemical stability of the drug was also studied by exposing the 300µg/ml&10µg/ml&25µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/$m^2$ in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 10µg/ml, 120µg/ml and 10µg/ml of all components and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Neutral Degradation Studies:**

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60º. For HPLC study, the resultant solution was diluted to obtain 10µg/ml, 120µg/ml and 10µg/ml of all components and 10µl were injected into the system and the chromatograms were recorded to

assess the stability of the sample**.**

**3.Figures**:

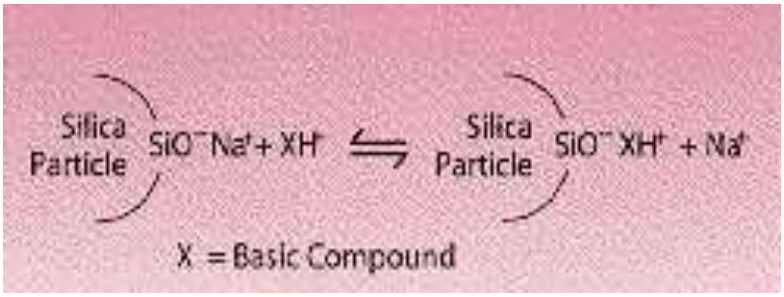


**Fig. 1.1: Peak Tailing Interaction**

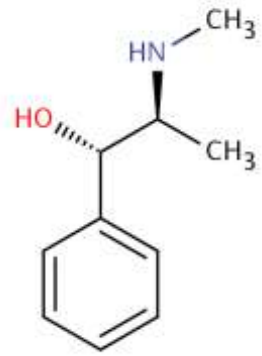


Fig2.1 pseudoephedrine

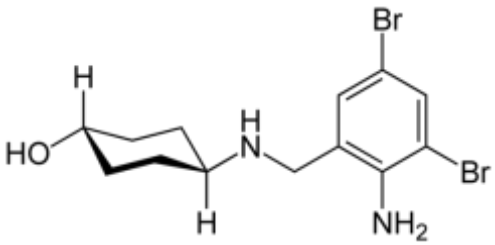


**Fig 2.2 Ambroxol**

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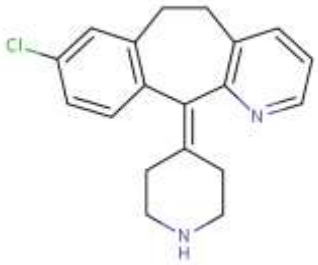



**Fig 2.3 Desloratadine**

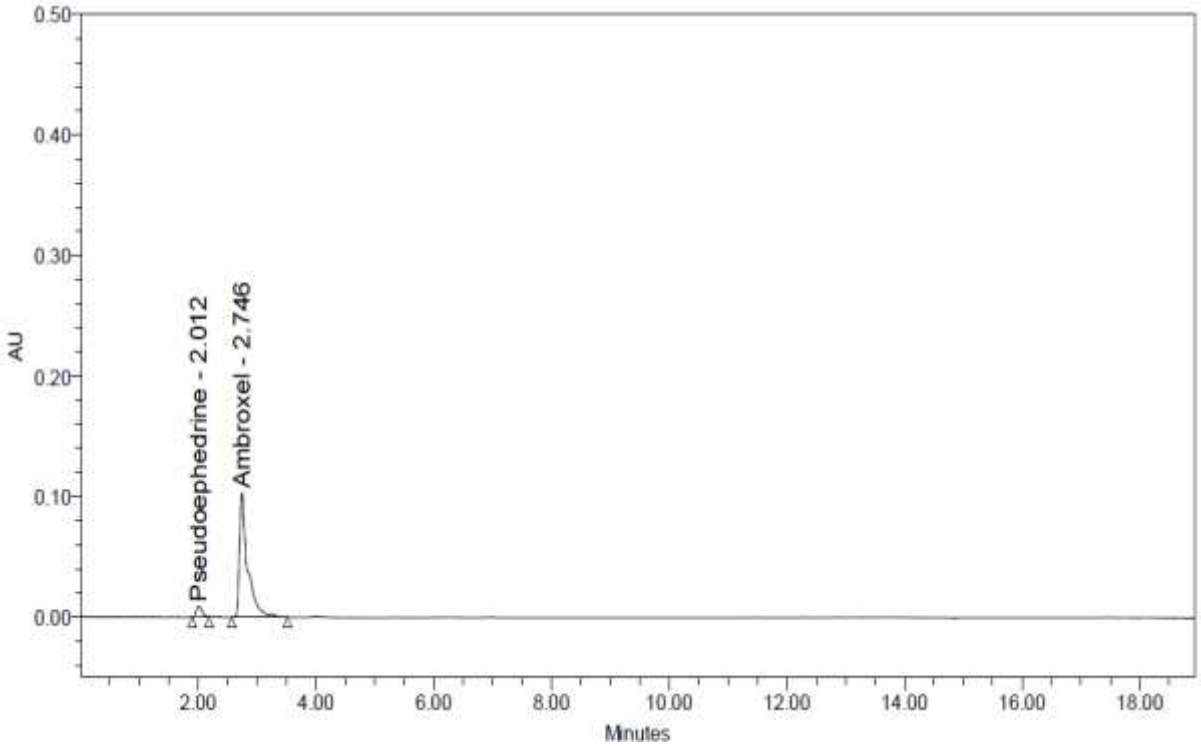



**Fig 6.1 Trial chromatogram 1**

**Observation:** peak shape was not good and desloratidine was not detected so further trails are carried out.

**Trial 2:**

Column : Kromosil 250mm x 4.6 mm, 5µ.
Mobile phase : 50:25A:25M
Buffer : $KH_2PO_4$ 3pH
Flow rate : 1ml/min
Temperature : 30°C
Wavelength : 225nm

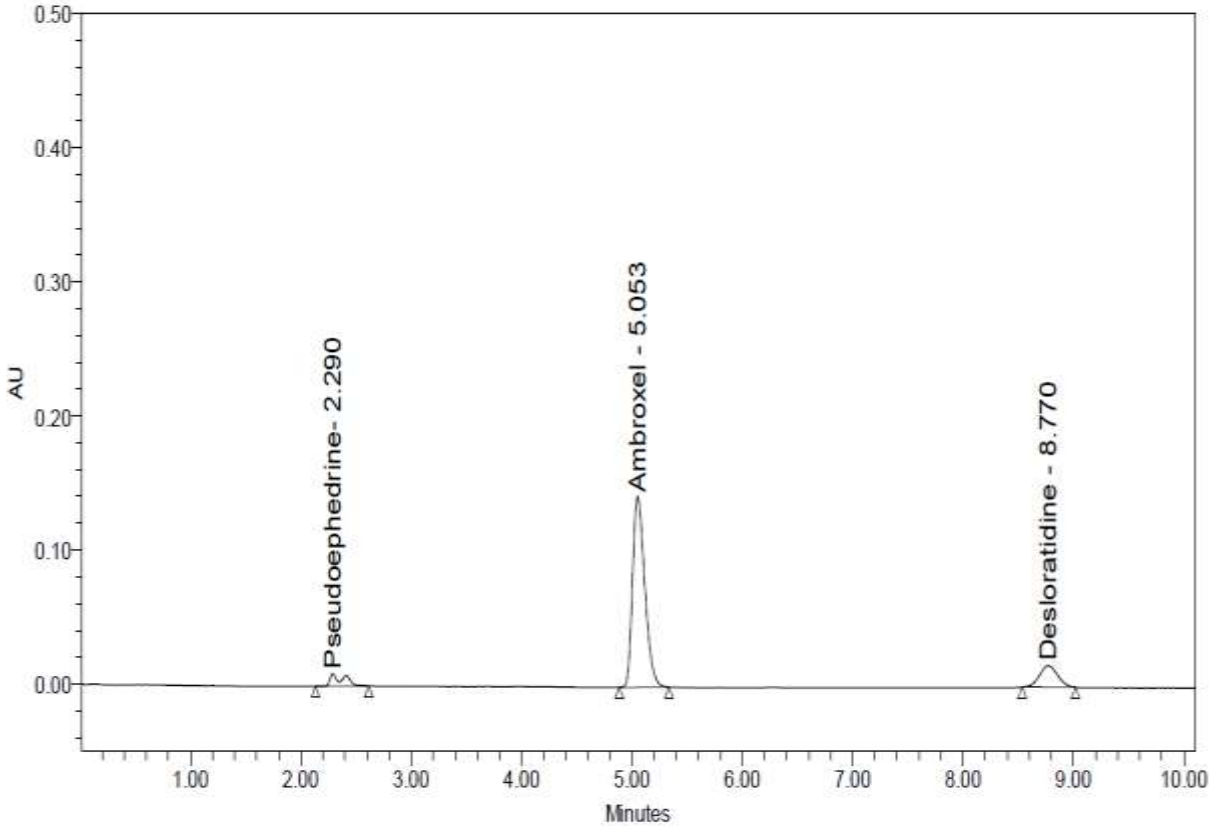



**Fig 6.2 Trial chromatogram 2**

**Observation:** Pseudoephedrine and desloratidine peak shape was not good and plate count was not good, so further trials are carried out.

**Trial 3:**

Column : ODS 250mm x 4.6 mm, 5µ.
Mobile phase : 45:20:35M
Buffer : $KH_2PO_4$ 5pH
Flow rate : 1ml/min
Temperature : 30°C
Wavelength : 225nm

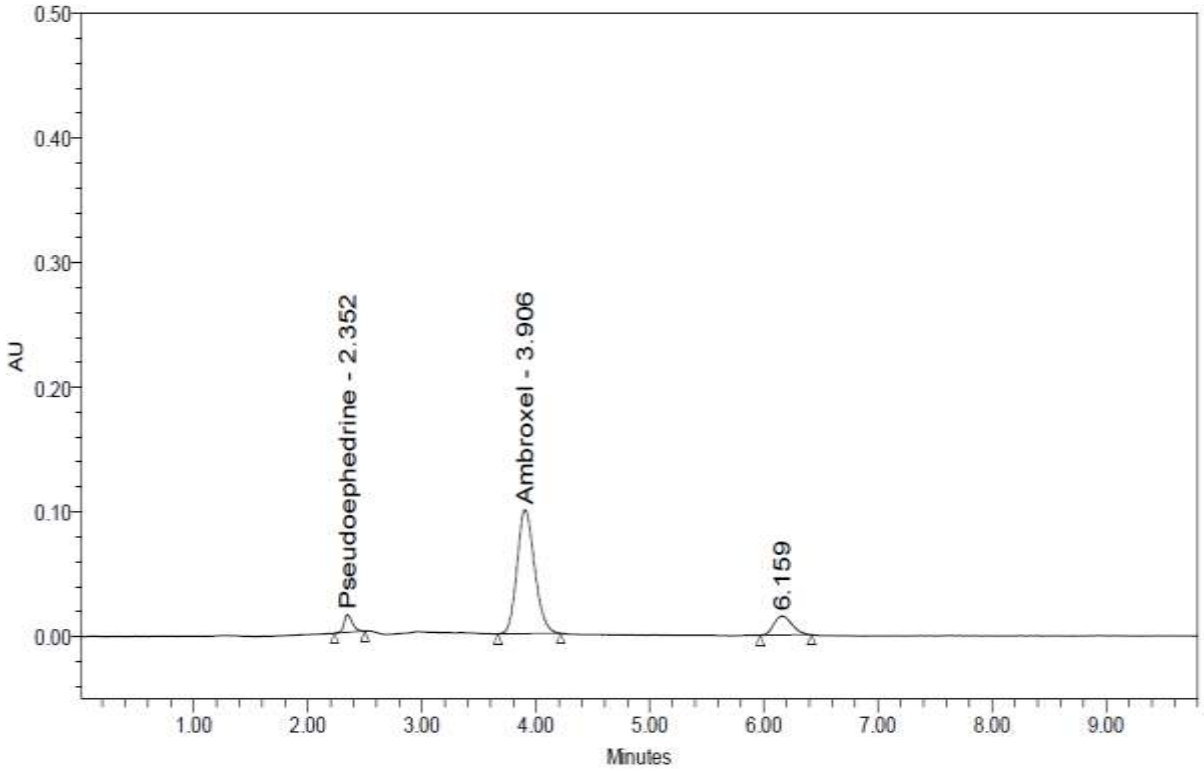



**Fig 6.3 Trial chromatogram 3**

**Observation:** Pseudoephedrine and desloratidine peak shape was not good and plate count was not good, and pseudoephedrine and desloratidine peak shape was not good so further trials are carried out.

