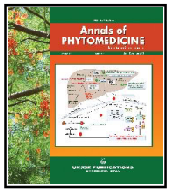
Annals of Phytomedicine 12(2): 321-326, 2023

DOI: http://dx.doi.org/10.54085/ap.2023.12.2.41

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Review Article : Open Access

Analytical insights: Reviewing established methods for ambroxol hydrochloride, levosalbutamol sulphate, and guaiphenesin

M. Archana◆, Afroz Patan and M. Selvakanimozhi

Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies, Chennai-600117, Tamil Nadu, India

Article Info

Article history
Received 5 November 2023
Revised 12 December 2023
Accepted 13 December 2023
Published Online 30 December 2023

Keywords
Cough
OTC medicines
Pulmoclear LS syrup
Respiratory diseases
Analytical methods
HPLC

Abstract

One important area of over-the-counter (OTC) medicine is the cough and cold segment. Traditional cough and cold remedies are available over-the-counter (OTC), but the market is strongly competitive. Ambroxol, levosalbutamol, and guaifenesin are ingredients in pulmoclear LS syrup, a medication classed as a mucolytic, bronchodilator, and expectorant. It is recommended for use in the treatment of mucus-producing productive cough in adults and also in children older than six years, who have bronchospasm, a condition in which the lung muscles spasm and cause difficulty breathing. Extreme respiratory diseases such as chronic obstructive pulmonary disease, cystic fibrosis, and asthma are characterized by increased mucus secretion. Levosalbutamol sulphate, ambroxol hydrochloride, and guaiphenesin work well together to treat asthmatic bronchoconstriction, productive and unproductive cough, common cold, and respiratory tract diseases. Ambroxol is a mucolytic agent that increases the volume of mucus while decreasing its viscosity to aid in its clearance. It eases productive coughing and helps with expectoration. An expectorant called guaiphenesin helps people spit out mucus by making lung secretions less viscous. Levosalbutamol is a beta-agonist that acts as an anti-inflammatory and helps to relax smooth muscles by activating the beta-2 adrenergic receptors in the lungs. This opens the airways for easier breathing. When combined, they assist patients with congestion in breathing deeply and freely.

The purpose of this article is to review the development and validation of analytical methods for the drugs ambroxol hydrochloride, guaiphenesin, and levosalbutamol sulphate in the pharmaceutical dosage form.

1. Introduction

Ambroxol hydrochloride functions as a mucolytic, levosalbutamol sulphate as a bronchodilator, and guaiphenesin as an expectorant. Cough syrups are made by combining these medications in one formulation. The literature reports fewer techniques for the simultaneous estimation of these medications in oral liquid dosage form (Rakesh Kumar *et al.,* 2020). An acute cough is frequently the result of acute bronchitis or common cold; however, differentiating between causes is frequently challenging (Ahmad Kantar *et al.,* 2020). Numerous respiratory diseases, including chronic bronchitis, asthma, bronchiectasis, and chronic obstructive pulmonary disease (COPD), are characterized by an excess of mucus in the airways (Mario Malerba and Beatrice Ragnoli, 2008). A common characteristic of many acute and chronic respiratory conditions is pathological hypersecretion of mucus. (Helmut Albrecht *et al.,* 2017). Coughing can be unproductive and productive. A productive cough produces secretions from the respiratory tract, whereas an unproductive cough just produces a cough with no secretions from the respiratory tract.

Corresponding author: Ms. M. Archana
Assistant Professor, Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Chennai-600117, Tamil Nadu, India
E-mail: archana.sps@velsuniv.ac.in
Tel.: +91-8778263132

A productive cough generates mucus, sputum, or phlegm (Mayuresh Dilip *et al.,* 2019). Childhood asthma is a common chronic illness that requires frequent medical attention and puts a significant financial strain on families. β_2 agonist medications are the most often utilized bronchodilators, which are used to relieve bronchospasm in asthma patients. For the majority of recorded medical history, respiratory disorders have been recognized, and currently, mortality and Morbidity from respiratory diseases represent a significant global health burden (Mukesh Kumar and Meenu Singh, 2007). Pharmacological methods for reducing mucus hypersecretion presently involve several agent classes, such as antioxidants, bronchodilators, mucoregulators, mucolytics, expectorants, and anti-inflammatory medications (Levon Melikyan *et al.,* 2014; Dandu Chaithra *et al.,* 2022).

