International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 13s, 2025
https://www.theaspd.com/ijes.php

Estimation of Salbutamol, Bromhexine, Guaifenesin, Methyl hydroxybenzoate and Propyl hydroxybenzoate in Multi-component Cough Expectorant syrup by HPLC

Dr. Parag Das (Ph.D.)[1], Mr. Ramesh Singh[2], Mr. Animesh Maity (M.Sc.)[3], Mr. Amrit Kumar Rath[4], Prof. (Dr.) Sruti Ranjan Mishra[5], Mrs. Manashi Das[6]

[1]Vice President – Technical, Oman Pharmaceutical Products Co LLC, Sultanate of Oman.
[2]Asst. Manager, Analytical Development Labs, Oman Pharmaceutical Products Co LLC, Sultanate of Oman.
[3]Sr.Manager, Analytical development Labs, Oman Pharmaceutical Products Co LLC, Sultanate of Oman.
[4]Associate Professor, Danteswari College of Pharmacy, Borpadar, Raipur Road, Jagdalpur, Chhattisgarh, Pin: 494221.
[5]Professor, Danteswari College of Pharmacy, Borpadar, Raipur Road, Jagdalpur, Chhattisgarh, Pin: 494221.
[6] Lecturer, Marathwada Mitra Mandal's College of Pharmacy, Pune, India.

ABSTRACT

Background: *Bronkovent syrup is a medication used to treat various respiratory conditions by addressing the underlying mucus and airflow issues. It contains a combination of active ingredients that work together to provide relief.*

The key components of Bronkovent syrup are:

- *Salbutamol (2 mg per 5 mL): This opens the constricted airways without affecting the heart.*
- *Bromhexine hydrochloride (4 mg per 5 mL): This thins out mucus in the airways to facilitate clearing.*
- *Guaifenesin (50 mg per 5 mL): This expectorant increases sputum volume and reduces its viscosity, making it easier to cough up.*
- *Methyl and propyl hydroxybenzoates: These are preservatives.*

Bronkovent syrup is indicated for use in acute and chronic respiratory conditions, including bronchial asthma, bronchiectasis, and whooping cough, where it helps clear thick, sticky mucus from the lungs.

Aim: *To develop a simple, rapid, and cost-effective HPLC methodology for the simultaneous determination of Salbutamol, Bromhexine hydrochloride, Guaifenesin, Methyl hydroxybenzoate, and Propyl hydroxybenzoate in multi-component cough expectorant syrup, and to validate the method.*

The method involves diluting the sample with a simple diluent and analyzing it using an HPLC system equipped with a UV detector and a Waters X-Bridge column (250 x 4.6 mm, 5 µm) coupled with a Ghost buster column (50 x 4.6 mm). The flow rate was set at 1.2 mL/min with a gradient elution. The column oven and autosampler temperatures were maintained at 40°C and 10°C, respectively, with an injection volume of 10 µL. The retention times for the analytes were approximately 11 minutes for Salbutamol, 28 minutes for Bromhexine hydrochloride, 16 minutes for Guaifenesin, 19 minutes for Methyl hydroxybenzoate, and 26 minutes for Propyl hydroxybenzoate.

Results and discussion: *The developed method was validated according to the ICH guidelines and the values of accuracy, method precision and other statistical analysis were found to be in good accordance with the specified acceptance criteria.*

Conclusion: *The proposed methodology was successfully applied to the determination of Salbutamol, Bromhexine hydrochloride, Guaifenesin, Methyl hydroxybenzoate, and Propyl hydroxybenzoate in Bronkovent syrup by GC-FID for routine analysis.*

KEYWORDS

Salbutamol, Bromhexine hydrochloride, Guaifenesin, Methyl hydroxybenzoate, Propyl hydroxybenzoate, HPLC and Validation.

INTRODUCTION

Salbutamol [Fig. 1], also known as albuterol and sold under the brand name Ventolin, is a medication that opens the airways in the lungs. It is a short-acting β2 adrenergic receptor agonist, meaning it causes

relaxation of airway smooth muscle. Salbutamol is primarily used to treat asthma, including attacks and exercise-induced bronchoconstriction, as well as chronic obstructive pulmonary disease (COPD). It may also be used to address high blood potassium levels.

Salbutamol is typically administered via inhaler or nebulizer, but is also available in pill, liquid, and intravenous forms. The inhaled version begins working within 15 minutes and provides relief for 2-6 hours. Common side effects include shakiness, headache, fast heart rate, dizziness, and anxiety. Serious side effects may include worsening bronchospasm, irregular heartbeat, and low blood potassium. While it can be used during pregnancy and breastfeeding, safety is not fully established.

Salbutamol was patented in 1966 in Britain and approved for medical use in the US in 1982. It is on the World Health Organization's List of Essential Medicines and available as a generic. In 2022, it was the 7th most prescribed medication in the US, with over 59 million prescriptions.

Bromhexine [Fig. 2] is a mucolytic drug developed in the late 1950s by Boehringer Ingelheim and introduced in 1963 under the trademark Bisolvon®. It is used to treat respiratory disorders associated with thick or excessive mucus.

Bromhexine is intended to support the body's natural mechanisms for clearing mucus from the respiratory tract. It is secretolytic, meaning it increases the production of thin, serous mucus, which is more easily transported out of the lungs by cilia. This contributes to a secretomotoric effect, allowing the cilia to more easily expel the phlegm. For this reason, bromhexine is often added to cough syrups.

Bromhexine is available in various formulations, including high and low strength syrups, tablets, and oral solutions, to meet the needs of patients of all ages. It is a well-established and well-tolerated medication.[2]

Guaifenesin [Fig. 3], also known as glyceryl guaiacolate, is an expectorant medication taken orally and marketed to help eliminate sputum from the respiratory tract. Chemically, it is an ether of guaiacol and glycerin. It may be used in combination with other medications.

A 2014 study found that guaifenesin does not affect sputum volume in upper respiratory infections. Potential side effects include dizziness, drowsiness, skin rash, and nausea. While its safety during pregnancy has not been thoroughly studied, it appears to be safe.

Despite claims that it works by making airway secretions more liquid, research has demonstrated that guaifenesin is not effective. It has been used medically since at least 1933 and is available as both generic and over-the-counter medication.

Guaifenesin is used to help with coughing up thick mucus. It is sometimes combined with the cough suppressant dextromethorphan, as well as with ephedrine or phenylephrine and paracetamol in certain formulations.

The proposed mechanism of action is that guaifenesin may increase the volume and reduce the viscosity of respiratory secretions, aiding their removal via the cough reflex. However, the evidence on its effectiveness as an expectorant is mixed, with a Cochrane review finding conflicting results across clinical trials.

MATERIALS AND METHODS

Chemicals and reagents:

Disodium phosphate anhydrous, pentane sulfonic acid, methanol (gradient grade) and orthophosphoric acid were procured from Merck.

Methodology:

Instrument	:	HPLC
Column	:	Waters X-Bridge 250 x 4.6mm; 5µm (Part # 186003117) + Ghost buster column 50 x 4.6mm (Part # 06100-31000)
Flow	:	1.2 mL/min
Column Oven Temp.	:	40°C
Auto sampler Temp.	:	15°C
Injection volume	:	10 µL

Wavelength	:	Salbutamol, Guaifenesin and Bromhexine: 224 nm Methyl hydroxybenzoate and Propyl hydroxybenzoate: 256 nm
Retention time	:	Salbutamol: About 11 min Guaifenesin: About 16 min Methyl hydroxybenzoate: About 19 min Propyl hydroxybenzoate: About 26 min Bromhexine: About 28 min
Run time	:	50 minutes

Preparation of Buffer:
Weigh and transfer accurately 3.55 gm of disodium hydrogen orthophosphate anhydrous and 0.85 gm of pentane sulfonic acid sodium salt in a beaker containing 1000 mL of Milli-Q water. Sonicate to dissolve. Adjust the pH of the buffer solution to 3.5 with orthophosphoric acid. Filter through 0.45µ nylon membrane filter and degas.
Mobile Phase A:
Buffer (100%)
Mobile Phase B:
Methanol (100%)
Gradient Program:

Time (min)	**%A**	**%B**
0	95	5
30	25	75
35	25	75
35.1	95	5
50	95	5

Diluent:
Mobile phase A and Mobile phase B in the ratio 90:10
Standard stock solution preparation:
Salbutamol sulphate standard stock solution (Solution A):
Weigh and transfer accurately about 50 mg Salbutamol sulphate working standard into a clean and dry 100 mL volumetric flask. Add 10 mL of methanol, sonicate to dissolve, and then dilute up to the mark with diluent. (500 µg/mL)
Bromhexine HCl standard stock solution (Solution B):
Weigh and transfer accurately about 50 mg Bromhexine HCl working standard into a clean and dry 100 mL volumetric flask. Add 10 mL of methanol, sonicate to dissolve, and then dilute up to the mark with diluent. (500 µg/mL)
Methyl hydroxybenzoate standard stock solution (Solution C):
Weigh and transfer accurately about 50 mg Methyl hydroxybenzoate working standard into a clean and dry 20 mL volumetric flask. Add 2 mL of methanol, sonicate to dissolve, and then dilute up to the mark with diluent. (2500 µg/mL)
Propyl hydroxybenzoate standard stock solution (Solution D):
Weigh and transfer accurately about 25 mg Propyl hydroxybenzoate working standard into a clean and dry 100 mL volumetric flask. Add 10 mL of methanol, sonicate to dissolve, and then dilute up to the mark with diluent. (250 µg/mL)
Guaifenesin standard stock solution (Solution E):
Weigh and transfer accurately about 50 mg Guaifenesin working standard into a clean and dry 20 mL volumetric flask. Add 2 mL of methanol, sonicate to dissolve, and then dilute up to the mark with diluent. (2500 µg/mL)

Preparation of Standard solution:

Transfer accurately 2 mL of Solution A, 4 mL of Solution B, 2 mL of Solution C, 2 mL of Solution D and 10mL of Solution E into a clean and dry 50 mL volumetric flask. Dilute up to the mark with diluent and mix well. (Salbutamol sulfate = 20 µg/mL, Bromhexine HCl = 40 µg/mL, Guaifenesin = 500 µg/mL, Methyl hydroxybenzoate = 100 µg/mL, Propyl hydroxybenzoate = 10 µg/mL)

Sample Preparation:

Weigh and transfer accurately 5 mL of sample (Wt: Approx. 6.5gm) into a clean and dry 100 mL volumetric flask. Add 60-70 mL of diluent, sonicate to dissolve, dilute up to the mark with diluent and mix well. Filter through 0.45µm syringe filter after discarding the first 5 mL of the filtrate.

Procedure

Inject 10µL of diluent (single injection), standard solution (six replicate injections) and sample solution (single injection) into the chromatograph & record the results obtained. Calculate the % Assay of each moiety by using the formulae as given below in the calculation.

System Suitability:

a. Tailing factor for Salbutamol, Bromhexine, Guaifenesin, Methyl hydroxybenzoate and Propyl hydroxybenzoate peaks from standard preparation should not be more than 2.0.

b. Theoretical plate count for Salbutamol, Bromhexine, Guaifenesin, Methyl hydroxybenzoate and Propyl hydroxybenzoate peak from standard preparation should not be less than 2000.

% RSD for the peak areas of Salbutamol, Bromhexine, Guaifenesin, Methyl hydroxybenzoate and Propyl hydroxybenzoate peak from six replicate injections of standard preparation should not be more than 2.0%.