**Trial 4:**

Column : ODS 250mm x 4.6 mm, 5µ.
Mobile phase : 50:10A:40M
Buffer : $KH_2PO_4$ 3pH
Flow rate : 1ml/min
Temperature : 30°C
Wavelength : 225nm

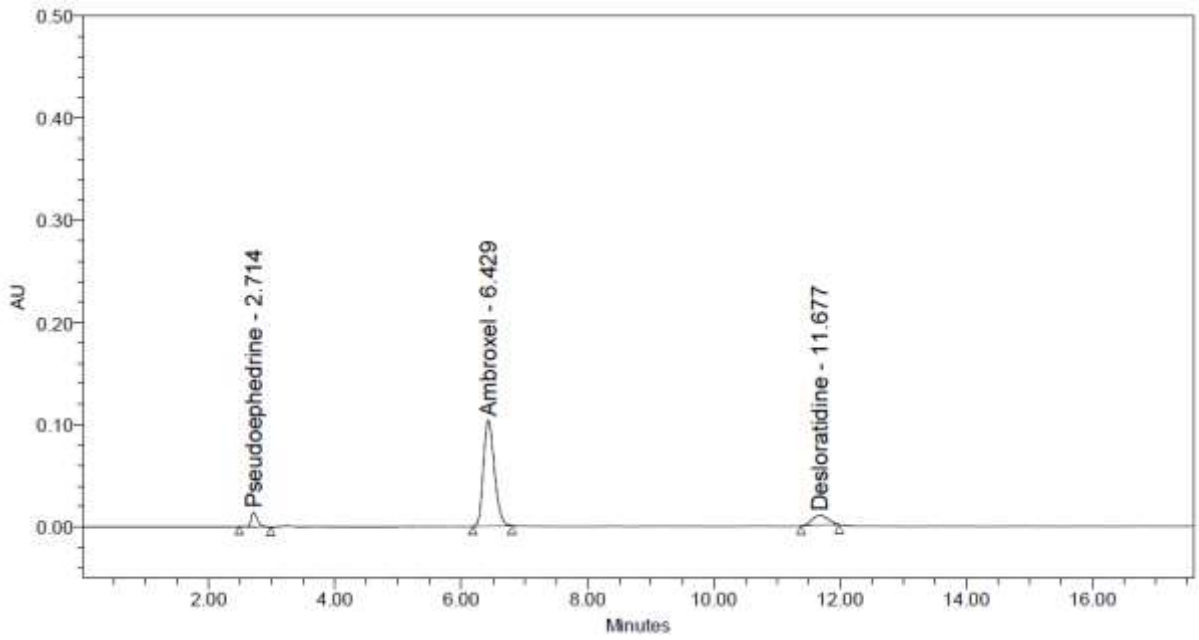



**Fig 6.4 Trial chromatogram 4**

**Observation:** Pseudoephedrine and desloratidine peak shape was not good, and plate count was not good, and pseudoephedrine and desloratidine peak shape was not good so further trials are carried out.

**Trial 5:**

Column : STD ODS 250mm x 4.6 mm, 5µ.
Mobile phase : 55:20A:25M
Buffer : $KH_2PO_4$ 3pH
Flow rate : 1ml/min
Temperature : 30°C
Wavelength : 225nm

DOI: 10.18535/ijsrm/v5i7.23

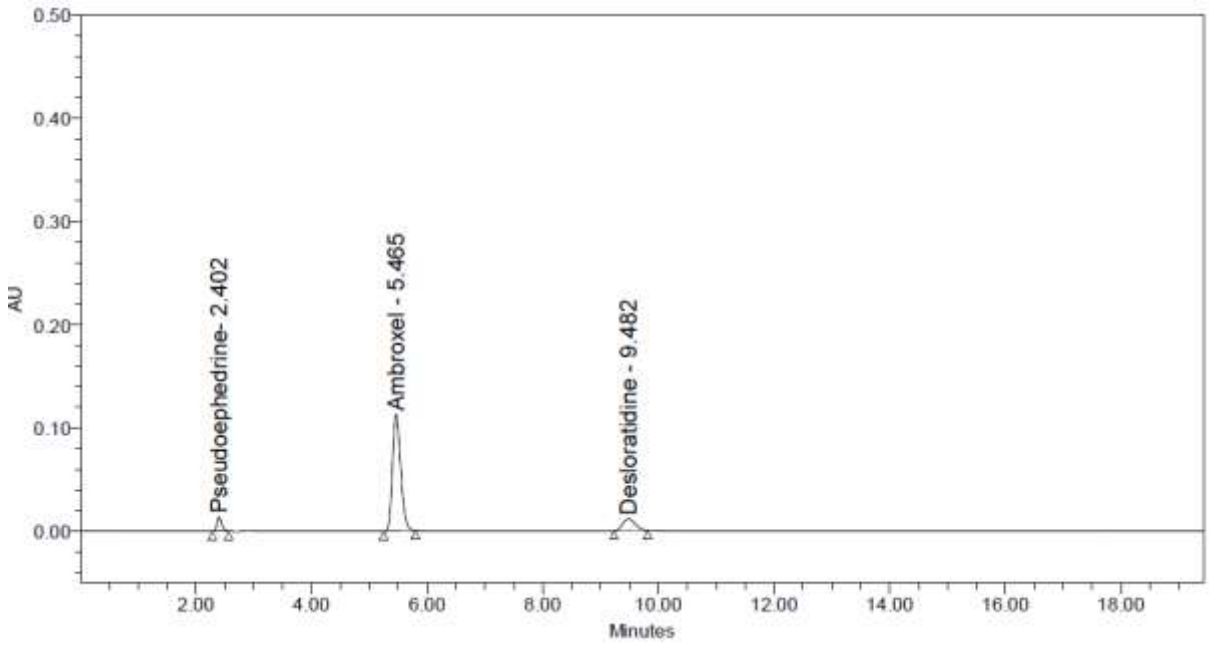



**Fig 6.5 Trial chromatogram 5**

**Observation:** Pseudoephedrine and desloratidine peak shape was not good, and plate count was not good, and pseudoephedrine and desloratidine peak shape was not good so further trials are carried out.

**Optimized Method**

Column : ODS 250mm x 4.6 mm, 5µ.
Mobile phase : 50:20A:30M
Buffer : $Na_2HPO_4$ 3pH
Flow rate : 1ml/min
Temperature : 30°C
Wavelength : 225nm

DOI: 10.18535/ijsrm/v5i7.23

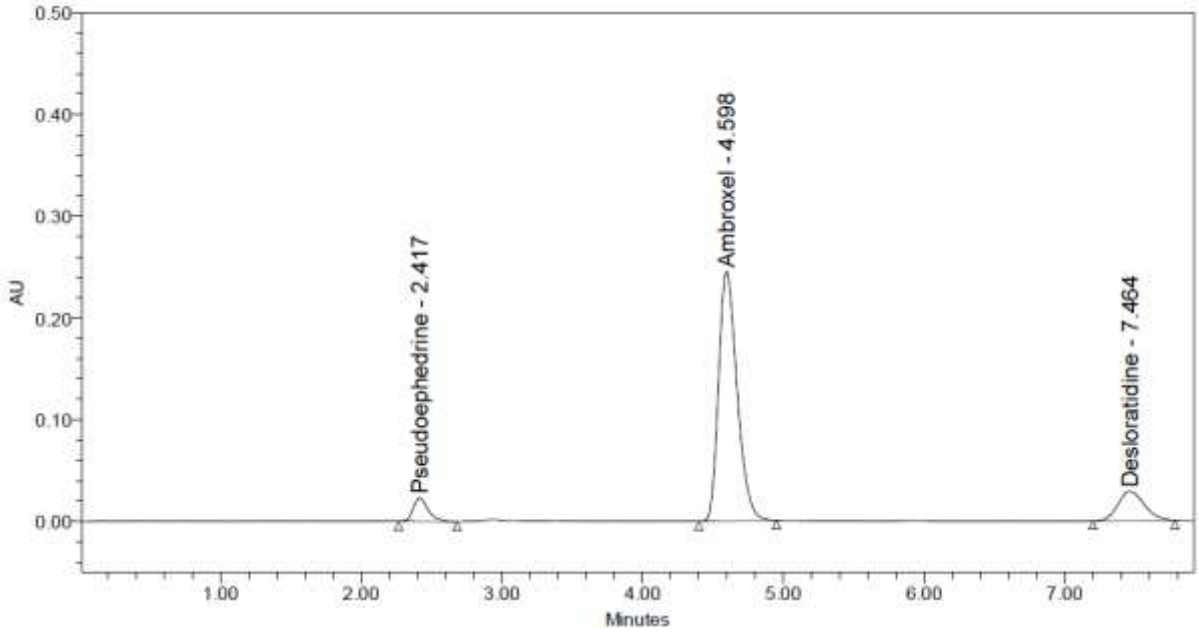
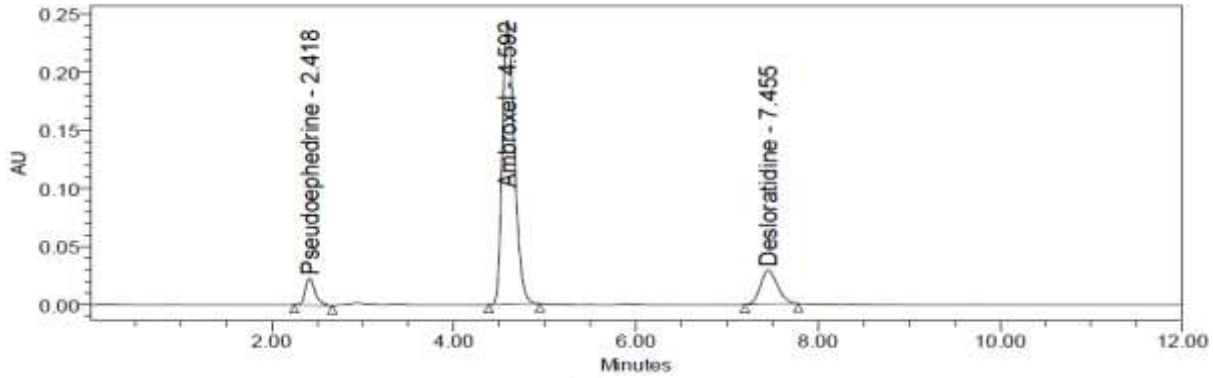



**Fig 6.6 Trial chromatogram 6**

**Observation:** retention time of pseudoephedrine (2.417min), Ambroxol (4.598min) and Desloratidine (7.464min) were good and tailing was passed plate count was good so this method was concluded as a optimized method.