The present recommendations for the treatment of mucus hypersecretion include the combination of expectorants, mucoregulators, mucolytics, and even bronchodilators in different multidrug component pharmaceutical formulations. As an expectorant, guaifenesin lowers surface tension and adhesiveness, increasing the production of phlegm and bronchial secretions (Prayas Acharyal *et al.,* 2017). Due to its strong bronchodilator action and quick onset of action, levosalbutamol is still the recommended medication for treating acute bronchospasm linked to asthma (Mukesh Kumar and Meenu Singh, 2007). A mucoactive drug's main effect is its capacity to alter mucus production, composition, and/or interaction with the mucociliary epithelium (Malerba and Beatrice Ragnoli, 2008).

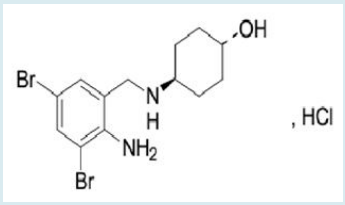
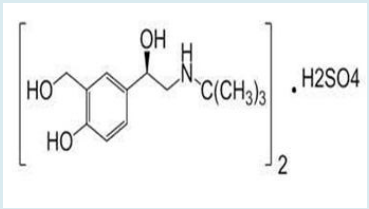
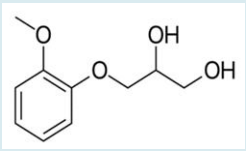
2. Benefits of analysis of drugs in combination

Because the HPLC method has many advantages over other methods, including specificity, precision, ease of automation, accuracy, and rapidity, it can be used to analyze the majority of drugs in multi-component dosage forms. The laborious extraction and isolation processes are eliminated by the HPLC method (Dhritimoni Devi and Sumithra, 2023). Among the benefits are:

- Greater sensitivity (different detectors can be used); speed (the analysis can be finished in 20 min or less); greater resolution.
- Trouble-free sample recovery, handling, and maintenance; ideal for substances with low volatility.
- Reusable columns, (costly columns that are suitable for multiple analyses) instrumentation is accurate and repeatable.
- Automation and quantification (reduced labor and time), suitable for much larger-scale preparative liquid chromatography (Akella Anuradha *et al*., 2023).

3. Drug profile

Table 1: Drug profile details of ambroxol hydrochloride, levosalbutamol sulphate, guaiphenesin

Contents	Ambroxol hydrochloride	Levosalbutamol sulphate	Guaiphenesin
Structure			
	Figure 1: Structure of ambroxol hydrochloride (Sumithra *et al*., 2016).	Figure 2: Structure of levosalbutamol sulphate (Narendra Nyola and Jeyabalan, 2014).	Figure 3: Structure of guaiphenesin (Salomi *et al*., 2018).
Iupac name	4-[(2-amino-3,5- dibromobenzyl) amino]cyclohexanol hydrochloride	4-[(1R)-2-(tert-butylamine)-1-hydroxy ethyl]-2-(hydroxymethyl)phenol;sulfuric acid	3-(2-methoxyphenyl) propane-1, 2-diol
Description	A white or yellowish crystalline powder	Crystalline powder that is white or almost white.	Crystalline powder, white or nearly white.
Molecular formula	$C_{13}H_{19}Br_2ClN_2O$	$C_{13}H_{21}NO_3$	$C_{10}H_{14}O_4$
Molecular weight	414.566 g/mol	239.311 g/mol	198.2 g/mol
Melting point	233-234.5°C	228°C	78.5 - 79°C
pKa	Acidic pKa: 15.26Basic pKa: 9.01	Acidic pKa : 10.12Basic pKa: 9.4	13.53 ± 0.20
Solubility	It is very slightly soluble in methylene chloride and 96% ethanol, but freely soluble in water (Padmavathi Prabhu *et al*., 2018).	Levosalbutamol sulfate was only very slightly soluble in dichloromethane and 95% ethanol, but it was freely soluble in water (Nikhil Patil *et al*., 2019).	It dissolves easily in ethanol, is practically insoluble in petroleum ether, soluble in propylene glycol, glycerol, and chloroform, and moderately soluble in benzene (Teja Kumar *et al*., 2019).
Uses	It helps patients expel mucus, facilitates expectoration, and lessens productive coughing, enabling them to breathe deeply (Schulz *et al*., 2006).	It is used as a bronchodilator for the treatment of COPD and asthma (Narendra Nyola and Jeyabalan, 2014).	Guaifenesin is used to treat bronchitis, respiratory illnesses, and common cold-related coughs and congestion (Azizollah Nezhadali *et al*., 2019).
Drug interaction	The concentrations of antibiotics like erythromycin, cefuroxime, and amoxicillin in sputum and bronchial secretions rise following the use of ambroxol.	Levosalbutamol should be used cautiously in combination with other short-acting sympathomimetic bronchodilators or epinephrine.	The rate of absorption of paracetamol may be increased while administering guaiphenesin.