Calculations:

1) Salbutamol sulfate (Label Claim: 2.0 mg per 5 ml)

$$= \frac{Au}{As} \times \frac{\text{Std Wt.}}{100} \times \frac{2}{50} \times \frac{478.6}{576.7} \times \frac{100}{\text{Spl .Wt.}} \times \frac{5}{\text{L.C.}} \times \frac{P}{100} \times \text{Wt.ml} \times \frac{100}{}$$

2) Guaifenesin (Label Claim: 50.0 mg per 5 ml)

$$= \frac{Au}{As} \times \frac{\text{Std Wt.}}{20} \times \frac{10}{50} \times \frac{100}{\text{Spl. Wt.}} \times \frac{5}{\text{L.C.}} \times \frac{P}{100} \times \text{Wt.ml} \times 100$$

3) Bromhexine HCl (Label Claim: 4.0 mg per 5 ml)

$$= \frac{Au}{As} \times \frac{\text{Std Wt.}}{100} \times \frac{4}{50} \times \frac{P}{100} \times \frac{100}{\text{Spl. Wt.}} \times \frac{5}{\text{L.C.}} \times \text{Wt.ml} \times 100$$

4) Methyl Hydroxybenzoate (Label Claim: 10.0 mg per 5 ml)

$$= \frac{Au}{As} \times \frac{\text{Std Wt.}}{20} \times \frac{2}{50} \times \frac{100}{\text{Spl. Wt.}} \times \frac{P}{100} \times \frac{5}{\text{L.C.}} \times \text{Wt.ml} \times 100$$

5) Propyl Hydroxybenzoate (Label Claim: 1.0 mg per 5 ml)

$$= \frac{Au}{As} \times \frac{\text{Std Wt.}}{100} \times \frac{2}{50} \times \frac{100}{\text{Spl. Wt.}} \times \frac{P}{100} \times \frac{5}{\text{L.C.}} \times \text{Wt.ml} \times 100$$

Where,

Au = Peak area in sample preparation

As = Average peak area in standard preparation

P = Potency of working standard on as is basis
LC = Label Claim

Chromatographic study:

Salbutamol, Bromhexine, Guaifenesin, Methyl hydroxybenzoate and Propyl hydroxybenzoate content in all solutions were determined by HPLC using the chromatographic conditions as mentioned above.
The Chromatographic data were analyzed, and Specificity, Force degradation, Precision, Linearity, accuracy, Range, Robustness, Ruggedness and solution stability were determined.

Results and discussion:

The developed method for the estimation of Salbutamol, Bromhexine, Guaifenesin, Methyl hydroxybenzoate and Propyl hydroxybenzoate in multi-component cough expectorant cough syrup by HPLC was validated by using the following parameters:

System suitability:

For establishing the system suitability, the procedure described in the methodology was followed before starting the analysis. System suitability data has been presented in Table No – 1 and 2.

Specificity:

Specificity of the method was evaluated with respect to interference from blank and placebo at the retention time of Salbutamol, Bromhexine, Guaifenesin, Methyl hydroxybenzoate and Propyl hydroxybenzoate peaks in standard and sample. Refer Fig. 5, 6, 7 and 8 for the chromatograms and specificity data has been presented in Table No – 3 to 29.

Linearity and range:

Standard solutions containing Salbutamol, Bromhexine, Guaifenesin, Methyl hydroxybenzoate and Propyl hydroxybenzoate were prepared. The linearity study was performed from 50% to 150% of the target concentration - Salbutamol sulfate = 20 µg/mL, Bromhexine HCl = 40 µg/mL, Guaifenesin = 500 µg/mL, Methyl hydroxybenzoate = 100 µg/mL, Propyl hydroxybenzoate = 10 µg/mL.
The linearity was evaluated using the calibration curve plotted by average peak areas against concentrations to calculate coefficient of correlation and slope. In general, the value of correlation coefficient (r) > 0.990 is considered as the evidence of an acceptable fit for the data to the regression line. The results obtained are presented in Table No – 30 to 34 which demonstrates that the current method was linear for all the five analytes in the range specified above with a correlation coefficient better than 0.990. The plots have been represented in Fig.

Method Precision:

Precision was determined by preparing the standard and sample as per the methodology. The sample was prepared in six replicates and injected into the chromatography system. The content of Salbutamol, Bromhexine, Guaifenesin, Methyl hydroxybenzoate and Propyl hydroxybenzoate in each of the preparation was calculated and finally the %RSD of the six replicate preparations data was deduced. The results are presented in Table No - 35.

Intermediate Precision:

Intermediate Precision was determined by preparing the standard and sample as per the methodology by a different analyst, on a different day and using a different HPLC column and HPLC system. The sample was prepared in six replicates and injected into the chromatography system. The content of Salbutamol, Bromhexine, Guaifenesin, Methyl hydroxybenzoate and Propyl hydroxybenzoate in each of the preparation was calculated and finally the %RSD of the six replicate preparations data was deduced. The results are presented in Table No – 36, 37 and 38.

Accuracy:

Accuracy study was conducted by spiking the known amount of Salbutamol sulfate, Guaifenesin, Methyl hydroxybenzoate, Propyl hydroxybenzoate and Bromhexine HCl in the placebo. Accuracy study was conducted in triplicate at three different levels - 50%, 100% and 150% of target concentration. The samples were analyzed as per methodology and % recovery at each spiked level was calculated. The results are presented in Table No – 41 to 45.

Filter compatibility:

Prepared the standard and sample in three replicates as per methodology. The standard solution was

injected in six replicates to establish the system suitability and each individual sample preparation was filtered through the following filters to demonstrate filter compatibility and establish the saturation volume by discarding different volumes of 3mL, 5mL and 10mL-

1. 0.45 µm Nylon GD-X filter.
2. 0.45 µm PTFE filter.
3. 0.45 µm PVDF filter.

The filtered samples were evaluated against the sample centrifuged at 10000 rpm for 5 minutes. The results are presented in Table No – 46 to 55.

Robustness:

Robustness of the analytical was evaluated by making deliberate changes and evaluating the system suitability parameters and %Assay of Salbutamol sulfate, Bromhexine HCl, Guaifenesin, Methyl hydroxybenzoate and Propyl hydroxybenzoate vis-à-vis Precision parameter results.

The following changes were performed –

Sr. No.	Actual Parameter	Change-1	Change-2
1.	Flow rate (1.2 mL/min)	1.0 mL/ min	1.4 mL/ min
2.	Column oven temperature (40°C)	35°C	45°C
3.	pH of Mobile phase – A (pH 3.5)	pH 3.3	pH 3.7
4.	HPLC Column	Column # 1	Column # 2
5.	Diluent composition (Mobile Phase – A: Mobile Phase – B = 90 : 10)	Mobile Phase – A: Mobile Phase – B = 92 : 08	Mobile Phase – A: Mobile Phase – B = 88 : 12

CONCLUSION

This intended study concludes that the proposed method is economical, simple, sensitive and reliable. Also, it is found to be specific, linear, precise, robust, rugged and accurate. Hence, it can be employed for the routine estimation of Assay of Salbutamol sulfate, Bromhexine HCl, Guaifenesin and Preservatives – Methyl hydroxybenzoate and Propyl hydroxybenzoate in multicomponent expectorant cough syrup by HPLC.

ACKNOWLEDGEMENT:

Authors wish to thank the management of Oman Pharmaceutical Products Co. LLC, for providing library and laboratory facility to carry out this analytical method validation for this multicomponent cough expectorant syrup formulation.

CONFLICT OF INTEREST: The authors declare no conflict of interest.

REFERENCES

1. "Salbutamol". Drugs.com. Archived from the original on 30 March 2016. Retrieved 11 April 2016.
2. Thereaputic Goods Administration (19 December 2018). "Prescribing medicines in pregnancy database". Australian Government.
3. "Albuterol Use During Pregnancy". Drugs.com. 8 March 2019. Retrieved 21 December 2019.
4. "FDA-sourced list of all drugs with black box warnings (Use Download Full Results and View Query links.)". nctr-crs.fda.gov. FDA. Retrieved 22 October 2023.
5. Thereaputic Goods Administration. "Poisons Standard October 2017". Australian Government.
6. "Prescription Drug List". Government of Canada. 23 October 2014.
7. "Respiratory health". Health Canada. 9 May 2018. Retrieved 13 April 2024.
8. "Albuterol". Drugs.com. The American Society of Health-System Pharmacists. Archived from the original on 8 December 2015. Retrieved 2 December 2015.
9. Mahoney BA, Smith WA, Lo DS, Tsoi K, Tonelli M, Clase CM (April 2005). "Emergency interventions for hyperkalaemia". The Cochrane Database of Systematic Reviews. 2005 (2): CD003235. doi:10.1002/14651858.CD003235.pub2. PMC 6457842. PMID 15846652.
10. Starkey ES, Mulla H, Sammons HM, Pandya HC (September 2014). "Intravenous salbutamol for childhood asthma: evidence-based medicine?" (PDF). Archives of Disease in Childhood. 99 (9): 873–7. doi:10.1136/archdischild-2013-

304467. PMID 24938536. S2CID 2070868. Archived from the original (PDF) on 8 September 2017.
11. Yaffe SJ (2011). Drugs in pregnancy and lactation: a reference guide to fetal and neonatal risk (9th ed.). Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins. p. 32. ISBN 9781608317080. Archived from the original on 8 December 2015.
12. Landau R (1999). Pharmaceutical innovation: revolutionizing human health. Philadelphia: Chemical Heritage Press. p. 226. ISBN 9780941901215. Archived from the original on 8 December 2015.
13. Fischer J, Ganellin CR (2006). Analogue-based Drug Discovery. John Wiley & Sons. p. 542. ISBN 9783527607495.
14. World Health Organization (2023). The selection and use of essential medicines 2023: web annex A: World Health Organization model list of essential medicines: 23rd list (2023). Geneva: World Health Organization. hdl:10665/371090. WHO/MHP/HPS/EML/2023.02.
15. "The Top 300 of 2022". ClinCalc. Archived from the original on 30 August 2024. Retrieved 30 August 2024.
16. "Albuterol Drug Usage Statistics, United States, 2013 - 2022". ClinCalc. Retrieved 30 August 2024.
17. Morton I, Hall J (1999). Concise Dictionary of Pharmacological Agents. Springer. p. 55. ISBN 0-7514-0499-3. Retrieved 2009-06-03.
18. Fischer J, Ganellin CR (2006). Analogue-based Drug Discovery. John Wiley & Sons. p. 544. ISBN 9783527607495.
19. "Guaifenesin Definition & Meaning". Merriam-Webster.
20. "FDA-sourced list of all drugs with black box warnings (Use Download Full Results and View Query links.)". nctr-crs.fda.gov. FDA. Retrieved 22 October 2023.
21. Aluri JB, Stavchansky S (1993). "Determination of guaifenesin in human plasma by liquid chromatography in the presence of pseudoephedrine". J Pharm BiomedAnal. 11 (9): 803–808. doi:10.1016/0731-7085(93)80072-9. PMID 8218524.
22. "Guaifenesin: Monograph for Professionals". Drugs.com, American Society of Health-System Pharmacists. 23 January 2023. Retrieved 30 July 2023.
23. Hoffer-Schaefer A, Rozycki HJ, Yopp MA, Rubin BK (May 2014). "Guaifenesin has no effect on sputum volume or sputum properties in adolescents and adults with acute respiratory tract infections". Respiratory Care. 59 (5): 631–636. doi:10.4187/respcare.02640. PMID 24003241.
24. O'Connell OJ (May 2014). "Is extended-release guaifenesin no better than a placebo?". RespirCare. 59 (5): 788– doi:10.4187/respcare.03319. PMID 24789023.
25. Weiner CP, Rope K (2013). The Complete Guide to Medications During Pregnancy and Breastfeeding: Everything You Need to Know to Make the Best Choices for You and Your Baby. St. Martin's Press. p. PT282. ISBN 9781250037206.
26. Riviere JE, Papich MG (2013). Veterinary Pharmacology and Therapeutics. John Wiley & Sons. p. 287. ISBN 9781118685907.
27. "The Top 300 of 2022". ClinCalc. Archived from the original on 30 August 2024. Retrieved 30 August 2024.
28. "Guaifenesin Drug Usage Statistics, United States, 2013 - 2022". ClinCalc. Retrieved 30 August 2024.
29. "Dextromethorphan; Guaifenesin Drug Usage Statistics, United States, 2013 - 2022". ClinCalc. Retrieved 30 August 2024.
30. "Guaifenesin DM". WebMD.com.
31. "Lemsip Max All in One". LemsipUK. Retrieved 4 November 2024.
32. Smith SM, Schroeder K, Fahey T (November 2014). "Over-the-counter (OTC) medications for acute cough in children and adults in community settings". Cochrane Database Syst Rev. 2014 (11): CD001831. doi:10.1002/14651858.CD001831.pub5. PMC 7061814. PMID 25420096.