**Fig:6. System suitability chromatogram**




**Validation:**

**Specificity:**

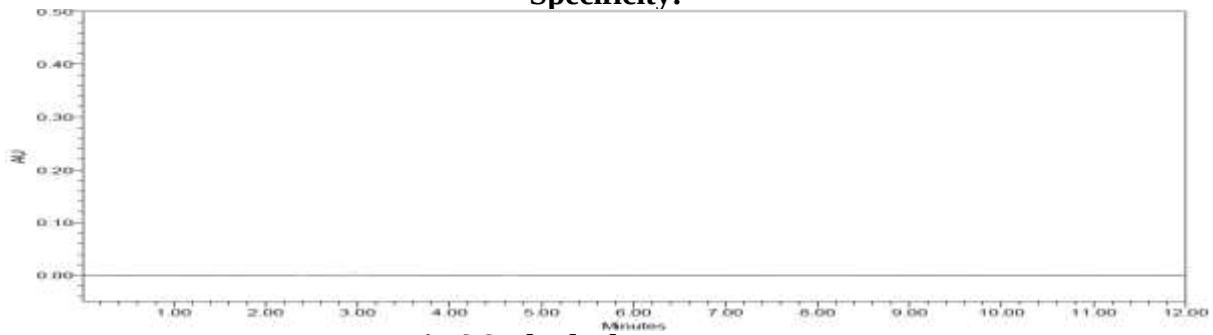



**Fig 6.8 Blank chromatogram.**

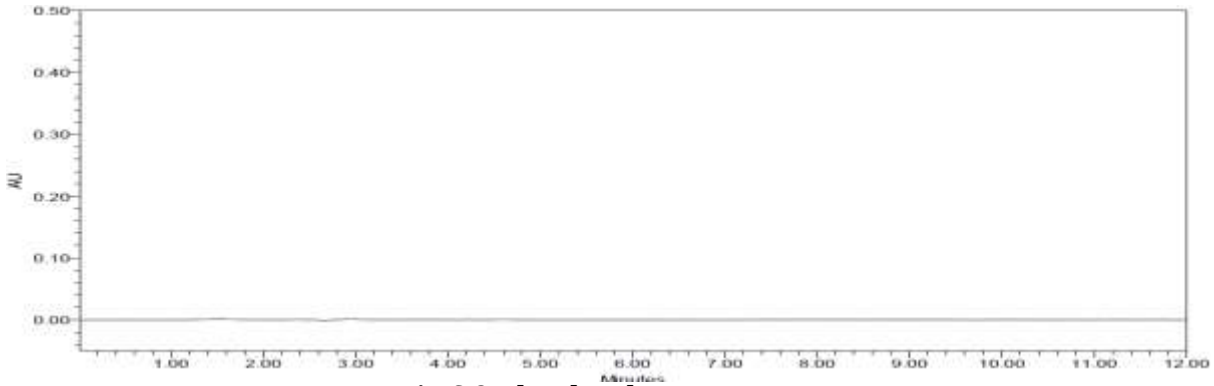



**Fig 6.9 Placebo chromatogram**

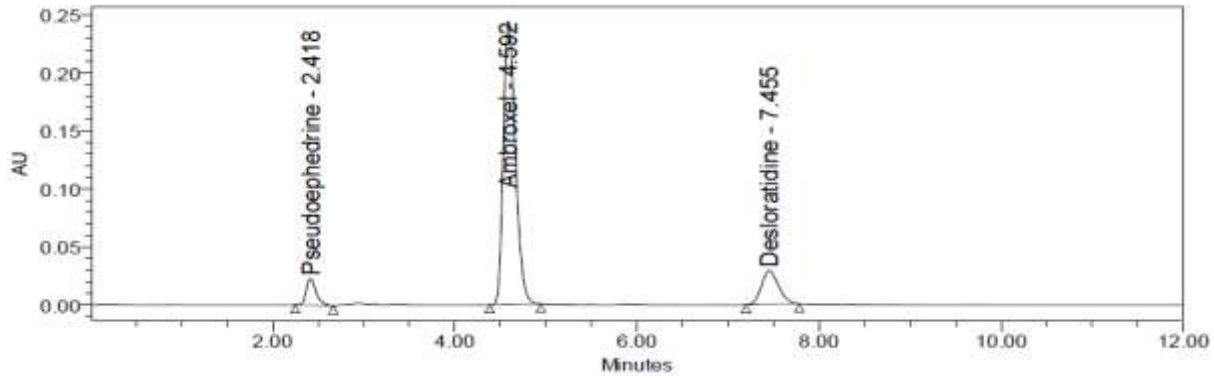



**Fig 6.10 Optimized chromatogram**

**Discussion:** Retention times of Pseudoephedrine, Ambroxol and Desloratidine were 2.418min, 4.592min and 7.455min respectively. We did not found and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