3.1 Mechanism of action

3.1.1 Ambroxol hydrochloride

Ambroxol functions as a mucolytic: Overproduction of nitric oxide (NO) has been linked to various airway function disturbances, including inflammation. NO improves the soluble guanylate cyclase and cGMP activation build-up. Ambroxol has demonstrated the ability to guanylate cyclase activation in soluble form is dependent on NO. Well, it is moreover, it is feasible that the suppression of NO-dependent soluble guanylate cyclase activation can reduce excessive mucus production, which reduces phlegm viscosity and enhances the transfer of mucociliary respiratory secretions (Mario Malerba *et al.,* 2008; Mounika and Hymavathi, 2021).

3.1.2 Levosalbutamol sulphate

Levosalbutamol (LEV) exhibits a nearly two-fold higher affinity for the β_2 adrenergic receptor in comparison to racemic salbutamol (RAC). About 100 times more binding affinity than salbutamol. Levosalbutamol raises the amount of intracellular adenyl cyclase that is activated to produce 3'5'-cyclic AMP (cAMP). During the airways, higher cAMP concentrations relax by decreasing intracellular bronchial smooth muscle calcium and inhibiting hyperresponsive muscle contraction pathways. A higher cAMP concentration also inhibits the discharge of mediators of inflammation from mast cells and thus, through their interaction with β_2 adrenoceptors, eosinophils. In addition to its bronchodilator, broncho protective, and antiedematous qualities, LEV also prevents mast cell activation of eosinophils (Mukesh Kumar and Meenu Singh, 2007, Amisha Sharma *et al.,* 2021).

3.1.3 Guaiphenesin

It functions as an expectorant by making secretions in the trachea and bronchi more volumetric and less viscous. It may improve the effectiveness of the cough reflex and respiratory tract secretion flow by facilitating ciliary movements that carry the secretions upward towards the pharynx and help the secretions to be removed. In addition to its anticonvulsant and muscle relaxant qualities, guaifenesin may also function as an NMDA receptor agonist (Teja Kumar *et al*., 2019; Hymavathi *et al*., 2021).

3.2 Pharmacokinetic properties

Table 2: Pharmacokinetic properties of ambroxol hydrochloride, levosalbutamol sulphate, and guaiphenesin

Drug parmeters	Ambroxol hydrochloride	Levosalbutamol sulphate	Guaiphenesin
Absorption	It is absorbed in the GI tract. Its bio-availability after oral dosage form is found to be 79%.	Levosalbutamol sulphate is efficiently absorbed from the GI tract.	The human gastrointestinal system absorbs guaifenesin quite well.
Distribution	The lungs have the highest concentration of ambroxol when it comes to transport from blood to tissue; in human lung tissue.	Plasma protein binding is minimal and levosalbutamol tissue binding is not enantioselective.	The drug reached its t-max around 15 min after administration, and the C max was around 1.4 mcg/ml.
Metabolism	It is metabolized by cytochrome P450 3A4 in the liver.	The intestine is the main site of enantio-selective presystemic metabolism for levosalbutamol, a medication absorbed through the gastrointestinal tract. Sulfo-transferase 1A3 (SULT1A3) transforms racemic salbutamol in human tissues into an inactive metabolite.	Fast oxidation of the drug to β-(2-methoxyphenyl)-lactic acid occurs in the liver during drug metabolism. Liver microsomes resident O-demethy lase is responsible for GGE (hydroxy guaifenesin) demethylation.
Half-life	10 h	4.6 h	1 h
Excretion	Dibromo anthranilic acid is the main Phase I metabolite that primarily removes ambroxol during biotransformation. Furthermore, Phase II metabolic processes take place, producing glucuronides (Ahmad Kantar *et al.,* 2020).	As a result, urine has comparatively higher drug concentrations than plasma. Levosalbutamol sulphate is eliminated in the urine. A small amount is excreted in the feces (Narendra Nyola and Jeyabalan, 2014).	Within three hours, 40% of the dosage is eliminated in the urine (Helmut Albrecht *et al.,* 2017).