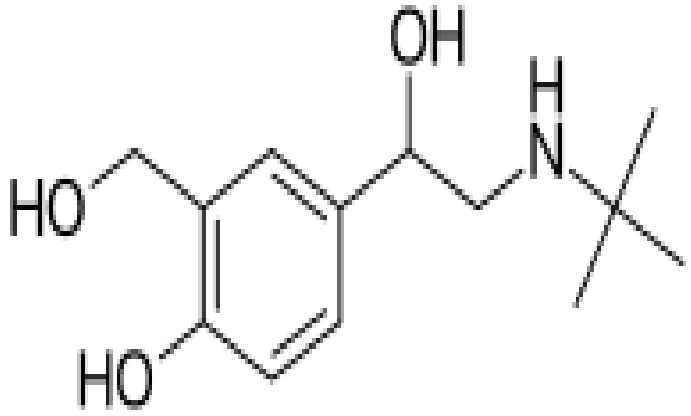

Fig. 1 – Salbutamol

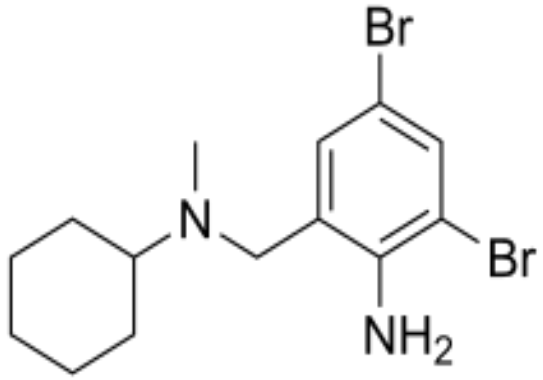

Fig. 2 – Bromhexine

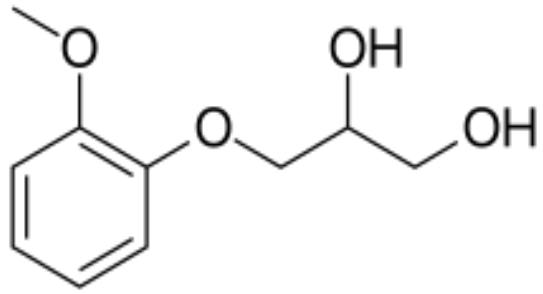

Fig. 3 – Guaifenesin

Table No. 1 – System suitability

Injection #	Area				
	Salbutamol	Guaifenesin	Methyl hydroxy benzoate	Propyl hydroxy benzoate	Bromhexine
1	267238	8683658	5101991	452838	594372
2	267269	8682080	5101430	452776	593993
3	267203	8688203	5103097	452856	593959
4	267470	8688480	5104185	452874	594442
5	266967	8691896	5101947	452732	594154
6	266826	8683861	5102814	452716	593393
Mean	**267162**	**8686363**	**5102577**	**452799**	**594052**
SD	**230.08296**	**3752.39913**	**996.80864**	**66.78523**	**377.17763**
%RSD	**0.1**	**0.0**	**0.0**	**0.0**	**0.1**

Table No. 2 – System suitability criteria's

Criteria	Limit	Peak	Results
% RSD for the peak areas of Salbutamol, Bromhexine, Guaifenesin, Methyl hydroxybenzoate and Propyl hydroxybenzoate peak from six replicate injections of standard preparation	NMT 2.0%	Salbutamol	0.1
		Guaifenesin	0.0
		Methyl hydroxybenzoate	0.0
		Propyl hydroxybenzoate	0.0
		Bromhexine	0.1
Tailing factor for Salbutamol, Bromhexine, Guaifenesin, Methyl hydroxybenzoate and	NMT 2.0	Salbutamol	1.01
		Guaifenesin	1.05

Propyl hydroxybenzoate peaks from standard preparation should not be more than 2.0		Methyl hydroxybenzoate	1.03
		Propyl hydroxybenzoate	1.02
		Bromhexine	1.02
Theoretical plate count for Salbutamol, Bromhexine, Guaifenesin, Methyl hydroxybenzoate and Propyl hydroxybenzoate peak from standard preparation should not be less than 2000	NLT 2000	Salbutamol	114376
		Guaifenesin	153771
		Methyl hydroxybenzoate	181957
		Propyl hydroxybenzoate	410842
		Bromhexine	377178

Table No. 3 – % Interference

Observation		**Interference**	
		Blank	Placebo
Standard	Salbutamol	x	x
	Guaifenesin	x	x
	Methyl hydroxybenzoate	x	x
	Propyl hydroxybenzoate	x	x
	Bromhexine	x	x
Sample	Salbutamol	x	x
	Guaifenesin	x	x
	Methyl hydroxybenzoate	x	x
	Propyl hydroxybenzoate	x	x
	Bromhexine	x	x

x – No interference

Table No. 4 – Retention Time & Peak purity data

Component		**Retention Time**	**Peak purity**
Standard	Salbutamol (@ 224nm)	11.090 min	1.0000
	Guaifenesin (@ 224nm)	16.290 min	0.9985
	Methyl hydroxybenzoate (@ 256 nm)	18.573 min	1.0000
	Propyl hydroxybenzoate (@ 256 nm)	26.440 min	1.0000
	Bromhexine (@ 224 nm)	27.637 min	1.0000
Sample	Salbutamol (@ 224nm)	11.090 min	0.9999
	Guaifenesin (@ 224nm)	16.300 min	0.9984
	Methyl hydroxybenzoate (@ 256 nm)	18.583 min	1.0000
	Propyl hydroxybenzoate (@ 256 nm)	26.453 min	1.0000
	Bromhexine (@ 224 nm)	27.647 min	1.0000

A. Salbutamol sulfate –

Table No. 5 – Acid degradation				
Degradation conditions	**Sample Area**	**% Assay**	**% Degradation**	**Peak Purity**
1M HCl,10mL, 100°C, 1 Hr	35268	11.3	85.7	0.6044
1M HCl,10mL, 100°C, 15 Min	98381	31.5	65.5	0.7544
1M HCl,10mL, 100°C, 30 Min	76844	24.3	72.7	0.6412
1M HCl,10mL, 100°C, 5 Min	177535	56.8	40.2	0.9993

Table No. 6 – Base degradation				
Degradation conditions	**Sample Area**	**% Assay**	**% Degradation**	**Peak Purity**
1M NaOH,10mL, 100°C, 1 Hr	285781	90.8	6.2	0.9996
1M NaOH,10mL, 100°C, 10 Min	306276	97.3	-0.3	0.9999
0.1M NaOH,10mL, 50°C, 5 Min	309371	98.5	-1.5	0.9998
1M NaOH,10mL, 100°C, 5 Min	307414	98.3	-1.3	0.9999
1M NaOH,10mL, 100°C, 3 Hrs	253058	80.5	16.5	0.9994

Table No. 7 – Peroxide degradation				
Degradation conditions	**Sample Area**	**% Assay**	**% Degradation**	**Peak Purity**
30% H_2O_2,10mL, 100°C, 1 Hr	91120	29.0	68.0	0.9991
30% H_2O_2,10mL, 100°C, 5 Min	303729	96.8	0.2	1.0000
30% H_2O_2,10mL, 100°C, 30 Min	198938	63.3	33.7	0.9999
30% H_2O_2,10mL, 100°C, 3 Hrs	10705	3.3	93.7	0.9932

Table No. 8 – Thermal degradation				
Degradation conditions	**Sample Area**	**% Assay**	**% Degradation**	**Peak Purity**
105°C, 24 Hrs	67155	21.3	75.7	0.9997
105°C, 72 Hrs	52223	16.5	80.5	0.9621

Table No. 9 – UV degradation				
Degradation conditions	**Sample Area**	**% Assay**	**% Degradation**	**Peak Purity**
1.2 million Lux Hours	305710	96.3	0.7	0.9999

B. Guaifenesin –

Table No. 10 – Acid degradation				
Degradation conditions	**Sample Area**	**% Assay**	**% Degradation**	**Peak Purity**
1M HCl,10mL, 100°C, 1 Hr	6736713	79.7	20.5	0.9987
1M HCl,10mL, 100°C, 15 Min	8207473	97.3	2.9	0.9985
1M HCl,10mL, 100°C, 30 Min	7806488	91.0	9.2	0.9986
1M HCl,10mL, 100°C, 5 Min	8521737	101.1	-0.9	0.9986

Table No. 11 – Base degradation				
Degradation conditions	**Sample Area**	**% Assay**	**% Degradation**	**Peak Purity**
1M NaOH,10mL, 100°C, 1 Hr	8702198	102.6	-2.4	0.9984
1M NaOH,10mL, 100°C, 10 Min	8719432	102.8	-2.6	0.9986
0.1M NaOH,10mL, 50°C, 5 Min	8668811	102.3	-2.1	0.9984
1M NaOH,10mL, 100°C, 5 Min	8645825	102.4	-2.2	0.9983
1M NaOH,10mL, 100°C, 3 Hrs	8600039	101.5	-1.3	0.9986

Table No. 12 – Peroxide degradation				
Degradation conditions	**Sample Area**	**% Assay**	**% Degradation**	**Peak Purity**
30% H_2O_2,10mL, 100°C, 1 Hr	2569259	30.4	69.8	0.9994
30% H_2O_2,10mL, 100°C, 5 Min	8541716	101.0	-0.8	0.9986
30% H_2O_2,10mL, 100°C, 30 Min	6524223	77.1	23.1	0.9988
30% H_2O_2,10mL, 100°C, 3 Hrs	157850	1.8	98.4	0.9991

Table No. 13 – Thermal degradation				
Degradation conditions	**Sample Area**	**% Assay**	**% Degradation**	**Peak Purity**
105°C, 24 Hrs	8058525	94.9	5.3	0.9985
105°C, 72 Hrs	8322051	98.1	2.1	0.9984

Table No. 14 – UV degradation				
Degradation conditions	**Sample Area**	**% Assay**	**% Degradation**	**Peak Purity**
1.2 million Lux Hours	8731528	101.4	-1.2	0.9984

C. Methyl hydroxybenzoate –

Table No. 15 – Acid degradation				
Degradation conditions	**Sample Area**	**% Assay**	**% Degradation**	**Peak Purity**
1M HCl,10mL, 100°C, 1 Hr	2102693	42.3	57.0	0.9279
1M HCl,10mL, 100°C, 15 Min	4018997	81.0	18.3	0.9959
1M HCl,10mL, 100°C, 30 Min	3376711	67.0	32.3	0.9731
1M HCl,10mL, 100°C, 5 Min	4831193	97.5	1.8	1.0000