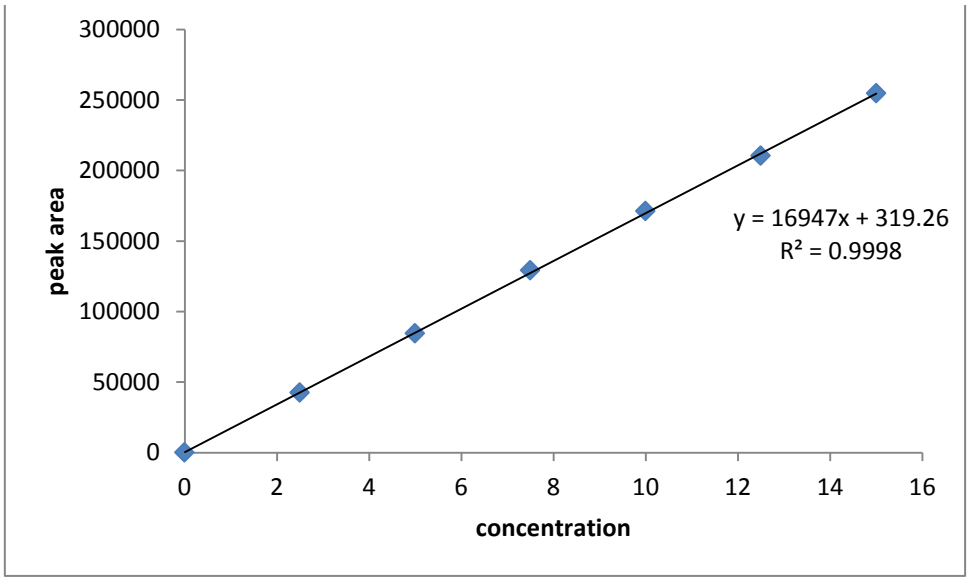



**Fig 6.11 Calibration curve of Pseudoephedrine**

DOI: 10.18535/ijsrm/v5i7.23

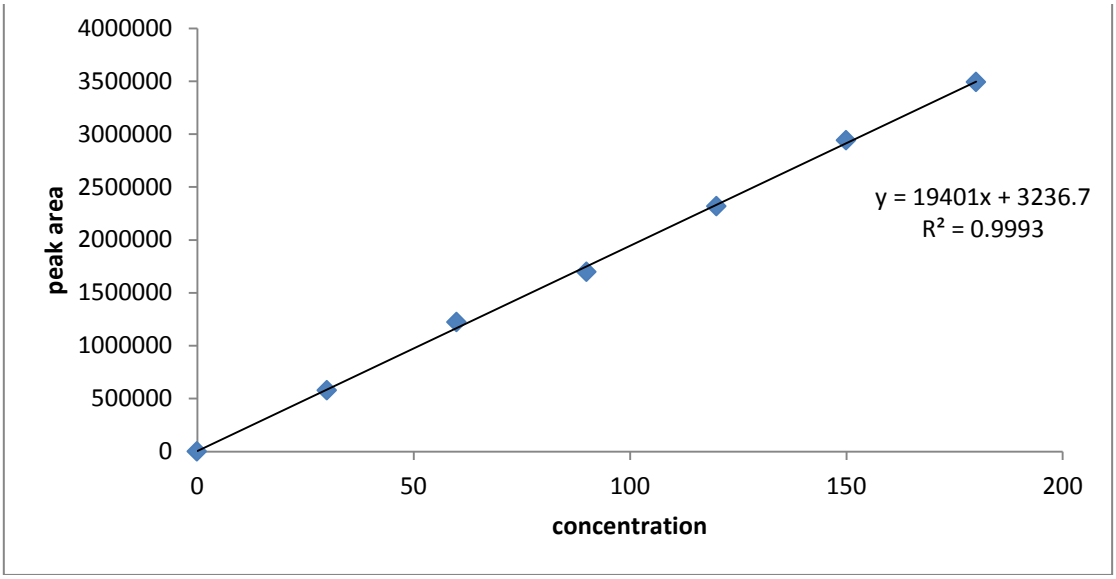



**Fig 6.12 calibration curve of Ambroxol**

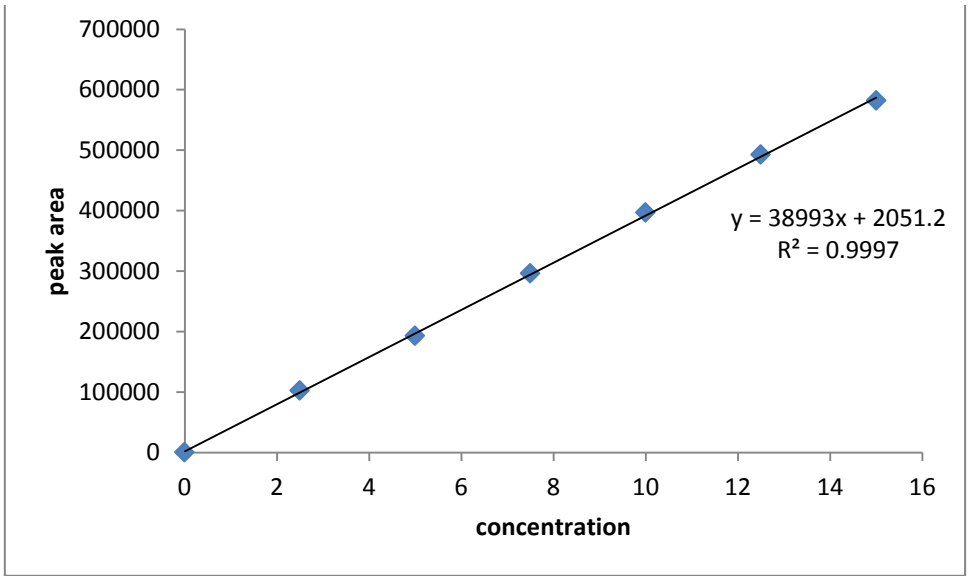



**Fig 6.13 calibration curve of Desloratidine**

**Fig 6.14 Linearity 25% Chromatogram**

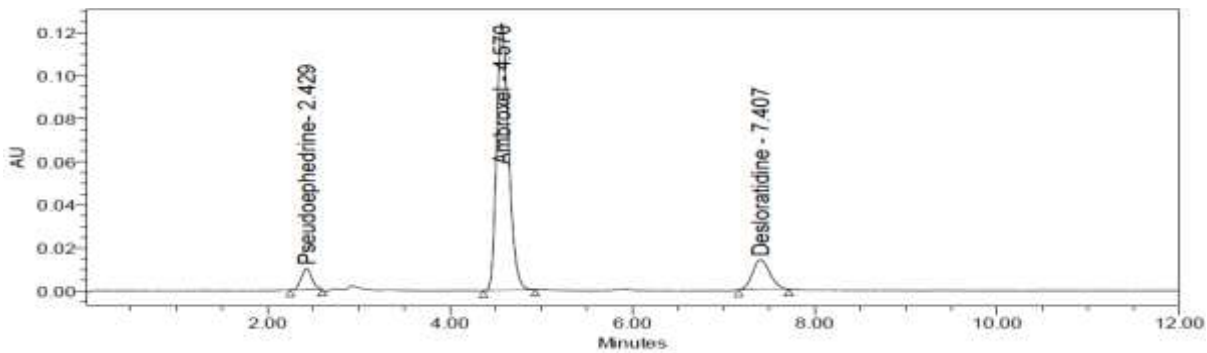



**Fig 6.15 Linearity 50% Chromatogram**

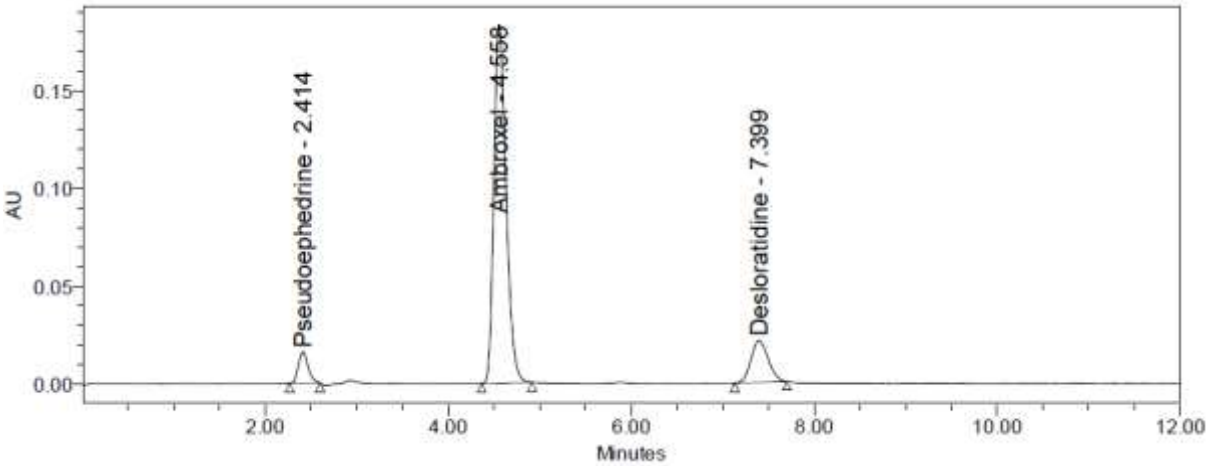



**Fig 6.16 Linearity 75% Chromatogram**

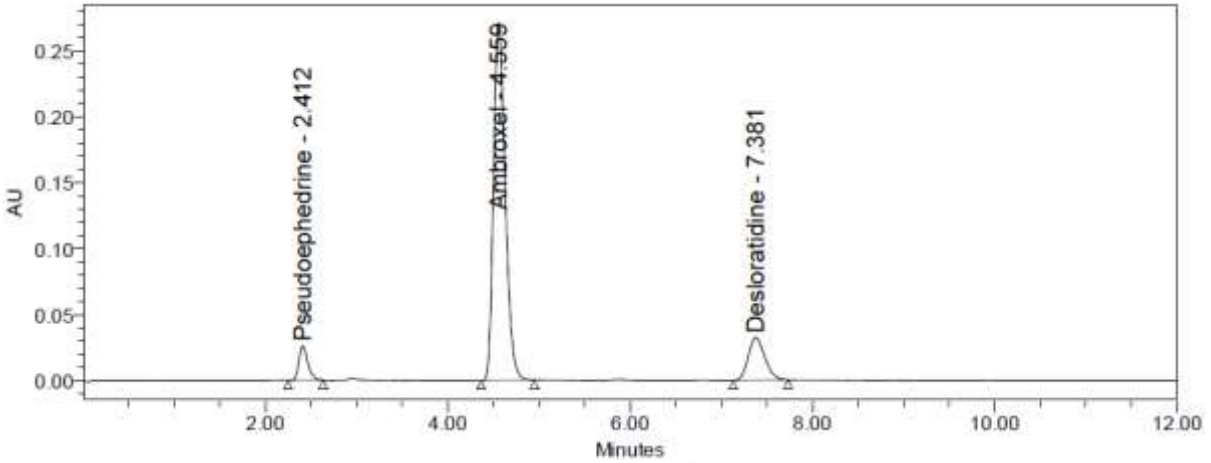



**Fig 6.17 Linearity 100% Chromatogram**

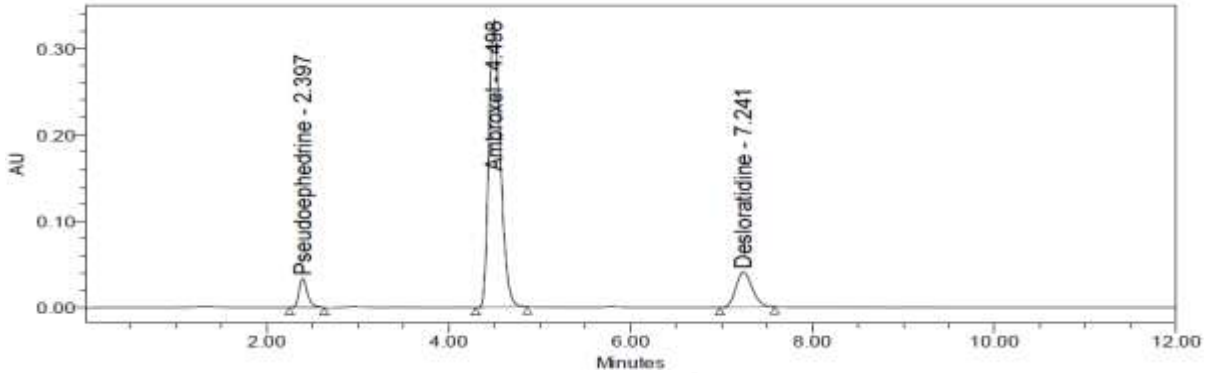



**Fig 6.18 Linearity 125% Chromatogram**

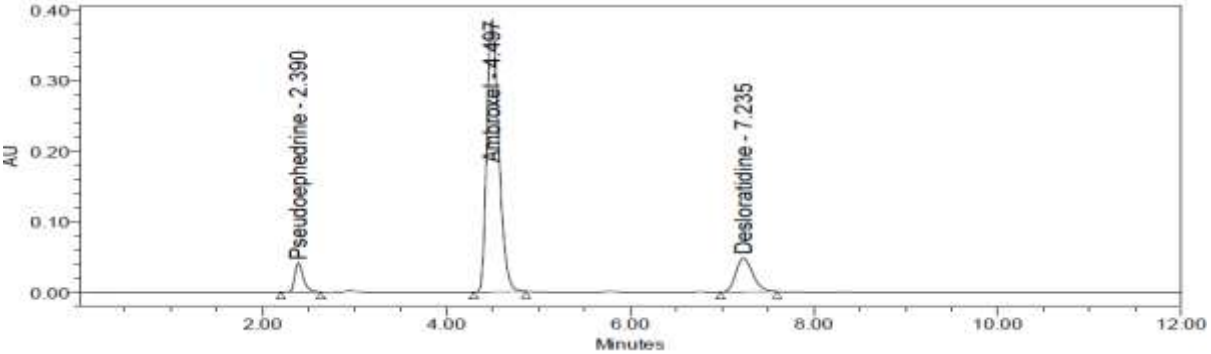



**Fig 6.19 Linearity 150% Chromatogram**

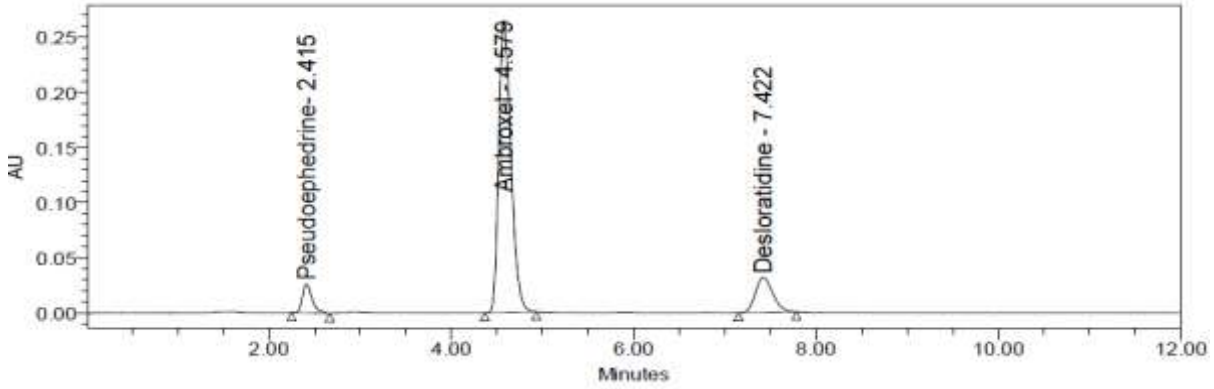



**Fig 6.20 System precision chromatogram**

**Fig 6.21 Repeatability chromatogram**

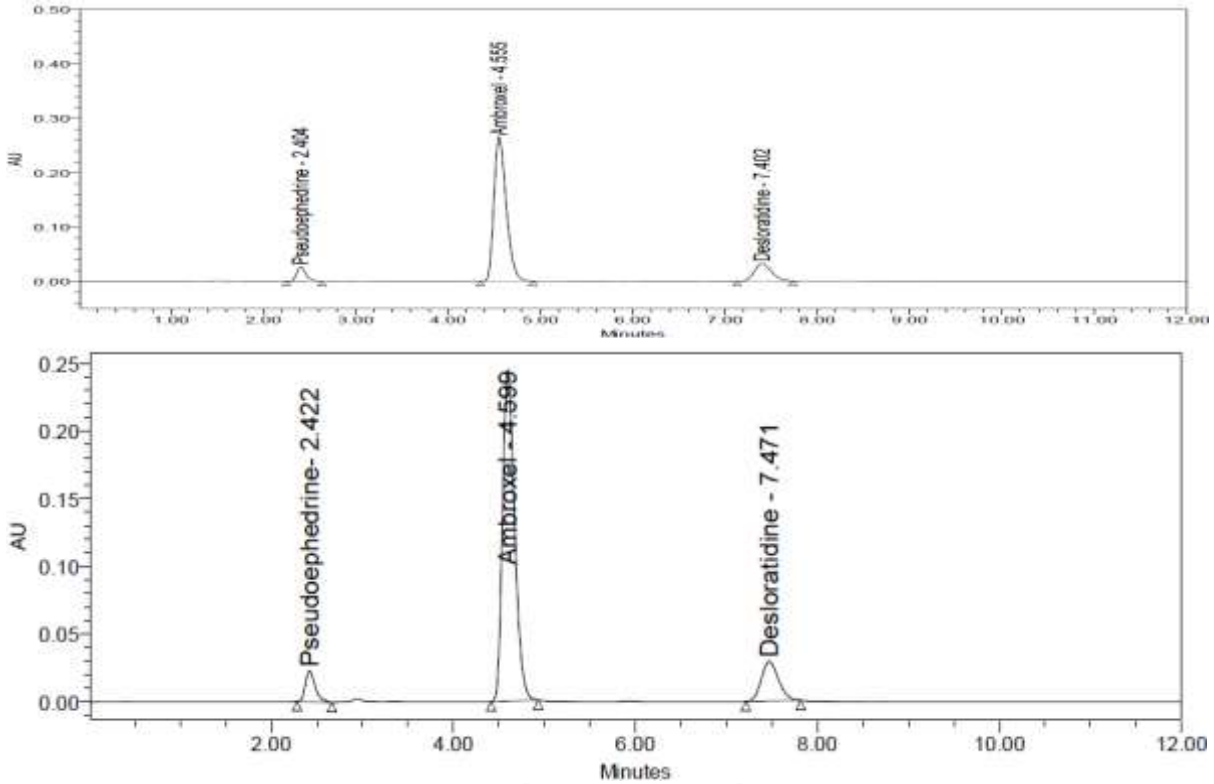



**Fig 6.22 Intermediate precision chromatogram**

Tables:

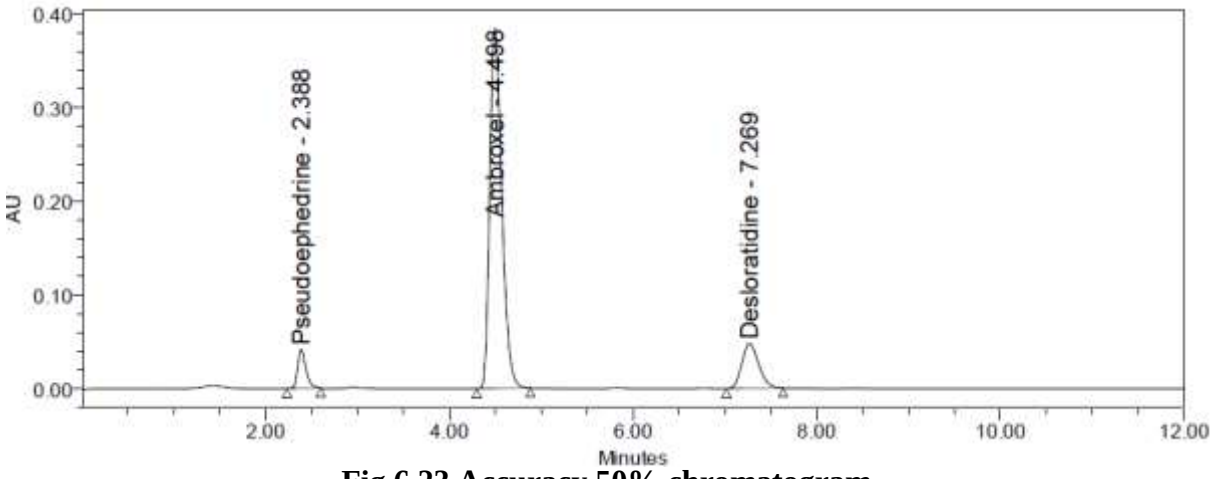



**Fig 6.23 Accuracy 50% chromatogram**

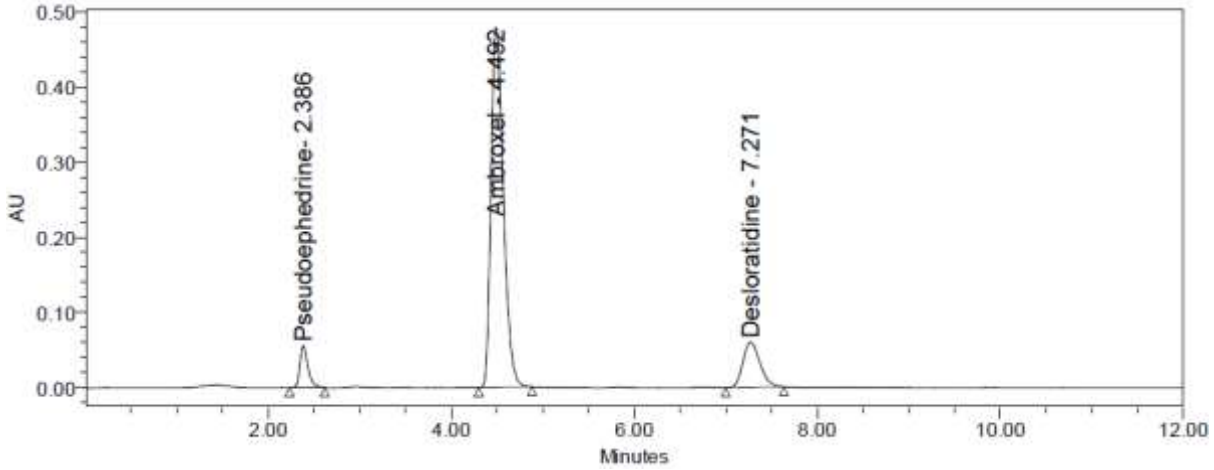



**Fig 6.24 Accuracy 100% chromatogram**

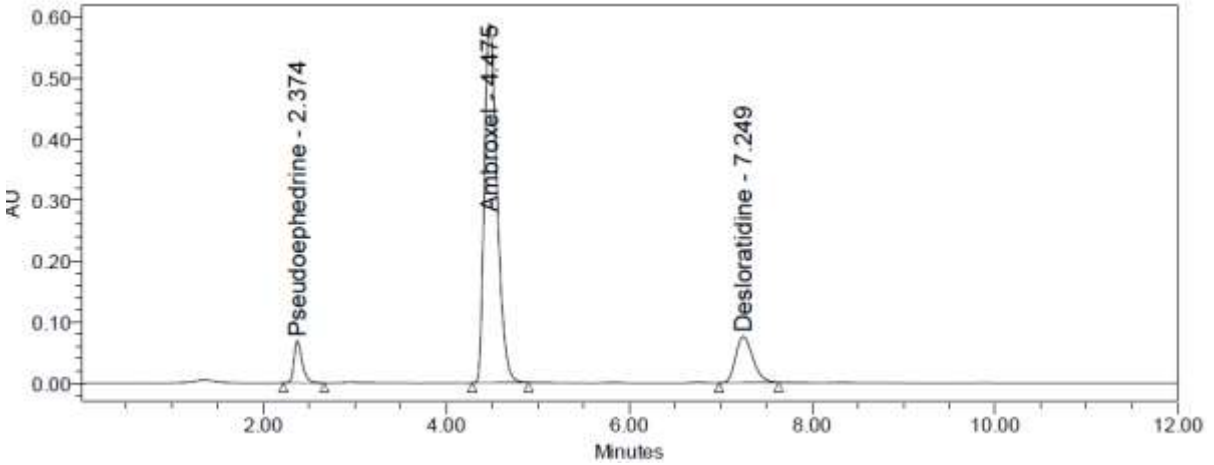



**Fig 6.25 Accuracy 150% chromatogram**

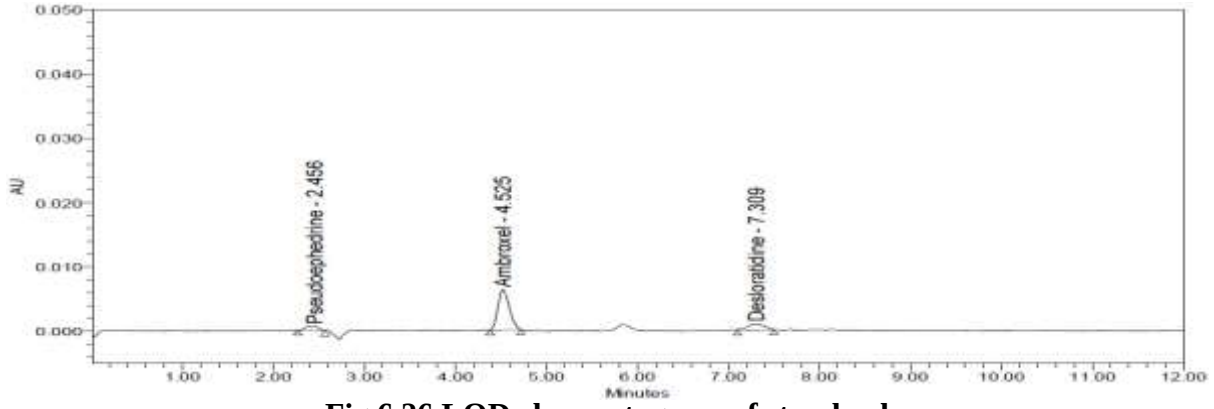



**Fig 6.26 LOD chromatogram of standard**

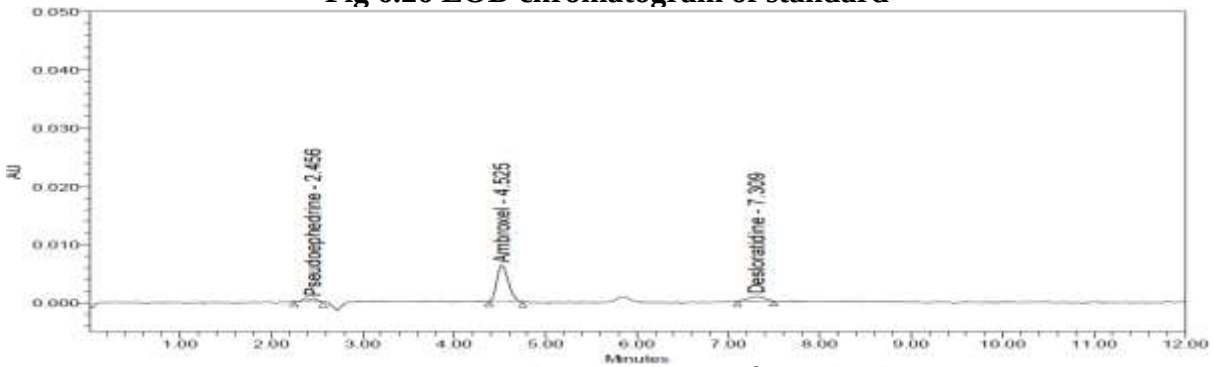



**Fig 6.27 LOQ chromatogram of standard**

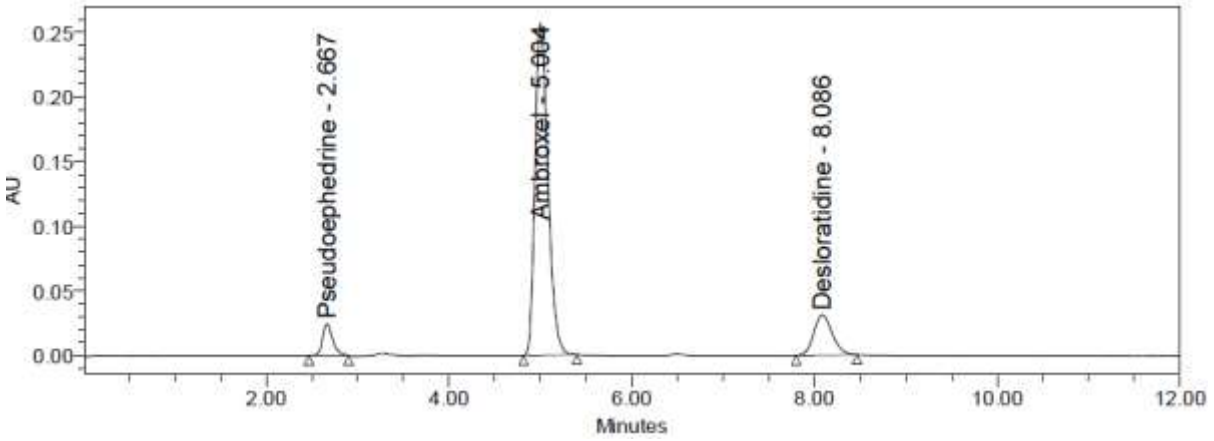



**Fig 6.28 Flow minus chromatogram injection**

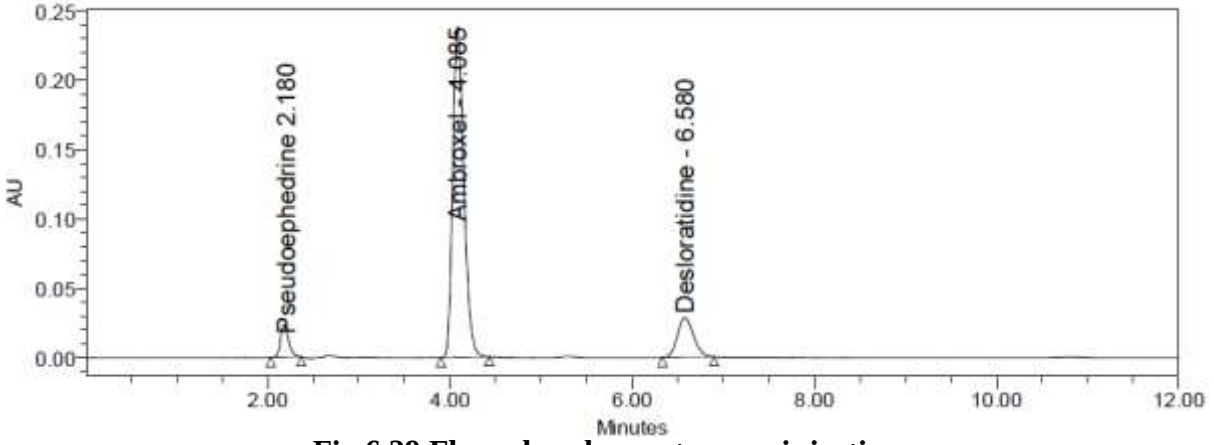