4. Method development and validation

Arkoti Chaitanya *et al*. (2019) have developed and validated a method for the simultaneous estimation of ambroxol, guaiphenesin, and levosalbutamol syrup formulation by using RP-HPLC. Stationary Phase: The process entails the water C18 column separation and analysis of ambroxol, guaiphenesin, and levosalbutamol. They used a mobile phase mixture in the ratio of 40% acetonitrile, and 60%, 0.1 M sodium dihydrogen phosphate (pH 5.0). Ambroxol, guaiphenesin, and levosalbutamol were eluted at 4.767 min, 9.850 min, and 6.409 min, in that order. The three linear ranges are 50-150 µg/ml, 0.5-1.5 µg/ml, and regression coefficient values of 0.9999, 0.9994, and 0.9991 were found for 15-45 µg/ml levosalbutamol, ambroxol, and guaiphenesin, in that order. The values of the determined LOD are: 0.022 µg/ml for levosalbutamol, 0.1115 µg/ml for guaiphenesin, and 0.072 µg/ml for ambroxol, the levosalbutamol LOQ value is 0.072 µg/ml, guaiphenesin LOQ value is 0.3716 µg/ml, and 0.239 µg/ml is ambroxol. Validation parameters are examined by regulations. The validity data will be useful for accurately quantifying guaiphenesin, ambroxol, and levosalbutamol in the combined dosage form. They stated that this technique works well for regular examination of ambroxol, levosalbutamol, and a combination of guaiphenesin.

Gagandeep *et al*. (2012) A method that allows for the simultaneous determination of levosalbutamol sulphate, guaiphenesin, and ambroxol in pharmaceutical formulations that are pure and do not require any prior purification or separation steps, four straightforward, quick, accurate, precise, dependable, and affordable spectrophotometric methods. The techniques include the double divisor ratio spectra derivative method, derivative ratio spectra zero crossing, simultaneous equation, and derivative zero crossing spectrophotometry. Best practices for linearity, accuracy, precision, LOD, and LOQ are found in developed procedures for standard laboratory mixtures of pure pharmaceuticals and commercial formulations. These APIs were determined without the influence of common additives and excipients. The student t-test was used to statistically compare the results produced by the suggested methods and they concluded that without first separating the individual drugs, derivative spectrophotometry can be successfully used for simultaneous estimation of AB, GF, and LS in the combined dosage form.

Nirav Patel *et al*. (2013) researched the estimation of levosalbutamol sulphate, guaifenesin, and ambroxol hydrochloride. The study was finished by combining colorimetry, first-order derivative, and three more spectrophotometric techniques. As guaifenesin and levosalbutamol sulphate were found to have zero absorption at 323 nm, ambroxol HCl could be measured using specific absorbance in a first-order spectrum. Since levosalbutamol sulphate and ambroxol HCl were found to have zero absorption at 276 nm, guaifenesin could be measured using specific absorbance in a first-order spectrum. Levosalbutamol sulphate was measured colorimetrically by reacting its oxidized product with 4-amino antipyrine and potassium ferricyanide to form a colored substance, the absorption of which was measured at 503 nm. According to ICH guidelines, the suggested approach was statistically validated, and the accuracy, precision, and specificity of the results were deemed adequate.