Table No. 16 – Base degradation				
Degradation conditions	**Sample Area**	**% Assay**	**% Degradation**	**Peak Purity**
1M NaOH,10mL, 100°C, 1 Hr	0	0.0	99.3	0
1M NaOH,10mL, 100°C, 10 Min	2191	0.1	99.2	0.8598
0.1M NaOH,10mL, 50°C, 5 Min	4667079	93.6	5.7	1.0000
1M NaOH,10mL, 100°C, 5 Min	80451	1.6	97.7	0.9981
1M NaOH,10mL, 100°C, 3 Hrs	0	0.0	99.3	0

Table No. 17 – Peroxide degradation				
Degradation conditions	**Sample Area**	**% Assay**	**% Degradation**	**Peak Purity**
30% H_2O_2,10mL, 100°C, 1 Hr	4615575	92.8	6.5	1.0000
30% H_2O_2,10mL, 100°C, 5 Min	4942188	99.4	-0.1	1.0000
30% H_2O_2,10mL, 100°C, 30 Min	4849446	97.4	1.9	0.9999
30% H_2O_2,10mL, 100°C, 3 Hrs	3798236	75.2	24.1	1.0000

Table No. 18 – Thermal degradation				
Degradation conditions	**Sample Area**	**% Assay**	**% Degradation**	**Peak Purity**
105°C, 24 Hrs	4753550	95.2	4.1	1.0000
105°C, 72 Hrs	4916498	98.6	0.7	1.0000

Table No. 19 – UV degradation				
Degradation conditions	**Sample Area**	**% Assay**	**% Degradation**	**Peak Purity**
1.2 million Lux Hours	5061537	100.8	-1.5	1.0000

D. Propyl hydroxybenzoate –

Table No. 20 – Acid degradation				
Degradation conditions	**Sample Area**	**% Assay**	**% Degradation**	**Peak Purity**
1M HCl,10mL, 100°C, 1 Hr	206100	47.0	52.5	0.9992
1M HCl,10mL, 100°C, 15 Min	365317	83.5	16.0	0.9996
1M HCl,10mL, 100°C, 30 Min	312467	70.0	29.5	0.9994
1M HCl,10mL, 100°C, 5 Min	426588	97.5	2.0	1.0000

Table No. 21 – Base degradation				
Degradation conditions	**Sample Area**	**% Assay**	**% Degradation**	**Peak Purity**
1M NaOH,10mL, 100°C, 1 Hr	0	0.0	99.5	0
1M NaOH,10mL, 100°C, 10 Min	29044	6.5	93.0	0.9989
0.1M NaOH,10mL, 50°C, 5 Min	433485	98.5	1.0	1.0000
1M NaOH,10mL, 100°C, 5 Min	147152	33.5	66.0	1.0000
1M NaOH,10mL, 100°C, 3 Hrs	0	0.0	99.5	0

Table No. 22 – Peroxide degradation				
Degradation conditions	**Sample Area**	**% Assay**	**% Degradation**	**Peak Purity**
30% H_2O_2,10mL, 100°C, 1 Hr	411016	93.5	6.0	0.9602
30% H_2O_2,10mL, 100°C, 5 Min	434474	99.0	0.5	1.0000
30% H_2O_2,10mL, 100°C, 30 Min	426102	97.0	2.5	0.9981
30% H_2O_2,10mL, 100°C, 3 Hrs	350045	78.5	21.0	0.8835**

Table No. 23 – Thermal degradation				
Degradation conditions	**Sample Area**	**% Assay**	**% Degradation**	**Peak Purity**
105°C, 24 Hrs	420684	95.5	4.0	0.9999
105°C, 72 Hrs	436412	99.0	0.5	1.0000

Table No. 24 – UV degradation				
Degradation conditions	**Sample Area**	**% Assay**	**% Degradation**	**Peak Purity**
1.2 million Lux Hours	443842	100.5	-1.0	1.0000

E. Bromhexine –

Table No. 25 – Acid degradation				
Degradation conditions	**Sample Area**	**% Assay**	**% Degradation**	**Peak Purity**
1M HCl,10mL, 100°C, 1 Hr	144328	24.4	75.0	0.9984
1M HCl,10mL, 100°C, 15 Min	450431	76.4	23.0	1.0000
1M HCl,10mL, 100°C, 30 Min	320579	53.5	45.9	1.0000
1M HCl,10mL, 100°C, 5 Min	574960	97.6	1.8	1.0000

Table No. 26 – Base degradation				
Degradation conditions	**Sample Area**	**% Assay**	**% Degradation**	**Peak Purity**
1M NaOH,10mL, 100°C, 1 Hr	602969	101.8	-2.4	1.0000
1M NaOH,10mL, 100°C, 10 Min	606216	102.3	-2.9	1.0000
0.1M NaOH,10mL, 50°C, 5 Min	596829	100.8	-1.4	1.0000
1M NaOH,10mL, 100°C, 5 Min	598653	101.5	-2.1	1.0000
1M NaOH,10mL, 100°C, 3 Hrs	597696	101.0	-1.6	1.0000

Table No. 27 – Peroxide degradation				
Degradation conditions	Sample Area	% Assay	% Degradation	Peak Purity
30% H_2O_2,10mL, 100°C, 1 Hr	6568	1.1	98.9	0.9980
30% H_2O_2,10mL, 100°C, 5 Min	543426	91.9	8.1	1.0000
30% H_2O_2,10mL, 100°C, 30 Min	26876	4.5	95.5	0.9986
30% H_2O_2,10mL, 100°C, 3 Hrs	2234	0.4	99.6	0.9159

Table No. 28 – Thermal degradation				
Degradation conditions	Sample Area	% Assay	% Degradation	Peak Purity
105°C, 24 Hrs	118549	1.0	98.4	0.9999
105°C, 72 Hrs	82451	0.5	98.9	0.9999

Table No. 29 – UV degradation				
Degradation conditions	Sample Area	% Assay	% Degradation	Peak Purity
1.2 million Lux Hours	502328	84.4	15.0	0.9999

Table No. 30 - Linearity of Salbutamol sulfate		
Level No.	Concentration (µg/mL)	Mean area
1	9.978	132740
2	14.966	192927
3	19.955	255437
4	24.944	319695
5	29.933	381452
Slope		12511.871
Intercept		6773.310
CC		0.9999
R^2		1.0000

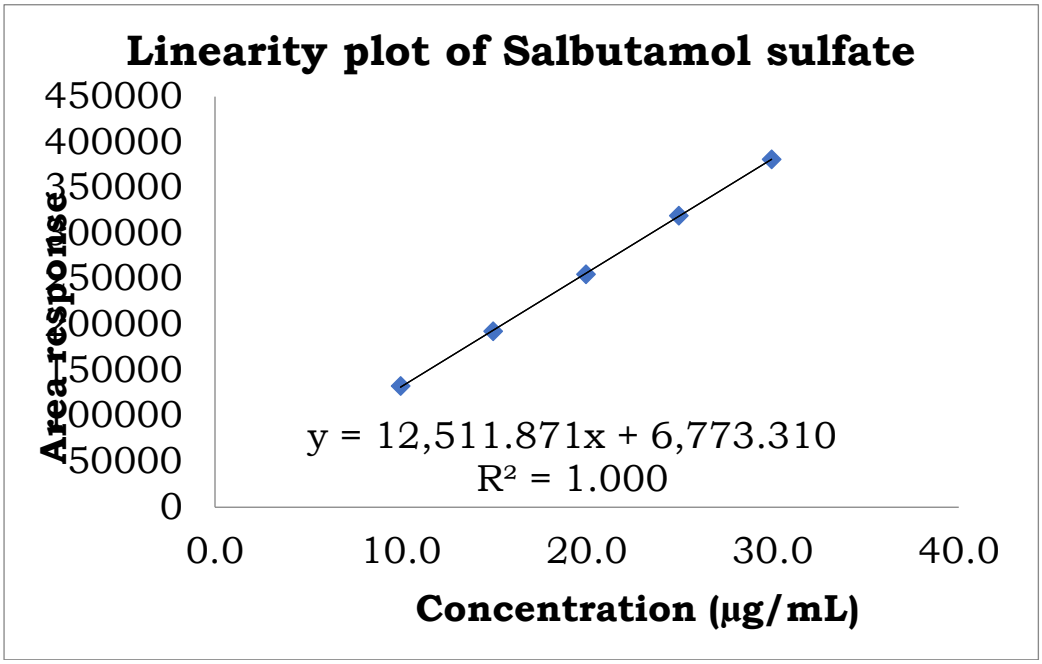

Figure-1

Table No. 31 - Linearity of Guaifenesin		
Level No.	Concentration (µg/mL)	Mean area
1	248.677	4312882
2	373.015	6381793
3	497.354	8363000
4	621.692	10380694
5	746.031	12187986
Slope		15883.341
Intercept		425630.757
CC		0.9997
R^2		0.999

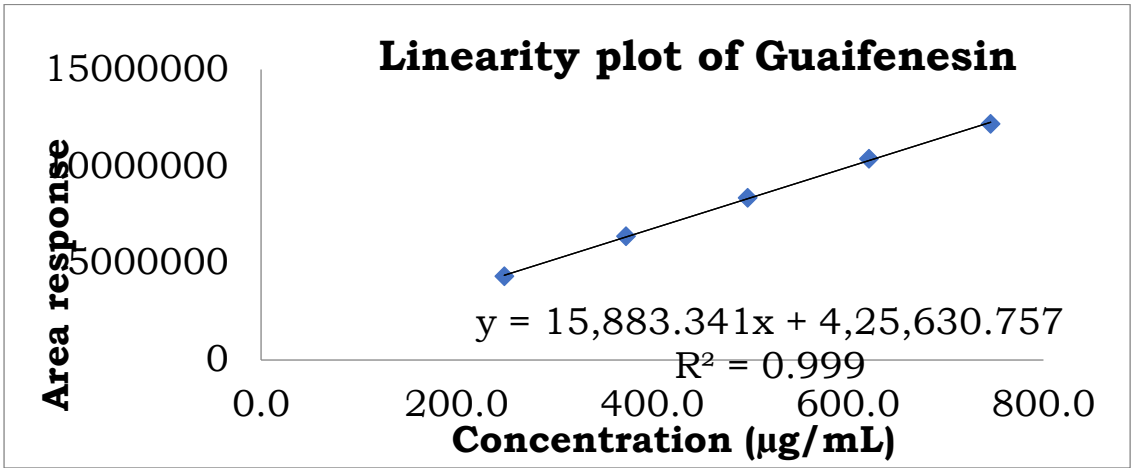

Table No. 32 - Linearity of Propyl hydroxybenzoate		
Level No.	Concentration (µg/mL)	Mean area
1	5.033	213687
2	7.549	325554
3	10.066	427996
4	12.582	542300
5	15.099	643259
Slope		42753.374
Intercept		212.288
CC		0.9998
R^2		1.000

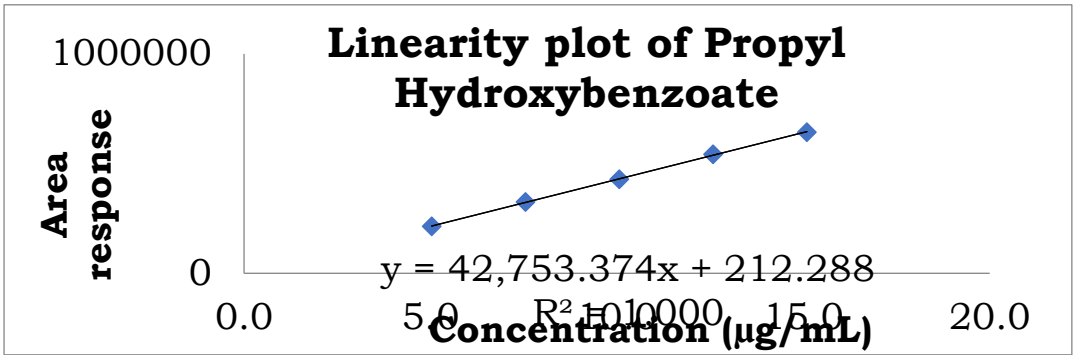

Figure-4

Table No. 33 - Linearity of Methyl hydroxybenzoate		
Level No.	Concentration (µg/mL)	Mean area
1	49.664	2427671
2	74.496	3742760
3	99.328	4865614
4	124.160	6148544
5	148.992	7326697
Slope		49145.602
Intercept		20722.800
CC		0.9998
R^2		1.000