**Fig 6.29 Flow plus chromatogram injection**

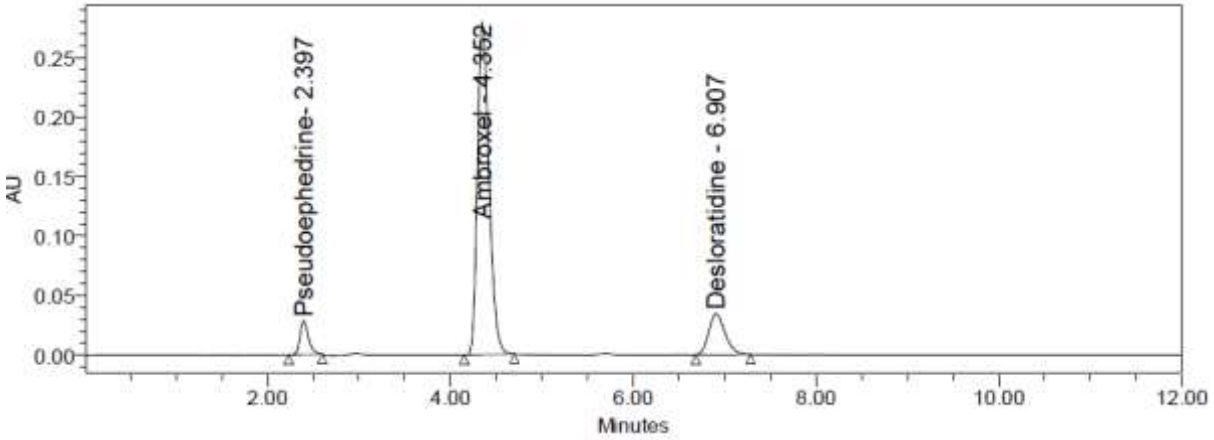



**Fig 6.30 Mobile phase minus chromatogram injection**

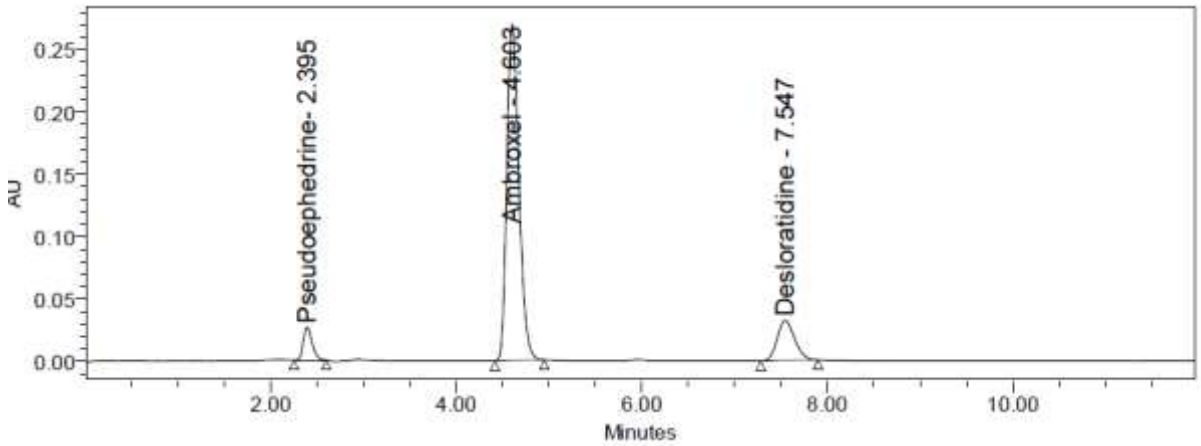



**Fig 6.31 Mobile phase plus chromatogram injection**

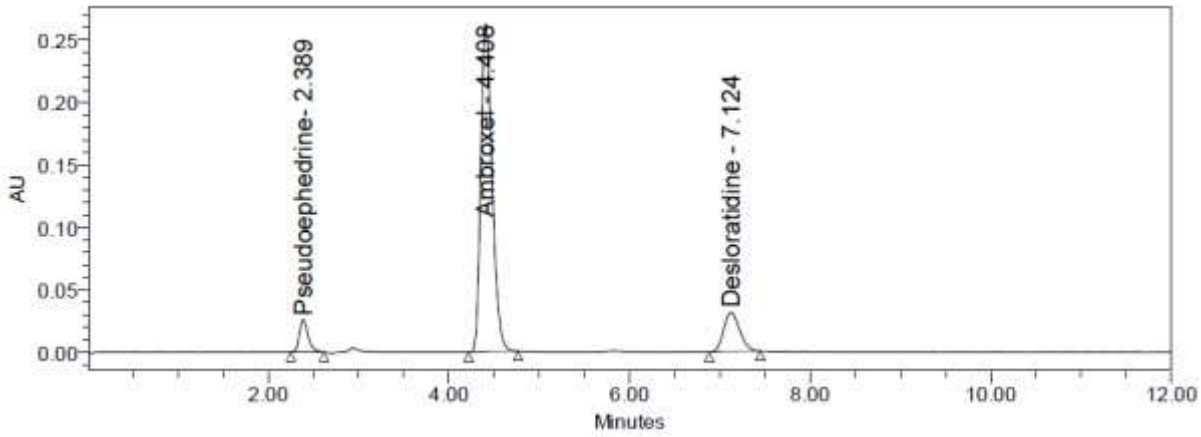



**Fig 6.32 Temperature minus chromatogram injection**

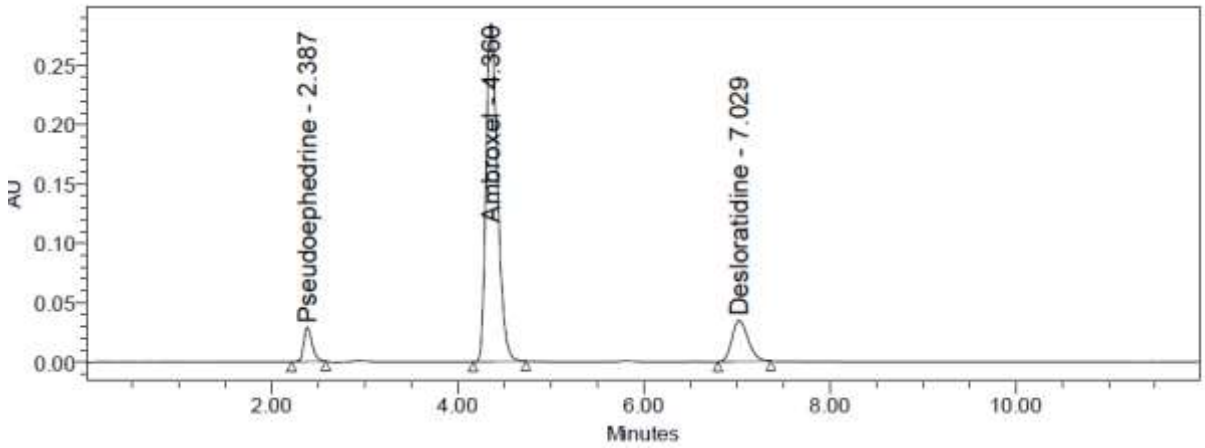



**Fig 6.33 Temperature plus chromatogram injection**

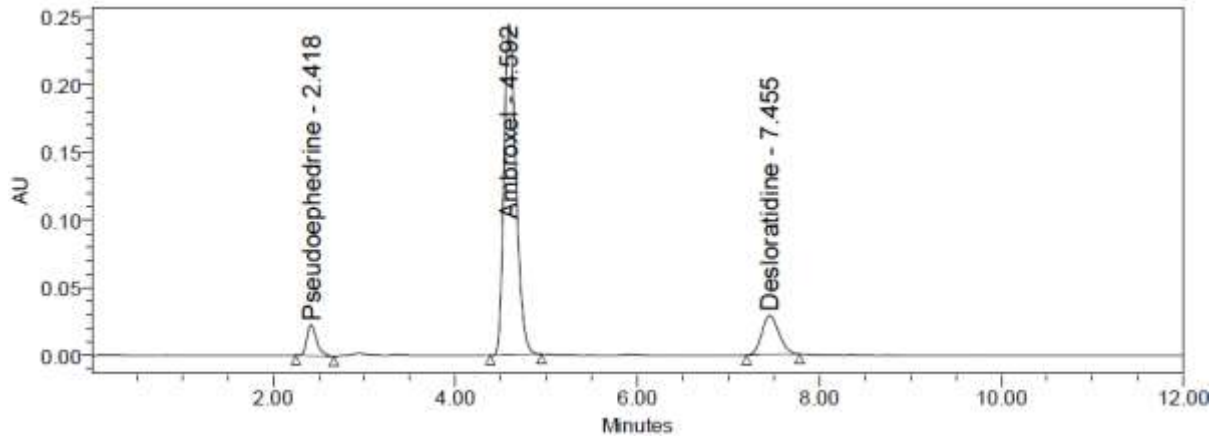


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**Fig 6.34 Chromatogram of working standard solution**