Itagimatha and Manjunatha, (2019) aimed to develop and validate an RP-HPLC method for a mixture of terbutaline sulphate (TSL), ambroxol hydrochloride (AML), and guaifenesin that was straightforward, sensitive, fast, and stable. High-performance liquid chromatography (HPLC) was used to analyze the combination of these drugs. They used a reverse phase C18 column (250 mm, 4.6 mm, 5 mm) and employed isocratic elution with a mobile phase of buffer: acetonitrile in the ratio 80:20 (buffer 0.1% v/v tri-ethylamine pH-3.0) and 1.0 ml/min flow rate resulted in successful separation. The detection wavelength was 220 nm. For TSL, AML, and GFN; the chromatographic retention times were 3.0, 10.5, and 13.8 min, respectively. The lower limits of quantification and detection for these three compounds were 3.3, 4.1, and 5.0 g/ml and 1.0, 1.25, and 1.5 g/ml, respectively. For TSL, AML, and GFN; the linearity concentrations were determined to be 1.0-7.0, 1.5-7.5, and 4.0-14.0 g/ml, respectively. It was discovered that all of the drug correlation coefficients were higher than 0.999. Less than 2.0% separated the intra- and inter-day relative standard deviations. The drugs that have been chosen for the assay can now be quantified with the help of their method. Their suggested technique is easy to use, precise, repeatable, and effective for analyzing three compounds in both pure and dosage form.

Prabha Thangavelu *et al.* (2019) focused on creating and validating a reverse-phase HPLC method for the concurrent measurement of ambroxol hydrochloride (AMB), guaifenesin (GUA), and salbutamol sulfate (SAL) in the oral liquid dosage form. The drugs were separated chromatographically using a mobile phase system that included acetonitrile, methanol, and sodium dihydrogen phosphate buffer pH 3.0 in the ratio of 65:10:25, with a flow rate of 1 ml/min and an injection volume of 10 µl. An Inertsil C8-3 (250 × 4.6 mm, 5 µm) column was employed, and the detection wavelength was 276 nm. For SAL, GUA, and AMB, respectively, the system produced sharp peaks with good resolution, little tailing, and acceptable retention times of 3.157, 9.949, and 11.883 min, indicating its suitability. The developed method was validated for several parameters, including accuracy, precision, linearity, robustness, and specificity, in compliance with ICH guidelines.

Rakesh Kumar *et al.* (2020) developed a reverse-phase HPLC method and validated it to determine salbutamol sulfate (SAL) and ambroxol hydrochloride (AMB) in oral liquid dosage form simultaneously. An inertsil C8-3 column, measuring 250 × 4.6 mm and 5 µm, was utilized. With a mobile phase system comprising pH 3.0 sodium dihydrogen phosphate buffer, acetonitrile, and methanol in the ratio 65:10:25, the drugs were separated chromatographically at a flow rate of 1 ml/min and a volume for injection of 10 µl. The wavelength of detection was 276 nm. Acceptable retention times of 11.883 min and distinct peaks with minimal tailing were produced by the system for both SAL and AMB. These outcomes show that the system is appropriate. The developed method was validated as per ICH guidelines, taking into account several parameters such as accuracy, precision, linearity, robustness, and specificity (Varuni *et al*., 2023).

5. Conclusion

In conclusion, this medication combination is designed to manage, cure, enhance, and prevent the following conditions and symptoms: tendinitis, thick mucus cough, asthma, breathing disorders, respiratory tract diseases, arthritis pain, pain from sprains or strains in the muscles, lung disorder, congestion, and pain in the tendons.

This review offers a broad summary of the analytical techniques which are developed and validated for the drugs guaiphenesin, ambroxol hydrochloride, and levosalbutamol sulphate. The approaches which are demonstrated in this review are to be cost-effective and compliant with the validation requirements. These methods can be routinely applied to analyze this combination in future advancements. Consequently, an analyst can greatly benefit from the methods listed in the review article above when estimating pharmaceutical formulations and bulk drugs which contain ambroxol hydrochloride, levosalbutamol sulphate, and guaiphenesin.

There are not many methods available in the literature for simultaneously estimating drugs in combined dosage form. Therefore, a novel approach that can be developed in future research, validated, and compared with the current method, is used to perform regular analysis of levosalbutamol sulphate, ambroxol hydrochloride, and guaiphenesin in bulk and pharmaceutical dose form.

