



Received on 27 June, 2013; received in revised form, 02 August, 2013; accepted, 26 October, 2013; published 01 November, 2013

## A NOVEL STABILITY INDICATING HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF GUAIPHENESIN, CHLORPHENERAMINE MALEATE & DEXTROMETHORPHAN HBr

Tarun Goyal

Department of Quality Control, Overseas Health Care Pvt. Ltd., 335Km Milestone, N.H -1, P.O. Box 25, Phillaur-144410, Punjab, India

### Keywords:

Liquid chromatography, Cough syrup, Guaiphenesin, Chlorpheniramine Maleate, Dextromethorphan HBr

### Correspondence to Author:

**Tarun Goyal**

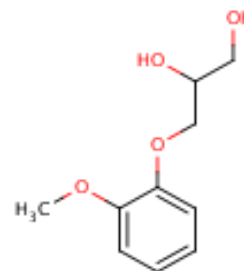
Manager Quality Control,  
Department of Quality Control,  
Overseas Health Care Pvt. Ltd.,  
335Km Milestone, N.H -1, P.O. Box  
25, Phillaur-144410, Punjab, India

E-mail: qcohcpl@gmail.com

**ABSTRACT:** An isocratic HPLC method with UV detection at 225nm is described for simultaneous determination of Guaiphenesin, Chlorpheniramine Maleate & Dextromethorphan HBr. Chromatographic separation of all three drugs were achieved on Purospher star RP-18e column (Merck) using Mobile phase as 74% Buffer (prepared by dissolving 1.8g dipotassium hydrogen orthophosphate in 1 litre of HPLC grade water, adjusting pH-3 with phosphoric acid) & 26% Acetonitrile. Method offers simplicity, good linearity, symmetric peak shape, and good resolution is achieved in a run time of 13 minutes. The method has been validated for Specificity, Linearity, Accuracy, and Precision. Stress testing which covered Oxidation, acid and alkali hydrolysis, photolysis and thermal degradation was performed on individual molecules under test to prove the specificity of the method and the degradation products formed were resolved effectively. It has a very good linearity with correlation coefficient value more than 0.999, % recovery for Guaiphenesin, Chlorpheniramine Maleate & Dextromethorphan HBr obtained were between 99-101%, and the relative standard deviation for 5 replicates obtained was less than 0.5%.

**INTRODUCTION:** Guaiphenesin (**figure 1**) chemically named as (RS)-3-(2-methoxyphenoxy) propan-1, 2-diol<sup>1, 2</sup>. It is an only expectorant recognized as safe and effective by the FDA. Often it is used with antihistamines, decongestants and antitussives in combination product. Guaiphenesin is an expectorant which increases the output of phlegm (sputum) and bronchial secretions by reducing adhesiveness and surface tension. The increased flow of less viscous secretions promotes ciliary action and changes a dry, unproductive cough to one that is more productive and less frequent.

By reducing the viscosity and adhesiveness of secretions, Guaiphenesin increases the efficacy of the mucociliary mechanism in removing accumulated secretions from the upper and lower airway<sup>3</sup>.



**FIGURE 1: GUAIFENESIN**

Dextromethorphan HBr (**figure 2**) is ent-3-methoxy-9a-methylmorphinan hydrobromide monohydrate, the d-isomer of the codeine analog of levorphanol. Dextromethorphan shows high affinity binding to several regions of the brain, including the medullary cough center.

<b>QUICK RESPONSE CODE</b> 	<b>DOI:</b> 10.13040/IJPSR.0975-8232.4(11).4435-41
	<b>Article can be accessed online on:</b> <a href="http://www.ijpsr.com">www.ijpsr.com</a>
<b>DOI link:</b> <a href="http://dx.doi.org/10.13040/IJPSR.0975-8232.4(11).4435-41">http://dx.doi.org/10.13040/IJPSR.0975-8232.4(11).4435-41</a>	

This compound is an NMDA receptor antagonist (receptors, N-methyl-D-aspartate) and acts as a non-competitive channel blocker. It is one of the widely used antitussives, and is also used to study the involvement of glutamate receptors in neurotoxicity [PubChem].

Dextromethorphan suppresses the cough reflex by a direct action on the cough center in the medulla of the brain. Dextromethorphan shows high affinity binding to several regions of the brain, including the medullary cough center. This compound is an NMDA receptor antagonist and acts as a non-competitive channel blocker. It is one of the widely used antitussives, and is also used to study the involvement of glutamate receptors in neurotoxicity<sup>3</sup>.