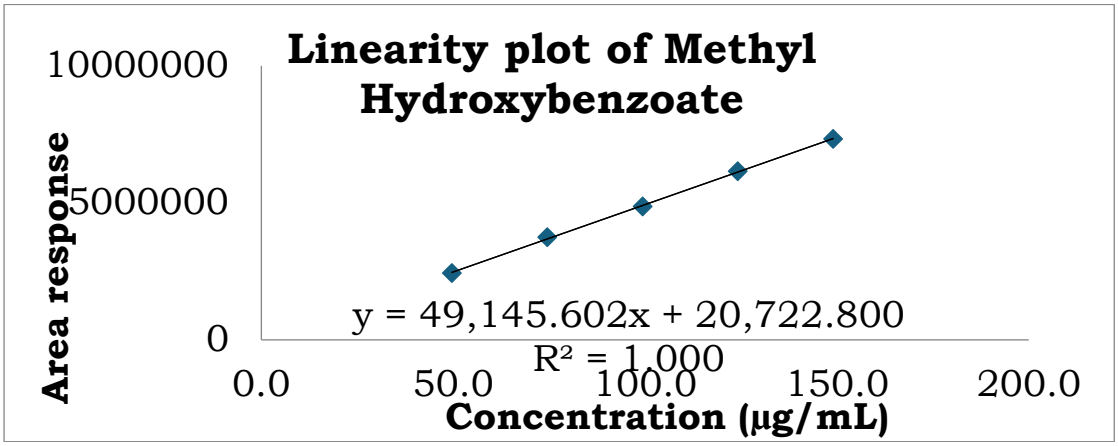

Figure-3

Table No. 34 - Linearity of Bromhexine HCl		
Level No.	Concentration (µg/mL)	Mean area
1	20.099	305499
2	30.149	447866
3	40.198	589374
4	50.248	738987
5	60.297	892636
Slope		14581.769
Intercept		8711.541
CC		0.9998
R^2		1.000

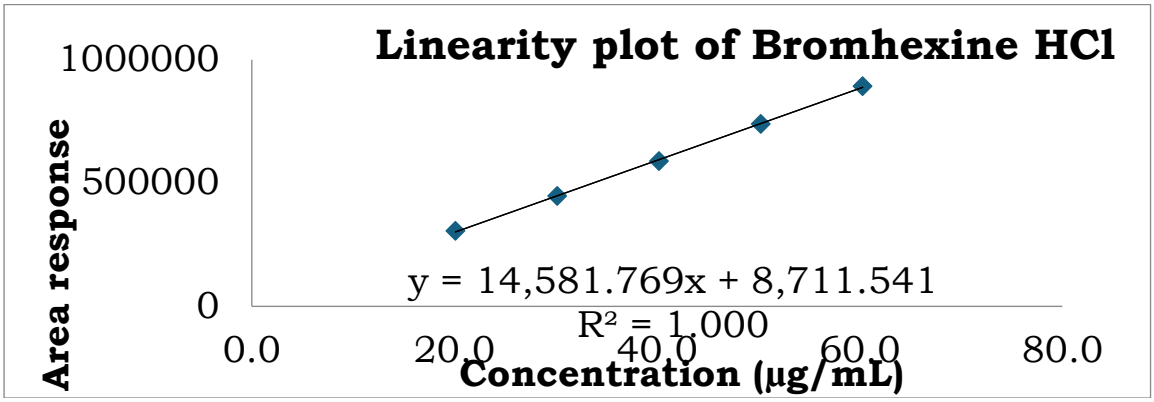

Figure-5

Table No. 35 - Method Precision					
Analysis date	**20.11.2023**				
Instrument ID	**QC/INS/213**				
Column No.	**ARND – 506**				
Analyst Name	**Ramesh Singh**				
Preparation #	**% Assay**				
	Salbutamol	Guaifenesin	Methyl hydroxy benzoate	Propyl hydroxy benzoate	Bromhexine
1	97.0	100.2	99.3	99.5	99.4
2	97.5	101.1	100.2	100.5	100.3
3	97.5	101.1	100.3	100.5	100.3
4	96.0	99.3	98.4	99.0	99.4
5	96.0	99.6	98.7	99.0	99.6
6	97.5	101.1	100.3	101.0	100.4
Mean	**96.9**	**100.4**	**99.5**	**99.9**	**99.9**
SD	**0.73598**	**0.81976**	**0.85479**	**0.86120**	**0.48166**
%RSD	**0.8**	**0.8**	**0.9**	**0.9**	**0.5**

Table No. 36 - Intermediate Precision					
Analysis date	**28.11.2023**				
Instrument ID	**QC/INS/162**				
Column No.	**ARND – 507**				
Analyst Name	**Prashantha Nayak**				
Preparation #	**% Assay**				
	Salbutamol	Guaifenesin	Methyl hydroxy benzoate	Propyl hydroxy benzoate	Bromhexine
1	98.8	102.0	100.4	100.5	100.9
2	98.8	102.0	100.4	100.5	100.9
3	98.5	101.8	100.1	100.0	100.3
4	98.5	101.9	100.3	100.5	100.6
5	99.0	102.1	100.5	101.0	100.9
6	98.5	101.7	100.0	100.0	100.5
Mean	**98.7**	**101.9**	**100.3**	**100.4**	**100.7**
SD	**0.21370**	**0.14720**	**0.19408**	**0.37639**	**0.25626**
%RSD	**0.2**	**0.1**	**0.2**	**0.4**	**0.3**

Table No. 37 – Cumulative – Method precision & Intermediate Precision						
	Method Precision		**Intermediate precision**			
Analysis date	**20.11.2023**		**28.11.2023**			
Instrument ID	**QC/INS/213**		**QC/INS/162**			
Column No.	**ARND – 506**		**ARND – 507**			
Analyst Name	**Ramesh Singh**		**Prashantha Nayak**			
Preparation #	**% Assay**					
	Salbutamol		Guaifenesin		Methyl hydroxy benzoate	
	MP	IP	MP	IP	MP	IP
1	97.0	98.8	100.2	102.0	99.3	100.4

2	97.5	98.8	101.1	102.0	100.2	100.4
3	97.5	98.5	101.1	101.8	100.3	100.1
4	96.0	98.5	99.3	101.9	98.4	100.3
5	96.0	99.0	99.6	102.1	98.7	100.5
6	97.5	98.5	101.1	101.7	100.3	100.0
Mean	**96.9**	**98.7**	**100.4**	**101.9**	**99.5**	**100.3**
SD	**0.73598**	**0.21370**	**0.81976**	**0.14720**	**0.85479**	**0.19408**
%RSD	**0.8**	**0.2**	**0.8**	**0.1**	**0.9**	**0.2**
Cumulative Mean	**97.8**		**101.2**		**99.9**	
Cumulative SD	**1.05744**		**0.97090**		**0.70898**	
Cumulative %RSD	**1.1**		**1.2**		**0.7**	

Table No. 38 – Cumulative – Method precision & Intermediate Precision				
	Method Precision		**Intermediate precision**	
Analysis date	**20.11.2023**		**28.11.2023**	
Instrument ID	**QC/INS/213**		**QC/INS/162**	
Column No.	**ARND – 506**		**ARND – 507**	
Analyst Name	**Ramesh Singh**		**Prashantha Nayak**	
Preparation #	**% Assay**			
	Propyl hydroxy benzoate		Bromhexine	
	MP	IP	MP	IP
1	99.5	100.5	99.4	100.9
2	100.5	100.5	100.3	100.9
3	100.5	100.0	100.3	100.3
4	99.0	100.5	99.4	100.6
5	99.0	101.0	99.6	100.9
6	101.0	100.0	100.4	100.5
Mean	**99.9**	**100.4**	**99.9**	**100.7**
SD	**0.86120**	**0.37639**	**0.48166**	**0.25626**
%RSD	**0.9**	**0.4**	**0.5**	**0.3**
Cumulative Mean	**100.2**		**100.3**	
Cumulative SD	**0.68534**		**0.55014**	
Cumulative %RSD	**0.7**		**0.8**	

Table No. 39 - Precision at Lower Level – 50% (Range)					
Injection No.	Salbutamol	Guaifenesin	Methyl hydroxy benzoate	Propyl hydroxy benzoate	Bromhexine
1	130401	4331864	2537707	221729	297319
2	129343	4316908	2527265	221063	295685
3	129162	4314961	2528112	221045	296023
4	129574	4313236	2525953	221023	295464
5	129956	4335520	2538475	222063	296887
6	130248	4333293	2536110	221941	296881
Mean	**129781**	**4324297**	**2532270**	**221477**	**296377**
SD	**500.19343**	**10278.59107**	**5745.46902**	**487.09985**	**753.55524**
% RSD	**0.4**	**0.2**	**0.2**	**0.2**	**0.3**

Table No. 40 - Precision at Lower Level – 150% (Range)					
Injection No.	Salbutamol	Guaifenesin	Methyl hydroxy benzoate	Propyl hydroxy benzoate	Bromhexine
1	399652	12733857	7751776	687490	893784
2	397828	12662712	7702279	683169	888463
3	399171	12710972	7741484	686646	893076
4	396979	12620132	7677056	681191	885534
5	396620	12607895	7695049	682520	886784
6	398067	12651494	7706151	683796	887900
Mean	**398053**	**12664510**	**7712299**	**684135**	**889257**
SD	**1188.65561**	**49628.69739**	**28593.80215**	**2444.77334**	**3391.95616**
% RSD	**0.3**	**0.4**	**0.4**	**0.4**	**0.4**

Table No. 41 - Accuracy of Salbutamol sulfate							
Sr. No.	Level	Sample Area	Amount recovered (µg/mL)	Amount added (µg/mL)	% Recovery		
1	50%-1	132137	9.971	9.987	99.8	Avg:	99.8
2	50%-2	132249	9.979	9.987	99.9	SD:	0.10000
3	50%-3	131934	9.955	9.987	99.7	%RSD:	0.1
4	100%-1	268961	20.295	19.975	101.6	Avg:	101.5
5	100%-2	268844	20.286	19.975	101.6	SD:	0.11547
6	100%-3	268360	20.249	19.975	101.4	%RSD:	0.1
7	150%-1	395907	29.874	29.962	99.7	Avg:	99.8
8	150%-2	396882	29.947	29.962	99.9	SD:	0.10000
9	150%-3	396283	29.902	29.962	99.8	%RSD:	0.1

Table No. 42 - Accuracy of Guaifenesin							
Sr. No.	Level	Sample Area	Amount recovered (µg/mL)	Amount added (µg/mL)	% Recovery		
1	50%-1	4378529	254.067	249.553	101.8	Avg:	101.7
2	50%-2	4370588	253.606	249.553	101.6	SD:	0.10000
3	50%-3	4372029	253.690	249.553	101.7	%RSD:	0.1
4	100%-1	8655450	502.238	499.106	100.6	Avg:	100.5
5	100%-2	8637213	501.180	499.106	100.4	SD:	0.10000
6	100%-3	8640985	501.399	499.106	100.5	%RSD:	0.1
7	150%-1	12662214	734.733	748.658	98.1	Avg:	98.3
8	150%-2	12705975	737.272	748.658	98.5	SD:	0.20817
9	150%-3	12697226	736.765	748.658	98.4	%RSD:	0.2