This review offers a thorough and current viewpoint on the analytical techniques for the combination of these drugs in the pharmaceutical dosage form.

Acknowledgments

The authors are thankful to the management of Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai-600117, Tamil Nadu, India, for providing research facilities and encouragement.

I would like to express my special thanks and gratitude to my inspiring guide for her motivation.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Ahmad Kantar; Ludger Klimek; Dorotheea Cazan; Annette Sperl; Ulrike Sent and Margarida Mesquita (2020). An overview of efficacy and safety of ambroxol for the treatment of acute and chronic respiratory diseases with special regard to children. Multidiscip. Respir. Med., **15**:511.

Akella Anuradha; Vijey Aanandhi, M. and Afroz Patan (2023). Analytical method development and validation for the simultaneous estimation of lopinavir and ritonavir by RP-HPLC method in tablet dosage form. Ann. Phytomed., **12**(1):573-580.

Amisha Sharma; Priyanka and Amarish Kumar Sharma. (2021). Comparative evaluation of pathophysiology of type 1 diabetes and gestational diabetes mellitus: A review. Ann. Phytomed., **10**(1):123-131.

Arkoti Chaitanya; Valli Kumari, R. V.; Marakatham, S. and Meruva Sathish Kumar (2019). A new RP-HPLC method development and validation of simultaneous estimation of levosalbutamol, ambroxol, and guaiphenesin by syrup formulation. Int. J. Pharm., **9**(4):18-28.

Azizollah Nezhadali; Mahmoud Reza Shapouri; Mitra Amoli-Diva; Amir Hossein Hooshangi and Farrokh Khodayari (2019). Method development for simultaneous determination of active ingredients in cough and cold pharmaceuticals by high-performance liquid chromatography. Heliyon, **5**(12):77-82.

Dandu Chaithra; Mohammed Abdul Rasheed Naikodi; Yasodamma Nimmanapalli; Baira Venkatesham; MohdNazeer; Javed Inam Siddiqui and Ahmed Minhajuddin (2022). Evaluation of antiulcer activity and GC-MS studies of *Curcuma neilgherrensis* Wt. on pyloric ligation induced wistar albino rats. Ann. Phytomed.,**11**(1):396-404.

Dhritimoni Devi and Sumithra, M. (2023). Development and validation of analytical technique for the evaluation of insulin glargine by RP-HPLC. Ann. Phytomed., **12**(1):611-615.

Gagandeep; Navdeep Kaur Gill; Karan; Sarma, G. S. and Arti Thakkar (2012). Simultaneous determination of ambroxol, guaiphenesin, and levosalbutamol sulphate in pharmaceutical formulations with the use of four rapid derivative spectrophotometric methods. J. Chil. Chem. Soc., **57**(4):1436-1441.

Helmut Albrecht, H.; Peter Dicpinigaitis, V. and Eric Guenin, P. (2017). Role of guaifenesin in the management of chronic bronchitis and upper respiratory tract infections. Multidiscip. Respir. Med., **12**(31):1-11.

Hymavathi, T.V. (2021). Nutrient and phytonutrient quality of nutri cereals incorporated flour mix suitable for diabetics. Ann. Phytomed., **10**(1):132-140.

Itagimatha, N. and Manjunatha, D.H. (2019). RP-HPLC-UV method development and validation for simultaneous determination of terbutaline sulfate, ambroxol HCl, and guaifenesin in pure and dosage forms. Ann. Pharm. Fr., **77**(4):295-301.

Levon, Melikyan, A.; Rosa, S. Grigoryan and Tigran Davtyan, K. (2014). Development and validation of RP-HPLC method for simultaneous determination of guaifenesin impurities in multi-drug combinations. Glob. J. Med. Res., **14**(2):27-34.

Mario Malerba and Beatrice Ragnoli (2008). Ambroxol in the 21st century: Pharmacological and clinical update. Expert Opin. Drug Metab. Toxicol., **4**(8):1119-1129.