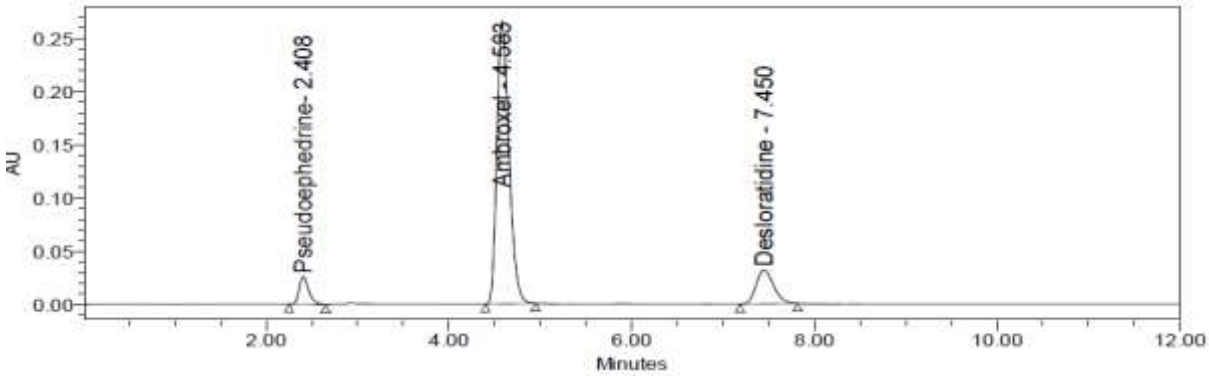


**Fig 6.35 Chromatogram of working sample solution**

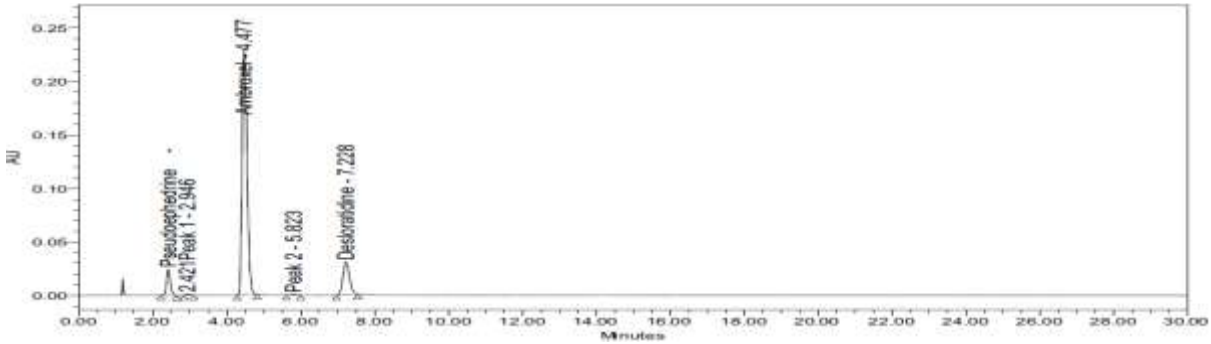


**Fig 6.36 Acid chromatogram**

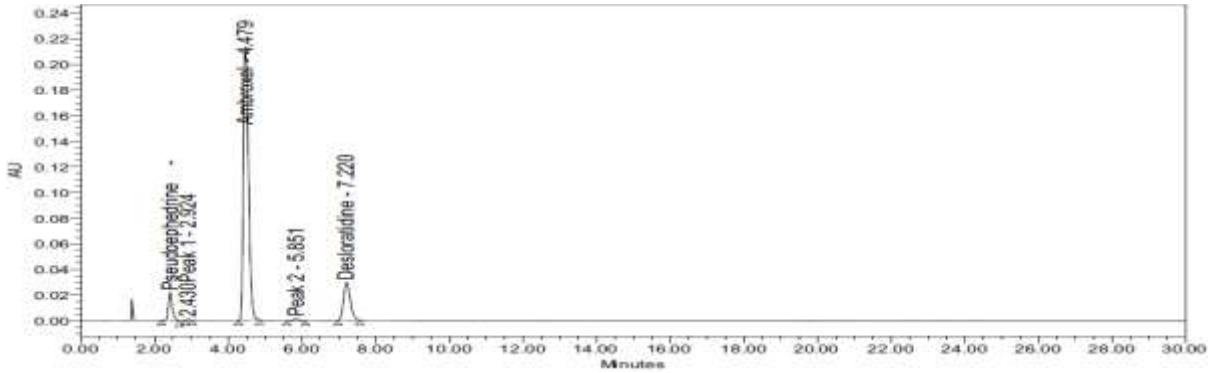


**Fig 6.37 Base chromatogram**

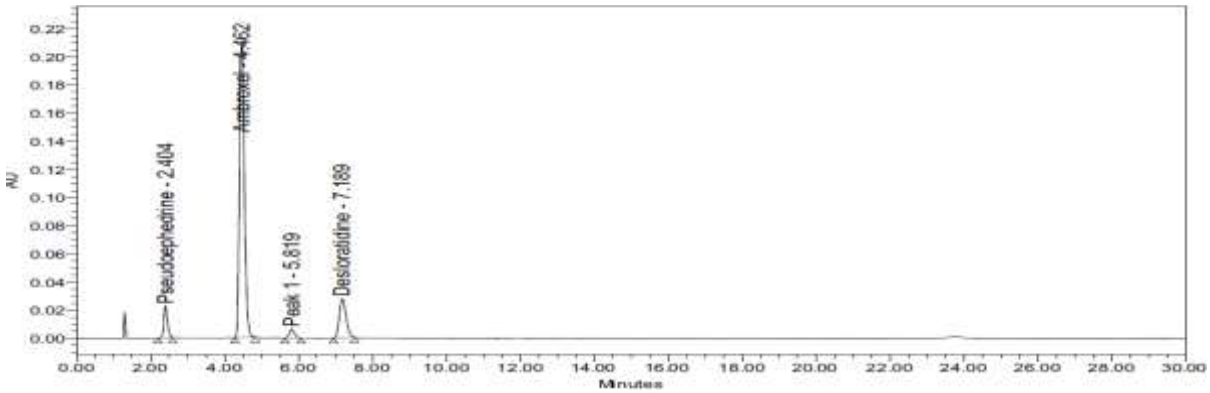


**Fig 6.38 Peroxide chromatogram**

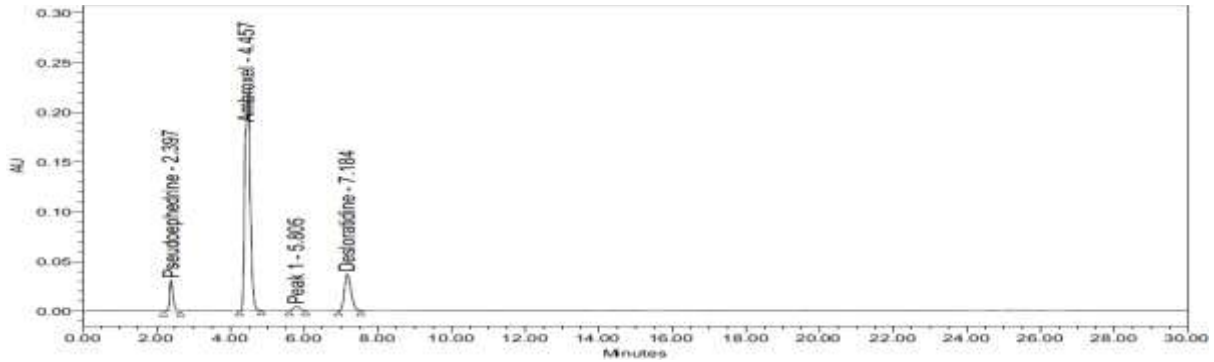


**Fig 6.39 Thermal chromatogram**

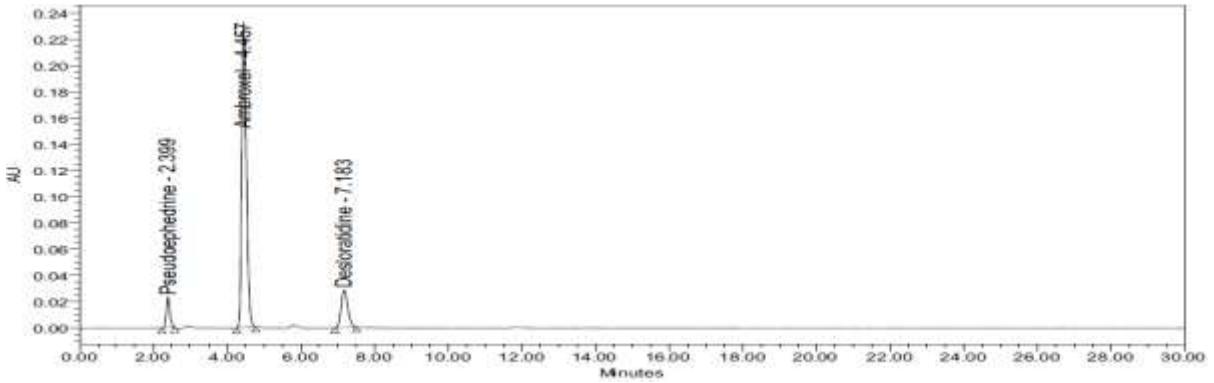


**Fig 6.40 UV chromatogram**

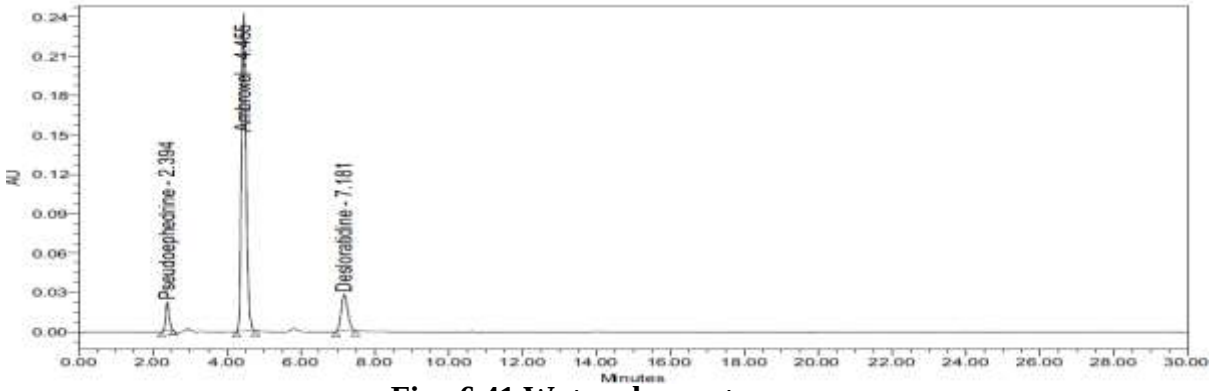


**Fig 6.41 Water chromatogram**

**4.Tables:**

**Table 6.1 System suitability parameters for Pseudoephedrine, Ambroxol and Desloratidine.**

| Property | Pseudoephedrine | Ambroxol | Desloratidine |
|---|---|---|---|
| Retention time ($t_R$) | 2.417± 0.3 min | 4.598±0.3min | 7.464±0.3min |
| Theoretical plates (N) | 2598± 163.48 | 5684± 163.48 | 7263± 163.48 |
| Tailing factor (T) | 1.45 ± 0.117 | 1.30± 0.117 | 1.15± 0.117 |

**Linearity:**

DOI: 10.18535/ijsrm/v5i7.23

**Table 6.2 Linearity table for Pseudoephedrine, Ambroxol and Desloratidine.**

| Pseudoephedrine | | Ambroxol | | Desloratidine | |
|---|---|---|---|---|---|
| Conc (µg/mL) | Peak area | Conc (µg/mL) | Peak area | Conc (µg/mL) | Peak area |
| 0 | 0 | 0 | 0 | 0 | 0 |
| 2.5 | 42354 | 30 | 576744 | 2.5 | 102026 |
| 5 | 84389 | 60 | 1222301 | 5 | 192892 |
| 7.5 | 129228 | 90 | 1697457 | 7.5 | 295860 |
| 10 | 171094 | 120 | 2316474 | 10 | 396722 |
| 12.5 | 210383 | 150 | 2941103 | 12.5 | 492280 |
| 15 | 254513 | 180 | 3491404 | 15 | 581733 |

**System Precision:**

**Table 6.3 System precision table of Pseudoephedrine, Ambroxol and Desloratidine**

| S. No | Area of Pseudoephedrine | Area of Ambroxol | Area of Desloratidine |
|---|---|---|---|
| 1. | 171063 | 2261866 | 386903 |
| 2. | 171799 | 2246853 | 379938 |
| 3. | 173575 | 2255152 | 386939 |
| 4. | 172309 | 2249571 | 387581 |
| 5. | 174661 | 2260763 | 385404 |
| 6. | 173924 | 2267323 | 383360 |
| Mean | 172889 | 2256921 | 385021 |
| S.D | 1381.3 | 7822.2 | 2916.1 |
| %RSD | 0.80 | 0.3 | 0.8 |

**Repeatability:**

**Table 6.4 Repeatability table of Pseudoephedrine, Ambroxol and Desloratidine**

| S. No | Area of Pseudoephedrine | Area of Ambroxol | Area of Desloratidine |
|---|---|---|---|

| | | | |
|---|---|---|---|
| 1. | 170946 | 2273699 | 384414 |
| 2. | 172610 | 2238529 | 392094 |
| 3. | 174788 | 2255110 | 388705 |
| 4. | 175995 | 2266768 | 380423 |
| 5. | 173350 | 2275116 | 385773 |
| 6. | 172644 | 2250262 | 391380 |
| Mean | 173389 | 2259914 | 387132 |
| S.D | 1782.1 | 14433.7 | 4458.4 |
| %RSD | 1.03 | 0.64 | 1.15 |