Table No. 43 - Accuracy of Methyl hydroxybenzoate							
Sr. No.	Level	Sample Area	Amount recovered (µg/mL)	Amount added (µg/mL)	% Recovery		
1	50%-1	2602116	51.722	50.910	101.6	Avg:	101.5
2	50%-2	2598265	51.645	50.910	101.4	SD:	0.10000
3	50%-3	2599279	51.665	50.910	101.5	%RSD:	0.1

4	100%-1	5118634	101.742	101.820	99.9	Avg:	99.8
5	100%-2	5112435	101.619	101.820	99.8	SD:	0.10000
6	100%-3	5106826	101.507	101.820	99.7	%RSD:	0.1
7	150%-1	7559214	150.253	152.730	98.4	Avg:	98.5
8	150%-2	7580158	150.669	152.730	98.7	SD:	0.15275
9	150%-3	7567015	150.408	152.730	98.5	%RSD:	0.2

Table No. 44 - Accuracy of Propyl hydroxybenzoate

Sr. No.	Level	Sample Area	Amount recovered (µg/mL)	Amount added (µg/mL)	% Recovery		
1	50%-1	229261	5.229	5.179	101.0	Avg:	100.9
2	50%-2	228615	5.215	5.179	100.7	SD:	0.17321
3	50%-3	229236	5.229	5.179	101.0	%RSD:	0.2
4	100%-1	453454	10.343	10.358	99.9	Avg:	99.8
5	100%-2	453159	10.336	10.358	99.8	SD:	0.15275
6	100%-3	452102	10.312	10.358	99.6	%RSD:	0.2
7	150%-1	689542	15.728	15.536	101.2	Avg:	101.4
8	150%-2	691951	15.783	15.536	101.6	SD:	0.20817
9	150%-3	690071	15.740	15.536	101.3	%RSD:	0.2

Table No. 45 - Accuracy of Bromhexine HCl

Sr. No.	Level	Sample Area	Amount recovered (µg/mL)	Amount added (µg/mL)	% Recovery		
1	50%-1	305753	20.367	20.119	101.2	Avg:	101.1
2	50%-2	305510	20.350	20.119	101.1	SD:	0.05774
3	50%-3	305392	20.343	20.119	101.1	%RSD:	0.1
4	100%-1	601251	40.050	40.238	99.5	Avg:	99.3
5	100%-2	600126	39.975	40.238	99.3	SD:	0.15275
6	100%-3	599328	39.922	40.238	99.2	%RSD:	0.2
7	150%-1	889842	59.274	60.357	98.2	Avg:	98.4
8	150%-2	892337	59.440	60.357	98.5	SD:	0.15275
9	150%-3	891411	59.378	60.357	98.4	%RSD:	0.2

Table No. 46 – Filter compatibility – Salbutamol sulfate

Procedure	**Sample Name**	**Sample details**	**% Assay**	**Mean % Assay**	**% Absolute difference**
Centrifuge	-	Sample # 1	98.5	98.6	-
		Sample # 2	98.7		
		Sample # 3	98.5		
0.45µ Nylon GD-X	3 ml	Sample # 1	98.2	98.5	-0.1
		Sample # 2	98.7		
		Sample # 3	98.7		
	5 ml	Sample # 1	98.5	98.6	0.0
		Sample # 2	98.5		
		Sample # 3	98.7		
	10 ml	Sample # 1	98.2	98.5	-0.1
		Sample # 2	98.7		

		Sample # 3	98.7		

Table No. 47 – Filter compatibility – Guaifenesin

Procedure	Sample Name	Sample details	% Assay	Mean % Assay	% Absolute difference
Centrifuge	-	Sample # 1	102.2	102.3	-
		Sample # 2	102.5		
		Sample # 3	102.2		
0.45µ Nylon GD-X	3 ml	Sample # 1	101.7	102.1	-0.2
		Sample # 2	102.3		
		Sample # 3	102.3		
	5 ml	Sample # 1	101.9	102.2	-0.1
		Sample # 2	102.3		
		Sample # 3	102.3		
	10 ml	Sample # 1	101.6	102.0	-0.3
		Sample # 2	102.1		
		Sample # 3	102.2		

Table No. 48 – Filter compatibility – Methyl hydroxybenzoate

Procedure	Sample Name	Sample details	% Assay	Mean % Assay	% Absolute difference
Centrifuge	-	Sample # 1	101.2	101.4	-
		Sample # 2	101.6		
		Sample # 3	101.3		
0.45µ Nylon GD-X	3 ml	Sample # 1	99.5	98.7	-2.7
		Sample # 2	97.3		
		Sample # 3	99.2		
	5 ml	Sample # 1	100.7	100.5	-0.9
		Sample # 2	99.9		
		Sample # 3	100.9		
	10 ml	Sample # 1	100.7	100.8	-0.6
		Sample # 2	100.5		
		Sample # 3	101.1		

Table No. 49 – Filter compatibility – Propyl hydroxybenzoate

Procedure	Sample Name	Sample details	% Assay	Mean % Assay	% Absolute difference
Centrifuge	-	Sample # 1	102.0	102.0	-
		Sample # 2	102.0		
		Sample # 3	102.0		
0.45µ Nylon GD-X	3 ml	Sample # 1	44.9	55.0	-47.0
		Sample # 2	53.5		
		Sample # 3	66.7		
	5 ml	Sample # 1	95.5	89.9	-12.1
		Sample # 2	83.3		
		Sample # 3	90.9		
	10 ml	Sample # 1	100.0	96.1	-5.9
		Sample # 2	92.9		
		Sample # 3	95.5		

Table No. 50 – Filter compatibility – Bromhexine HCl					
Procedure	**Sample Name**	**Sample details**	**% Assay**	**Mean % Assay**	**% Absolute difference**
Centrifuge	-	Sample # 1	102.5	102.7	-
		Sample # 2	102.8		
		Sample # 3	102.7		
0.45µ Nylon GD-X	3 ml	Sample # 1	100.4	101.0	-1.7
		Sample # 2	101.3		
		Sample # 3	101.3		
	5 ml	Sample # 1	101.8	101.3	-1.4
		Sample # 2	101.0		
		Sample # 3	101.1		
	10 ml	Sample # 1	100.5	101.5	-1.2
		Sample # 2	102.0		
		Sample # 3	101.9		

Table No. 51 – Filter compatibility – Salbutamol sulfate					
Procedure	**Sample Name**	**Sample details**	**% Assay**	**Mean % Assay**	**% Absolute difference**
Centrifuge	-	Sample # 1	98.5	98.6	-
		Sample # 2	98.7		
		Sample # 3	98.5		
0.45µ PVDF	3 ml	Sample # 1	98.5	98.6	0.0
		Sample # 2	98.7		
		Sample # 3	98.5		
	5 ml	Sample # 1	98.5	98.6	0.0
		Sample # 2	98.7		
		Sample # 3	98.7		
	10 ml	Sample # 1	94.7	97.4	-1.2
		Sample # 2	99.0		
		Sample # 3	98.5		

Table No. 52 – Filter compatibility – Guaifenesin					
Procedure	**Sample Name**	**Sample details**	**% Assay**	**Mean % Assay**	**% Absolute difference**
Centrifuge	-	Sample # 1	102.2	102.3	-
		Sample # 2	102.5		
		Sample # 3	102.2		
0.45µ PVDF	3 ml	Sample # 1	102.1	102.3	0.0
		Sample # 2	102.4		
		Sample # 3	102.3		
	5 ml	Sample # 1	102.0	102.3	0.0
		Sample # 2	102.3		
		Sample # 3	102.5		
	10 ml	Sample # 1	98.2	100.9	-1.4
		Sample # 2	102.3		
		Sample # 3	102.1		

Table No. 53 – Filter compatibility – Methyl hydroxybenzoate					
Procedure	**Sample Name**	**Sample details**	**% Assay**	**Mean % Assay**	**% Absolute difference**
Centrifuge	-	Sample # 1	101.2	101.4	-
		Sample # 2	101.6		
		Sample # 3	101.3		
0.45µ PVDF	3 ml	Sample # 1	101.1	101.3	-0.1
		Sample # 2	101.4		
		Sample # 3	101.3		
	5 ml	Sample # 1	101.1	101.3	-0.1
		Sample # 2	101.4		
		Sample # 3	101.5		
	10 ml	Sample # 1	97.3	100.0	-1.4
		Sample # 2	101.4		
		Sample # 3	101.3		

Table No. 54 – Filter compatibility – Propyl hydroxybenzoate					
Procedure	**Sample Name**	**Sample details**	**% Assay**	**Mean % Assay**	**% Absolute difference**
Centrifuge	-	Sample # 1	102.0	102.0	-
		Sample # 2	102.0		
		Sample # 3	102.0		
0.45µ PVDF	3 ml	Sample # 1	101.5	101.8	-0.2
		Sample # 2	102.0		
		Sample # 3	102.0		
	5 ml	Sample # 1	101.5	101.8	-0.2
		Sample # 2	102.0		
		Sample # 3	102.0		
	10 ml	Sample # 1	98.0	100.7	-1.3
		Sample # 2	102.0		
		Sample # 3	102.0		

Table No. 55 – Filter compatibility – Bromhexine HCl					
Procedure	**Sample Name**	**Sample details**	**% Assay**	**Mean % Assay**	**% Absolute difference**
Centrifuge	-	Sample # 1	102.5	102.7	-
		Sample # 2	102.8		
		Sample # 3	102.7		
0.45µ PVDF	3 ml	Sample # 1	102.3	102.2	-0.5
		Sample # 2	101.8		
		Sample # 3	102.5		
	5 ml	Sample # 1	102.3	102.5	-0.2
		Sample # 2	102.5		
		Sample # 3	102.7		
	10 ml	Sample # 1	97.5	100.2	-2.5
		Sample # 2	101.6		
		Sample # 3	101.5		

Low flow rate = 1.0 mL/min

System suitability results are presented in Table No. 56 to 59.

Table No. 56 – System suitability					
Injection #	Area				
	Salbutamol	Guaifenesin	Methyl hydroxy benzoate	Propyl hydroxy benzoate	Bromhexine
1	309904	10045694	5821280	515923	714781
2	308607	10021961	5811619	514847	716211
3	308239	10002131	5796476	513736	709892
4	308285	10022228	5802759	514703	714067
5	308478	10041529	5812739	514952	714624
6	307260	9997475	5791081	512245	709593
Mean	**308462**	**10021836**	**5805992**	**514401**	**713195**
SD	**851.64627**	**19692.29823**	**11262.79315**	**1264.97162**	**2767.60869**
%RSD	**0.3**	**0.2**	**0.2**	**0.2**	**0.4**

Table No. 57 – System suitability criteria's			
Criteria	**Limit**	**Peak**	**Results**
% RSD for the peak areas of Salbutamol, Bromhexine, Guaifenesin, Methyl hydroxybenzoate and Propyl hydroxybenzoate peak from six replicate injections of standard preparation	NMT 2.0%	Salbutamol	0.3
		Guaifenesin	0.2
		Methyl hydroxybenzoate	0.2
		Propyl hydroxybenzoate	0.2
		Bromhexine	0.4
Tailing factor for Salbutamol, Bromhexine, Guaifenesin, Methyl hydroxybenzoate and Propyl hydroxybenzoate peaks from standard preparation should not be more than 2.0	NMT 2.0	Salbutamol	1.01
		Guaifenesin	1.05
		Methyl hydroxybenzoate	1.03
		Propyl hydroxybenzoate	1.02
		Bromhexine	1.02
Theoretical plate count for Salbutamol, Bromhexine, Guaifenesin, Methyl hydroxybenzoate and Propyl hydroxybenzoate peak from standard preparation should not be less than 2000	NLT 2000	Salbutamol	141401
		Guaifenesin	183189
		Methyl hydroxybenzoate	214963
		Propyl hydroxybenzoate	442863
		Bromhexine	404854