Mayuresh Dilip Kiran; Lalit Jeevan Pawaskar and Shaheen Naseem Sheikh (2019). Efficacy and safety for the combination of levosalbutamol, ambroxol, and guaifenesin for the symptomatic treatment of productive cough: Phase IV clinical trial. Int. J. Innov. Res. Med. Sci., **4**(1):11-15.

Mounika, M. and Hymavathi, T.V. (2021). Nutrient and phytonutrient quality of nutricereals incorporated flour mix suitable for diabetics. Ann. Phytomed., **10**(1):132-140.

Mukesh Kumar Gupta and Meenu Singh (2007). Evidence-based review on levosalbutamol. Indian J. Pediatr., **74**(2):161-167.

Narendra Nyola and Govinda Samy Jeyabalan (2014). Method development and validation of levosalbutamol in pure and rota caps by UV-VIS spectroscopy. Int. J. Chemtech. Res., **1**(1):16-20.

Nikhil Patil, A.; Sandip Bandgar, A.; Sardar Shelake, S; Pravin Patil and Shitalkumar S Patil, S. (2019). Design, development, and evaluation of fast dissolving tablet of antiasthmatic drug. Res. J. Pharm. Technol., **12**(1):142-148.

Nirav Patel, C.; Dipti Patel, B. and Pruthviraj Chaudhari, K. (2013). Spectrophotometric estimation of ambroxol hydrochloride, guaifenesin, and levosalbutamol sulphatein syrup. Asian J. Research Chem., **6**(4):407-414.

Padmavathi Prabhu, P.; Paramita Das and Shukla Khushboo, N. (2018). Method development and validation for the estimation of ambroxol hcl in the pharmaceutical dosage form. IJPSR., **9**(6):2550-2553.

Prabha Thangavelu; Arul Caroline Grace and Jagadeeswaran Murugesan (2019). Analytical method development and validation for the simultaneous estimation of salbutamol sulphate, guaifenesin, and ambroxol hydrochloride by RP-HPLC method in the commercial oral liquid dosage form. IJPSR., **10**(1):260-265.

Prayas Acharya; Prasanth Kumar, T.; Immanuel Agasteen; Sreerama Rajasekhar and Neelima, G. (2017). A review of analytical methods for determination of guaifenesin alone and in combination with other drugs in pharmaceutical formulations. Saudi J. Med. Pharm. Sci.; **3**(3):148-159.

Rakesh Kumar; Mukesh Bansal and Dilip Agarwal (2020). Development and validation of the RP-HPLC method for the simultaneous estimation of salbutamol sulphate and ambroxol hydrochloride in the oral liquid dosage form. IJARW., **2**(1):22-31.

Salomi, P.; Mahendra Nayak, D.; Vimalakannan, T. and Ravindra Reddy, K. (2018). Method development and method validation of guaifenesin and dextromethorphan by RP-HPLC. Int. J. Res. Pharm. Chem and Analy., **1**(1):18-24.

Schulz, M.; Hämmerlein, A.; Hinkel, U.; Weis, G. and Gillissen, A. (2006). Safety and usage pattern of an over-the-counter ambroxol cough syrup: A community pharmacy-based cohort study. Int. J. Clin. Pharmacol. Ther.; **44**(9):409-421.

Sumithra, M.; Yuvanesh, P. and Anamika Mistry (2016). Analytical method development and validation of ambroxol hydrochloride by UV spectroscopy and forced degradation study and detection of stability. Res. J. Pharm. Technol., **9**(7):794-800.

Teja Kumar Reddy Konatham; Jyothsna, V. and Pavan, N. (2019). Development and validation of guaifenesin by using the HPLC method. J. Emerg. Technol. Innov. Res., **6**(6):144-183.

Varuni, K.; Vijey Aanandhi, M. and Gandhimathi, R. (2023). Estimation of biphasic isophane insulin 30/70 by using validated RP-HPLC technique. Ann. Phytomed., **12**(1):606-610.

Citation **M. Archana, Afroz Patan and M. Selvakanimozhi (2023). Analytical insights: Reviewing established methods for ambroxol hydrochloride, levosalbutamol sulphate, and guaiphenesin. Ann. Phytomed., 12(2):321-326. http://dx.doi.org/10.54085/ap.2023.12.2.41.**