**Intermediate precision (Day_ Day Precision):**

**Table 6.5 Intermediate precision table of Pseudoephedrine, Ambroxol and Desloratidine**

| S. No | Area of Pseudoephedrine | Area of Ambroxol | Area of Desloratidine |
|---|---|---|---|
| 1. | 171063 | 2254703 | 378366 |
| 2. | 171409 | 2242034 | 376604 |
| 3. | 173575 | 2255546 | 386939 |
| 4. | 172309 | 2246924 | 382840 |
| 5. | 170334 | 2260763 | 385294 |
| 6. | 172259 | 2267323 | 382308 |
| Mean | 171825 | 2254549 | 382059 |
| S.D | 1136.8 | 9138.6 | 3958.5 |
| %RSD | 0.66 | 0.4 | 1.0 |

**Accuracy:**

**Table 6.6 Accuracy table of Pseudoephedrine**

| % Level | Amount Spiked (µg/mL) | Total amount found (µg/mL) | Amount recovered (µg/mL) | % Recovery | Mean %Recovery |
|---|---|---|---|---|---|
| 50% | 5 | 15.02 | 5.02 | 100.32 | 99.67% |
| | 5 | 14.99 | 4.99 | 99.78 | |
| | 5 | 14.94 | 4.94 | 98.71 | |
| 100% | 10 | 19.99 | 9.99 | 99.91 | |
| | 10 | 20.05 | 10.05 | 100.47 | |
| | 10 | 19.94 | 9.94 | 99.41 | |
| 150% | 15 | 25.06 | 15.06 | 100.42 | |
| | 15 | 25.09 | 15.09 | 100.60 | |
| | 15 | 24.95 | 14.95 | 99.67 | |

**Table 6.7 Accuracy table of ambroxol**

| % Level | Amount Spiked (µg/mL) | Total amount found (µg/mL) | Amount recovered (µg/mL) | % Recovery | Mean %Recovery |
|---|---|---|---|---|---|
| 50% | 60 | 180.28 | 60.28 | 100.47 | 100.21% |
| | 60 | 179.23 | 59.23 | 98.72 | |
| | 60 | 180.37 | 60.38 | 100.63 | |
| 100% | 120 | 238.09 | 118.09 | 98.41 | |
| | 120 | 241.22 | 121.22 | 101.01 | |
| | 120 | 242.08 | 122.08 | 101.74 | |
| 150% | 180 | 298.79 | 178.79 | 99.33 | |
| | 180 | 302.91 | 182.91 | 101.62 | |
| | 180 | 299.96 | 179.96 | 99.98 | |

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**Table 6.8 Accuracy table of Desloratidine**

| % Level | Amount Spiked (µg/mL) | Total amount found (µg/mL) | Amount recovered (µg/mL) | % Recovery | Mean %Recovery |
|---|---|---|---|---|---|
| 50% | 5 | 15.02 | 5.02 | 100.36 | 99.92% |
| | 5 | 14.92 | 4.92 | 98.44 | |
| | 5 | 14.98 | 4.98 | 99.53 | |
| 100% | 10 | 20.04 | 10.04 | 100.42 | |
| | 10 | 20.03 | 10.03 | 100.27 | |
| | 10 | 20.00 | 10.00 | 99.98 | |
| 150% | 15 | 25.13 | 15.13 | 100.85 | |
| | 15 | 25.07 | 15.07 | 100.48 | |
| | 15 | 24.84 | 14.84 | 98.95 | |

**Sensitivity:**

**Table 6.9 Sensitivity table of Pseudoephedrine, ambroxol and Desloratidine**

| Molecule | LOD(µg/ml) | LOQ(µg/ml) |
|---|---|---|
| **Pseudoephedrine** | 0.01 µg/ml | 0.02 µg/ml |
| **Ambroxol** | 0.15 µg/ml | 0.44 µg/ml |
| **Desloratidine** | 0.06µg/ml | 0.17 µg/ml |

**Robustness:**

**Table 6.10 Robustness data for Pseudoephedrine, Ambroxol and Desloratidine.**

| S.no | Condition | %RSD of Pseudoephedrine | %RSD of Ambroxol | %RSD of Desloratidine |
|---|---|---|---|---|
| 1 | Flow rate (-) | 0.2 | 0.1 | 0.2 |

| | | | | |
|---|---|---|---|---|
| 2 | Flow rate (+) | 0.6 | 0.2 | 0.7 |
| 3 | Mobile phase (-) | 0.6 | 0.4 | 0.9 |
| 4 | Mobile phase (+) | 0.7 | 0.3 | 1.0 |
| 5 | Temperature (-) | 0.3 | 0.2 | 0.4 |
| 6 | Temperature (+) | 0.6 | 0.3 | 0.3 |

**Table 6.11 Assay Data of Pseudoephedrine**

| S.no | Standard Area | Sample area | % Assay |
|---|---|---|---|
| 1 | 171063 | 170946 | 98.48 |
| 2 | 171799 | 172610 | 99.44 |
| 3 | 173575 | 174788 | 100.69 |
| 4 | 172309 | 175995 | 101.39 |
| 5 | 174661 | 173350 | 99.87 |
| 6 | 173924 | 172644 | 99.46 |
| Avg | 172889 | 173389 | 99.89 |
| Stdev | 1381.3 | 1782.1 | 1.027 |
| %RSD | 0.80 | 1.03 | 1.03 |

**Table 6.12 Assay Data of Ambroxol**

| S.no | Standard Area | Sample area | % Assay |
|---|---|---|---|
| 1 | 2261866 | 2273699 | 100.34 |
| 2 | 2246853 | 2238529 | 98.79 |
| 3 | 2255152 | 2255110 | 99.52 |
| 4 | 2249571 | 2266768 | 100.03 |
| 5 | 2260763 | 2275116 | 100.40 |
| 6 | 2267323 | 2250262 | 99.31 |
| Avg | 2256921 | 2259914 | 99.73 |
| Stdev | 7822.2 | 14433.7 | 0.64 |
| %RSD | 0.3 | 0.64 | 0.64 |

**Table 6.13 Assay Data of Desloratidine**

| S.no | Standard Area | Sample area | % Assay |
|---|---|---|---|
| 1 | 386903 | 384414 | 99.44 |
| 2 | 379938 | 392094 | 101.43 |
| 3 | 386939 | 388705 | 100.55 |
| 4 | 387581 | 380423 | 98.41 |
| 5 | 385404 | 385773 | 99.79 |
| 6 | 383360 | 391380 | 101.25 |
| Avg | 385021 | 387132 | 100.15 |
| Stdev | 2916.1 | 4458.4 | 1.153 |
| %RSD | 0.8 | 1.15 | 1.15 |

**Table 6.14 Degradation Data of Pseudoephedrine**

| S.NO | Degradation Condition | % Drug Degraded | Purity Angle | Purity Threshold |
|---|---|---|---|---|
| 1 | Acid | 6.28 | 1.977 | 2.190 |
| 2 | Alkali | 5.46 | 2.067 | 1.420 |
| 3 | Oxidation | 7.92 | 1.202 | 1.298 |
| 4 | Thermal | 3.81 | 1.955 | 2.116 |
| 5 | UV | 1.63 | 1.012 | 1.260 |
| 6 | Water | 0.61 | 1.473 | 1.746 |

**Table 6.15 Degradation Data of Ambroxol**

| S.NO | Degradation Condition | % Drug Degraded | Purity Angle | Purity Threshold |
|---|---|---|---|---|
| 1 | Acid | 6.27 | 0.378 | 1.677 |
| 2 | Alkali | 5.21 | 0.393 | 0.311 |
| 3 | Oxidation | 7.76 | 0.125 | 0.306 |
| 4 | Thermal | 2.11 | 0.125 | 0.322 |
| 5 | UV | 1.99 | 0.089 | 0.314 |
| 6 | Water | 0.67 | 0.101 | 0.322 |

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**Table 6.16 Degradation Data of Desloratidine**

| S.NO | Degradation Condition | % Drug Degraded | Purity Angle | Purity Threshold |
|---|---|---|---|---|
| 1 | Acid | 5.92 | 1.363 | 2.625 |
| 2 | Alkali | 6.52 | 0.457 | 0.683 |
| 3 | Oxidation | 7.11 | 1.089 | 2.657 |
| 4 | Thermal | 3.93 | 0.384 | 0.610 |
| 5 | UV | 1.64 | 0.505 | 0.721 |
| 6 | Water | 0.74 | 0.710 | 0.918 |

## 7. SUMMARY AND CONCLUSION

**Table 7.1 Summary**

| Parameters | Pseudoephedrine | Ambroxol | Desloratidine |
|---|---|---|---|
| ration range (mcg / ml) | 2.5-15ppm | 30-180ppm | 2.5-15ppm |
| Optimized wavelength | 225nm | 225nm | 225nm |
| Retention time | 2.417min | 4.598min | 7.464min |
| Regression equation (Y*) | y = 16947x + 319.2 | y = 19401x + 3236. | y = 38993x +2051. |
| Correlation coefficient($r^2$) | 0.999 | 0.999 | 0.999 |
| Precision (% RSD*) | 1.03 | 0.64 | 1.15 |
| % Recovery | 99.92% | 100.21% | 99.92% |
| Limit of Detection (mcg / ml) | 0.01ppm | 0.15ppm | 0.06ppm |
| Limit of Quantitation (mcg / ml) | 0.02ppm | 0.44ppm | 0.17ppm |

**5.Equations:**

$N = 16 (t / w)^2$

Where, t is retention time and w is width at the base of the peak.

HETP = L / N
Where L=length of column.

$$2 (t_2 - t_1) \quad Rs = \quad \text{----------}$$
$$W_1 + W_2$$

Where, $t_2$ and $t_1$ is the retention time of second and first compound respectively, whereas $W_2$ and $W_1$ are the corresponding widths at the bases of peak obtained by extrapolating straight sides of the peaks to baselines.

R should be more than 2 between peaks of interest and the closest eluted potential interferences (impurities, excipients, degradation products or internal standard).

**D. Tailing Factor (T):** It is the measure of peak symmetry, is unity for perfectly symmetrical peaks and its value increases as tailing become more pronounced.

$$T = \frac{W_{0.05}}{2F}$$

Where, $W_{0.05}$ is the width of peak at 5% height and F is the distance from the peak maximum to the leading edge of the peak height from baseline.

Tailing factor should be less than 2.

**E. Capacity Factor (K'):** It is calculated by using the formula,

$$K' = \frac{t}{t_a} - 1$$

Where, t is the retention time of drug and $t_a$ is the retention time of non-retarded component, air with thermal conductivity detection.

**6.Conclusion:**

A simple, Accurate, precise method was developed for the simultaneous estimation of the Pseuduephedrine, Ambroxol and Desloratidine in Tablet dosage form. Retention time of Pseuduephedrine, Ambroxol and Desloratidine were found to be 2.417 min, 4.518min. 7.464min %RSD of system precision for Pseuduephedrine , Ambroxol and Desloratidine were and found to be 1.03 , 0.64 and 1.15 respectively.

Recovery was obtained as 99.92% 100.21% and 99.92% for Pseuduephedrine, Ambroxol and Desloratidine respectively. LOD, LOQ values are obtained from regression equations of Pseuduephedrine, Ambroxol and Desloratidine were 0.01ppm, 0.02ppm, 0.15ppm, 0.44ppm and 0.06ppm, 0.17ppm respectively. Regression equation of Pseuduephedrine is y = 16947x + 319.2 Ambroxol is y = 19401x + 3236.and Desloratidine y = 38993x +2051. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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