Table No. 58 – Cumulative – Method precision & Robustness – Flow rate						
	Method Precision			**Robustness**		
Flow rate	**1.2 ml/min**			**1.0 ml/min**		
Preparation #	% Assay					
	Salbutamol		**Guaifenesin**		**Methyl hydroxy benzoate**	
	MP	Robustness	MP	Robustness	MP	Robustness
1	97.0	98.3	100.2	101.8	99.3	101.0
2	97.5	98.8	101.1	101.7	100.2	100.9

3	97.5	98.5	101.1	101.9	100.3	101.1
4	96.0	-	99.3	-	98.4	-
5	96.0	-	99.6	-	98.7	-
6	97.5	-	101.1	-	100.3	-
Mean	**96.9**	**98.5**	**100.4**	**101.8**	**99.5**	**101.0**
SD	**0.73598**	**0.25166**	**0.81976**	**0.10000**	**0.85479**	**0.10000**
%RSD	**0.8**	**0.3**	**0.8**	**0.1**	**0.9**	**0.1**
Cumulative Mean	**97.5**		**100.9**		**100.0**	
Cumulative SD	**1.00388**		**0.95525**		**0.99847**	
Cumulative %RSD	**1.0**		**0.9**		**1.0**	

Table No. 59 – Cumulative – Method precision & Robustness – Flow rate				
	Method Precision			**Robustness**
Flow rate	**1.2 ml/min**			**1.0 ml/min**
Preparation #	% Assay			
	Propyl hydroxybenzoate		**Bromhexine**	
	MP	Robustness	MP	Robustness
1	99.5	102.0	99.4	100.4
2	100.5	102.0	100.3	99.9
3	100.5	102.0	100.3	100.5
4	99.0	-	99.4	-
5	99.0	-	99.6	-
6	101.0	-	100.4	-
Mean	**99.9**	**102.0**	**99.9**	**100.3**
SD	**0.86120**	**0.00000**	**0.48166**	**0.32146**
%RSD	**0.9**	**0.0**	**0.5**	**0.3**
Cumulative Mean	**100.6**		**100.0**	
Cumulative SD	**1.24443**		**0.45216**	
Cumulative %RSD	**1.2**		**0.5**	

Low flow rate = 1.4 mL/min

System suitability results are presented in Table No. 60 to 63

Table No. 60 – System suitability					
Injection #	**Area**				
	Salbutamol	**Guaifenesin**	**Methyl hydroxy benzoate**	**Propyl hydroxy benzoate**	**Bromhexine**
1	208530	6847204	3921691	346113	480830
2	209507	6845689	3925643	346424	480587
3	208341	6826804	3914506	346291	480195
4	209435	6854392	3925674	346214	481427
5	207967	6832409	3916395	345394	479442
6	209477	6832361	3915260	345121	478541
Mean	**208876**	**6839810**	**3919862**	**345926**	**480170**
SD	**678.83206**	**10782.27285**	**5146.45069**	**534.82536**	**1036.70510**
%RSD	**0.3**	**0.2**	**0.1**	**0.2**	**0.2**

Table No. 61 – System suitability criteria's			
Criteria	**Limit**	**Peak**	**Results**
% RSD for peak areas of Salbutamol, Bromhexine, Guaifenesin, Methyl hydroxybenzoate and Propyl hydroxybenzoate from six injections of standard preparation	NMT 2.0%	Salbutamol	0.3
		Guaifenesin	0.2
		Methyl hydroxybenzoate	0.1
		Propyl hydroxybenzoate	0.2
		Bromhexine	0.2
Tailing factor for Salbutamol, Bromhexine, Guaifenesin, Methyl hydroxybenzoate and Propyl hydroxybenzoate peaks from standard preparation should not be more than 2.0	NMT 2.0	Salbutamol	1.01
		Guaifenesin	1.04
		Methyl hydroxybenzoate	1.02
		Propyl hydroxybenzoate	1.00
		Bromhexine	1.01
Theoretical plate count for Salbutamol, Bromhexine, Guaifenesin, Methyl hydroxybenzoate and Propyl hydroxybenzoate peak from standard preparation should not be less than 2000	NLT 2000	Salbutamol	124920
		Guaifenesin	158627
		Methyl hydroxybenzoate	183736
		Propyl hydroxybenzoate	427884
		Bromhexine	391567

Table No. 62 – Cumulative – Method precision & Robustness – Flow rate						
	Method Precision			**Robustness**		
Flow rate	**1.2 ml/min**			**1.4 ml/min**		
Preparation #	**% Assay**					
	Salbutamol		**Guaifenesin**		**Methyl hydroxy benzoate**	
	MP	Robustness	MP	Robustness	MP	Robustness
1	97.0	98.3	100.2	101.6	99.3	101.0
2	97.5	98.8	101.1	102.0	100.2	101.2
3	97.5	99.3	101.1	102.1	100.3	101.4
4	96.0	-	99.3	-	98.4	-
5	96.0	-	99.6	-	98.7	-
6	97.5	-	101.1	-	100.3	-
Mean	**96.9**	**98.8**	**100.4**	**101.9**	**99.5**	**101.2**
SD	**0.73598**	**0.50000**	**0.81976**	**0.26458**	**0.85479**	**0.20000**
%RSD	**0.8**	**0.5**	**0.8**	**0.3**	**0.9**	**0.2**
Cumulative Mean	**97.5**		**100.9**		**100.1**	
Cumulative SD	**1.13480**		**1.00000**		**1.07755**	
Cumulative %RSD	**1.2**		**1.0**		**1.1**	

Table No. 63 – Cumulative – Method precision & Robustness – Flow rate				
	Method Precision		**Robustness**	
Flow rate	**1.2 ml/min**		**1.4 ml/min**	
Preparation #	**% Assay**			
	Propyl hydroxybenzoate		**Bromhexine**	
	MP	Robustness	MP	Robustness

1	99.5	102.0	99.4	100.8
2	100.5	102.5	100.3	100.5
3	100.5	102.5	100.3	100.8
4	99.0	-	99.4	-
5	99.0	-	99.6	-
6	101.0	-	100.4	-
Mean	**99.9**	**102.3**	**99.9**	**100.6**
SD	**0.86120**	**0.28868**	**0.48166**	**0.20817**
%RSD	**0.9**	**0.3**	**0.5**	**0.2**
Cumulative Mean	**100.7**		**100.1**	
Cumulative SD	**1.39443**		**0.51667**	
Cumulative %RSD	**1.4**		**0.5**	

Low column oven temperature – 35°C

System suitability results are presented in Table No. 64 and 67

Table No. 64 – System suitability

Injection #	Area				
	Salbutamol	**Guaifenesin**	**Methyl hydroxy benzoate**	**Propyl hydroxy benzoate**	**Bromhexine**
1	254196	8361457	4800408	423997	591369
2	254461	8365951	4799363	424649	591441
3	254690	8380396	4802439	424481	590447
4	253746	8309082	4796337	424027	589014
5	253751	8311450	4795196	423454	587152
6	253000	8349518	4812020	425164	590275
Mean	**253974**	**8346309**	**4800961**	**424295**	**589950**
SD	**608.19372**	**29625.97080**	**6032.61100**	**597.15682**	**1630.59347**
%RSD	**0.2**	**0.4**	**0.1**	**0.1**	**0.3**

Table No. 65 – System suitability criteria's

Criteria	Limit	Peak	Results
% RSD for the peak areas of Salbutamol, Bromhexine, Guaifenesin, Methyl hydroxybenzoate and Propyl hydroxybenzoate peak from six replicate injections of standard preparation	NMT 2.0%	Salbutamol	0.2
		Guaifenesin	0.4
		Methyl hydroxybenzoate	0.1
		Propyl hydroxybenzoate	0.1
		Bromhexine	0.3
Tailing factor for Salbutamol, Bromhexine, Guaifenesin, Methyl hydroxybenzoate and Propyl hydroxybenzoate peaks from standard preparation should not be more than 2.0	NMT 2.0	Salbutamol	1.01
		Guaifenesin	1.04
		Methyl hydroxybenzoate	1.03
		Propyl hydroxybenzoate	1.01
		Bromhexine	1.02
Theoretical plate count for Salbutamol, Bromhexine, Guaifenesin, Methyl hydroxybenzoate and Propyl hydroxybenzoate peak from standard preparation should not be less than 2000	NLT 2000	Salbutamol	128035
		Guaifenesin	168627
		Methyl hydroxybenzoate	202661
		Propyl hydroxybenzoate	431109
		Bromhexine	396644

Table No. 66 – Cumulative – Method precision & Robustness – Column Oven Temperature - Low						
	Method Precision			**Robustness**		
Column oven temperature	**40°C**			**35°C**		
Preparation #	% Assay					
	Salbutamol		**Guaifenesin**		**Methyl hydroxy benzoate**	
	MP	Robustness	MP	Robustness	MP	Robustness
1	97.0	99.0	100.2	101.7	99.3	101.2
2	97.5	98.8	101.1	101.4	100.2	101.0
3	97.5	99.3	101.1	101.5	100.3	101.2
4	96.0	-	99.3	-	98.4	-
5	96.0	-	99.6	-	98.7	-
6	97.5	-	101.1	-	100.3	-
Mean	**96.9**	**99.0**	**100.4**	**101.5**	**99.5**	**101.1**
SD	**0.73598**	**0.25166**	**0.81976**	**0.15275**	**0.85479**	**0.11547**
%RSD	**0.8**	**0.3**	**0.8**	**0.2**	**0.9**	**0.1**
Cumulative Mean	**97.6**		**100.8**		**100.1**	
Cumulative SD	**1.21427**		**0.86426**		**1.04881**	
Cumulative %RSD	**1.2**		**0.9**		**1.0**	

Table No. 67 – Cumulative – Method precision & Robustness – Column Oven Temperature - Low				
	Method Precision			**Robustness**
Column oven temperature	**40°C**			**35°C**
Preparation #	% Assay			
	Propyl hydroxybenzoate		**Bromhexine**	
	MP	Robustness	MP	Robustness
1	99.5	102.0	99.4	100.3
2	100.5	102.0	100.3	100.0
3	100.5	102.0	100.3	100.3
4	99.0	-	99.4	-
5	99.0	-	99.6	-
6	101.0	-	100.4	-
Mean	**99.9**	**102.0**	**99.9**	**100.2**
SD	**0.86120**	**0.00000**	**0.48166**	**0.17321**
%RSD	**0.9**	**0.0**	**0.5**	**0.2**
Cumulative Mean	**100.6**		**100.0**	
Cumulative SD	**1.24443**		**0.41833**	
Cumulative %RSD	**1.2**		**0.4**	

High column Temperature = 45°C

System suitability results are presented in Table No. 68 and 71

Table No. 68 – System suitability

Injection #	Area				
	Salbutamol	Guaifenesin	Methyl hydroxy benzoate	Propyl hydroxy benzoate	Bromhexine
1	255072	8351081	4808917	425599	594146
2	255344	8341994	4803246	425819	593770
3	255284	8354850	4816614	427034	594318
4	255182	8339899	4808570	425559	593482
5	255790	8333880	4811036	426502	593728
6	255328	8325366	4811404	426488	592771
Mean	**255333**	**8341178**	**4809965**	**426167**	**593703**
SD	**245.89320**	**10868.07101**	**4373.01387**	**596.71548**	**547.18397**
%RSD	**0.1**	**0.1**	**0.1**	**0.1**	**0.1**

Table No. 69 – System suitability criteria's

Criteria	Limit	Peak	Results
% RSD for the peak areas of Salbutamol, Bromhexine, Guaifenesin, Methyl hydroxybenzoate and Propyl hydroxybenzoate peak from six replicate injections of standard preparation	NMT 2.0%	Salbutamol	0.1
		Guaifenesin	0.1
		Methyl hydroxybenzoate	0.1
		Propyl hydroxybenzoate	0.1
		Bromhexine	0.1
Tailing factor for Salbutamol, Bromhexine, Guaifenesin, Methyl hydroxybenzoate and Propyl hydroxybenzoate peaks from standard preparation should not be more than 2.0	NMT 2.0	Salbutamol	0.99
		Guaifenesin	1.03
		Methyl hydroxybenzoate	1.01
		Propyl hydroxybenzoate	0.99
		Bromhexine	0.99
Theoretical plate count for Salbutamol, Bromhexine, Guaifenesin, Methyl hydroxybenzoate and Propyl hydroxybenzoate peak from standard preparation should not be less than 2000	NLT 2000	Salbutamol	133129
		Guaifenesin	170872
		Methyl hydroxybenzoate	196224
		Propyl hydroxybenzoate	457004
		Bromhexine	406858

Table No. 70 – Cumulative – Method precision & Robustness – Column Oven Temperature - High

	Method Precision			**Robustness**		
Column oven temperature	**40°C**			**45°C**		
Preparation #	% Assay					
	Salbutamol		**Guaifenesin**		**Methyl hydroxy benzoate**	
	MP	Robustness	MP	Robustness	MP	Robustness
1	97.0	99.0	100.2	101.7	99.3	101.1
2	97.5	98.8	101.1	101.5	100.2	100.9
3	97.5	99.0	101.1	101.7	100.3	101.0
4	96.0	-	99.3	-	98.4	-
5	96.0	-	99.6	-	98.7	-
6	97.5	-	101.1	-	100.3	-
Mean	**96.9**	**98.9**	**100.4**	**101.6**	**99.5**	**101.0**

SD	**0.73598**	**0.11547**	**0.81976**	**0.11547**	**0.85479**	**0.10000**
%RSD	**0.8**	**0.1**	**0.8**	**0.1**	**0.9**	**0.1**
Cumulative Mean	**97.6**		**100.8**		**100.0**	
Cumulative SD	**1.16559**		**0.89644**		**0.99847**	
Cumulative %RSD	**1.2**		**0.9**		**1.0**	

Table No. 71 – Cumulative – Method precision & Robustness – Column Oven Temperature - High

	Method Precision			**Robustness**
Column oven temperature	**40°C**			**45°C**
Preparation #	**% Assay**			
	Propyl hydroxybenzoate		**Bromhexine**	
	MP	Robustness	MP	Robustness
1	99.5	102.5	99.4	99.0
2	100.5	102.0	100.3	99.0
3	100.5	102.0	100.3	99.0
4	99.0	-	99.4	-
5	99.0	-	99.6	-
6	101.0	-	100.4	-
Mean	**99.9**	**102.2**	**99.9**	**99.0**
SD	**0.86120**	**0.28868**	**0.48166**	**0.00000**
%RSD	**0.9**	**0.3**	**0.5**	**0.0**
Cumulative Mean	**100.7**		**99.6**	
Cumulative SD	**1.32288**		**0.58949**	
Cumulative %RSD	**1.3**		**0.6**	

Low mobile phase buffer pH – 3.3

System suitability results are presented in Table No. 72 and 65

Table No. 72 – System suitability

Injection #	**Area**				
	Salbutamol	**Guaifenesin**	**Methyl hydroxy benzoate**	**Propyl hydroxy benzoate**	**Bromhexine**
1	255809	8423302	4888990	430494	588444
2	251053	8294445	4801782	424253	580207
3	248860	8217559	4762297	419417	576727
4	248247	8196278	4738621	419565	572738
5	249562	8225059	4772342	420903	573976
6	249397	8240812	4765704	420682	575000
Mean	**250488**	**8266243**	**4788289**	**422552**	**577849**
SD	**2769.56632**	**83750.78863**	**53347.23193**	**4264.62799**	**5801.93629**
%RSD	**1.1**	**1.0**	**1.1**	**1.0**	**1.0**

Table No. 73 – System suitability criteria's

Criteria	Limit	Peak	Results
% RSD for the peak areas of Salbutamol, Bromhexine, Guaifenesin, Methyl hydroxybenzoate and Propyl hydroxybenzoate peak from six replicate injections of standard preparation	NMT 2.0%	Salbutamol	1.1
		Guaifenesin	1.0
		Methyl hydroxybenzoate	1.1
		Propyl hydroxybenzoate	1.0
		Bromhexine	1.0
Tailing factor for Salbutamol, Bromhexine, Guaifenesin, Methyl hydroxybenzoate and Propyl hydroxybenzoate peaks from standard preparation should not be more than 2.0	NMT 2.0	Salbutamol	1.01
		Guaifenesin	1.05
		Methyl hydroxybenzoate	1.04
		Propyl hydroxybenzoate	1.01
		Bromhexine	1.03
Theoretical plate count for Salbutamol, Bromhexine, Guaifenesin, Methyl hydroxybenzoate and Propyl hydroxybenzoate peak from standard preparation should not be less than 2000	NLT 2000	Salbutamol	129380
		Guaifenesin	165182
		Methyl hydroxybenzoate	193550
		Propyl hydroxybenzoate	439070
		Bromhexine	426406

Table No. 74 – Cumulative – Method precision & Robustness – Mobile phase buffer pH - Low

	Method Precision		**Robustness**			
Mobile phase buffer pH	**3.5**		**3.3**			
Preparation #	% **Assay**					
	Salbutamol		**Guaifenesin**		**Methyl hydroxy benzoate**	
	MP	Robustness	MP	Robustness	MP	Robustness
1	97.0	99.0	100.2	102.2	99.3	100.8
2	97.5	99.3	101.1	102.6	100.2	101.1
3	97.5	98.8	101.1	102.6	100.3	100.8
4	96.0	-	99.3	-	98.4	-
5	96.0	-	99.6	-	98.7	-
6	97.5	-	101.1	-	100.3	-
Mean	**96.9**	**99.0**	**100.4**	**102.5**	**99.5**	**100.9**
SD	**0.73598**	**0.25166**	**0.81976**	**0.23094**	**0.85479**	**0.17321**
%RSD	**0.8**	**0.3**	**0.8**	**0.2**	**0.9**	**0.2**
Cumulative Mean	**97.6**		**101.1**		**100.0**	
Cumulative SD	**1.21427**		**1.22520**		**0.96494**	
Cumulative %RSD	**1.2**		**1.2**		**1.0**	

Table No. 75 – Cumulative – Method precision & Robustness – Mobile phase buffer pH - Low				
	Method Precision		**Robustness**	
Mobile phase buffer pH	**3.5**		**3.3**	
Preparation #	% **Assay**			
	Propyl hydroxybenzoate		**Bromhexine**	
	MP	Robustness	MP	Robustness
1	99.5	100.0	99.4	100.4
2	100.5	100.5	100.3	100.9
3	100.5	100.5	100.3	100.9
4	99.0	-	99.4	-
5	99.0	-	99.6	-
6	101.0	-	100.4	-
Mean	**99.9**	**100.3**	**99.9**	**100.7**
SD	**0.86120**	**0.28868**	**0.48166**	**0.28868**
%RSD	**0.9**	**0.3**	**0.5**	**0.3**
Cumulative Mean	**100.1**		**100.2**	
Cumulative SD	**0.72648**		**0.58262**	
Cumulative %RSD	**0.7**		**0.6**	

Low mobile phase buffer pH – 3.7

System suitability results are presented in Table No. 76 and 69

Table No. 76 – System suitability					
Injection #	**Area**				
	Salbutamol	**Guaifenesin**	**Methyl hydroxy benzoate**	**Propyl hydroxy benzoate**	**Bromhexine**
1	249935	8235706	4798887	419886	577061
2	250048	8241683	4806390	421440	576609
3	250412	8254522	4809858	421307	577034
4	250399	8266162	4815998	422858	578602
5	251534	8267052	4818136	422330	577260
6	250646	8253109	4814759	421857	575623
Mean	**250496**	**8253039**	**4810671**	**421613**	**577032**
SD	**571.28510**	**12650.47914**	**7192.57233**	**1022.71638**	**967.67117**
%RSD	**0.2**	**0.2**	**0.1**	**0.2**	**0.2**

Table No. 77 – System suitability criteria's			
Criteria	**Limit**	**Peak**	**Results**
% RSD for the peak areas of Salbutamol, Bromhexine, Guaifenesin, Methyl hydroxybenzoate and Propyl hydroxybenzoate peak from six replicate	NMT 2.0%	Salbutamol	0.2
		Guaifenesin	0.2
		Methyl hydroxybenzoate	0.1

injections of standard preparation		Propyl hydroxybenzoate	0.2
		Bromhexine	0.2
Tailing factor for Salbutamol, Bromhexine, Guaifenesin, Methyl hydroxybenzoate and Propyl hydroxybenzoate peaks from standard preparation should not be more than 2.0	NMT 2.0	Salbutamol	1.02
		Guaifenesin	1.05
		Methyl hydroxybenzoate	1.03
		Propyl hydroxybenzoate	1.01
		Bromhexine	1.01
Theoretical plate count for Salbutamol, Bromhexine, Guaifenesin, Methyl hydroxybenzoate and Propyl hydroxybenzoate peak from standard preparation should not be less than 2000	NLT 2000	Salbutamol	140672
		Guaifenesin	166736
		Methyl hydroxybenzoate	195276
		Propyl hydroxybenzoate	453781
		Bromhexine	407697

Table No. 78 – Cumulative – Method precision & Robustness – Mobile phase buffer pH - High

	Method Precision		**Robustness**			
Mobile phase buffer pH	**3.5**		**3.7**			
Preparation #	**% Assay**					
	Salbutamol		**Guaifenesin**		**Methyl hydroxy benzoate**	
	MP	Robustness	MP	Robustness	MP	Robustness
1	97.0	99.5	100.2	102.8	99.3	101.1
2	97.5	99.8	101.1	102.9	100.2	101.3
3	97.5	99.5	101.1	102.9	100.3	101.2
4	96.0	-	99.3	-	98.4	-
5	96.0	-	99.6	-	98.7	-
6	97.5	-	101.1	-	100.3	-
Mean	**96.9**	**99.6**	**100.4**	**102.9**	**99.5**	**101.2**
SD	**0.73598**	**0.17321**	**0.81976**	**0.05774**	**0.85479**	**0.10000**
%RSD	**0.8**	**0.2**	**0.8**	**0.1**	**0.9**	**0.1**
Cumulative Mean	**97.8**		**101.2**		**100.1**	
Cumulative SD	**1.46496**		**1.39354**		**1.07406**	
Cumulative %RSD	**1.5**		**1.4**		**1.1**	

Table No. 79 – Cumulative – Method precision & Robustness – Mobile phase buffer pH - High

	Method Precision	**Robustness**
Mobile phase buffer pH	**3.5**	**3.7**
Preparation #	**% Assay**	

	Propyl hydroxybenzoate		**Bromhexine**	
	MP	Robustness	MP	Robustness
1	99.5	101.0	99.4	102.3
2	100.5	101.0	100.3	102.4
3	100.5	101.0	100.3	102.6
4	99.0	-	99.4	-
5	99.0	-	99.6	-
6	101.0	-	100.4	-
Mean	**99.9**	**101.0**	**99.9**	**102.4**
SD	**0.86120**	**0.00000**	**0.48166**	**0.15275**
%RSD	**0.9**	**0.0**	**0.5**	**0.1**
Cumulative Mean	**100.3**		**100.7**	
Cumulative SD	**0.87003**		**1.32487**	
Cumulative %RSD	**0.9**		**1.3**	