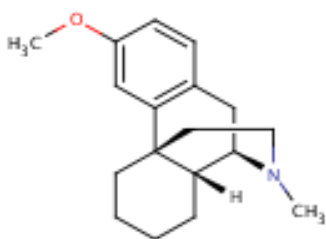


FIGURE 2: DEXTROMETHORPHAN

Chlorpheniramine Maleate (**figure 3**) is (RS)-3-(4-chlorophenyl)-3-(pyridin-2-yl)propyl dimethylamine hydrogen Maleate. It is histamine H1 antagonist used in allergic reactions, hay fever, rhinitis, urticaria, and asthma. It has also been used in veterinary applications. One of the most widely used of the classical antihistaminics, it generally causes less drowsiness and sedation. It is widely used in cough syrups in combination with other drugs [PubChem].

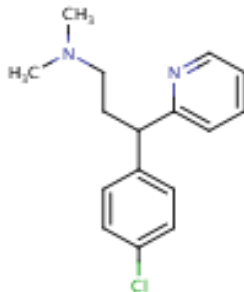


FIGURE 3: CHLORPHENERAMINE MALEATE

During literature survey, many methods for simultaneous estimation of Guaifenesin with dextromethorphan/acetaminophen/phenylephrine/chlorpheniramine/pseudoephedrine/phenylpropanolamine<sup>4, 5, 6, 7</sup> were reported but only one method for

simultaneous estimation this combination (Guaifenesin, Chlorpheniramine Maleate & Dextromethorphan HBr) of drugs was found, which employs methanol as organic solvent for elution<sup>8</sup>. Mobile phases with methanol show more backpressure, may have more noise at lower wavelengths, thus giving less S/N values and affecting the LOD/LOQ values.

The proposed method employs Acetonitrile instead of methanol for elution, which has low backpressure and less noise at the detection wavelength.

Moreover an attempt has also been made to prove the specificity of the method by performing stress studies on three drugs individually, and method is capable of separating the degradation products from the main peak of interest.

Hence, an attempt has been made to develop and validate the procedure in accordance with ICH guidelines.<sup>9</sup>

## Materials & Method

**Chemical Reagents:** Guaifenesin, Chlorpheniramine & Dextromethorphan HBr of IP grade were used for the test. Acetonitrile & Dipotassium hydrogen orthophosphate of chromatographic grade were obtained from Merck. All other chemicals were of Analytical grade.

**Apparatus:** Chromatographic separation was performed on Waters HPLC system equipped with 2489 U.V-Visible detector, 600E quaternary pump controller, 717 plus autosampler along with inline degasser. Empower 2 was used for data collection & processing.

**Chromatographic Conditions:** Chromatographic separation of all three drugs were achieved on Purospher star RP-18e column (Merck) 250\*4.6 (5um) stainless steel column using Mobile phase as 74% Buffer (prepared by dissolving 1.8g dipotassium hydrogen orthophosphate in 1 litre of HPLC grade water, adjusting pH-3 with phosphoric acid) & 26% Acetonitrile, filtered through 0.45um Nylon membrane. Separation was achieved at ambient temperature (25°C). Injection volume was 20ul & flow rate was 1.5ml/min

**Preparation of standard:** Accurately weigh 25mg of Guaiphenesin in 100ml volumetric flask. Separately weigh 100mg of Dextromethorphan HBr & 50mg of Chlorpheniramine Maleate in 100ml volumetric flask. Add about 70ml of mobile phase, sonicate to dissolve, and make up the volume with mobile phase.

Pipette 2.5ml of solution to 100ml flask containing 25mg Guaiphenesin add about 70ml mobile phase, sonicate to dissolve & make up the volume. This solution contains Guaiphenesin 250ppm, Dextromethorphan HBr 25ppm & Chlorpheniramine Maleate 12.5ppm.

**Preparation of sample:** 2.5ml of cough syrup was diluted to 100ml with mobile phase.

Separately inject 20ul each of standard & sample preparation & measure the responses for the major peaks.

## RESULTS & DISCUSSIONS:

**Method Development & Validation: Selectivity:** Selectivity is the ability of the analytical procedure

to differentiate & quantify the analytes in the presence of other components in sample. Forced degradation studies were done to build confidence in the analytical method.

Thermal, Hydrolytic, Oxidation & Photolytic reactions were covered under stress studies. Hydrolytic studies were performed using 0.1N HCL & 0.1N NaOH at 70deg C , Oxidation was performed by H<sub>2</sub>O<sub>2</sub> (1%) at 70°C, Photolysis was also performed by exposing the solutions of the drugs under study to U.V & Visible light for 200W hr & 1.2million lux respectively<sup>10</sup>.

The results suggest that drugs Chlorpheniramine & Dextromethorphan HBr are sensitive to oxidation (**figure 4**) & Photolysis (**figure 5**), and thus are unstable in these conditions. The proposed method is capable to differentiate between the analyte & degraded products.

Routine Excipients used in cough syrups such as Methyl Paraben sodium, Propyl Paraben sodium, Sodium Benzoate do not interfere.

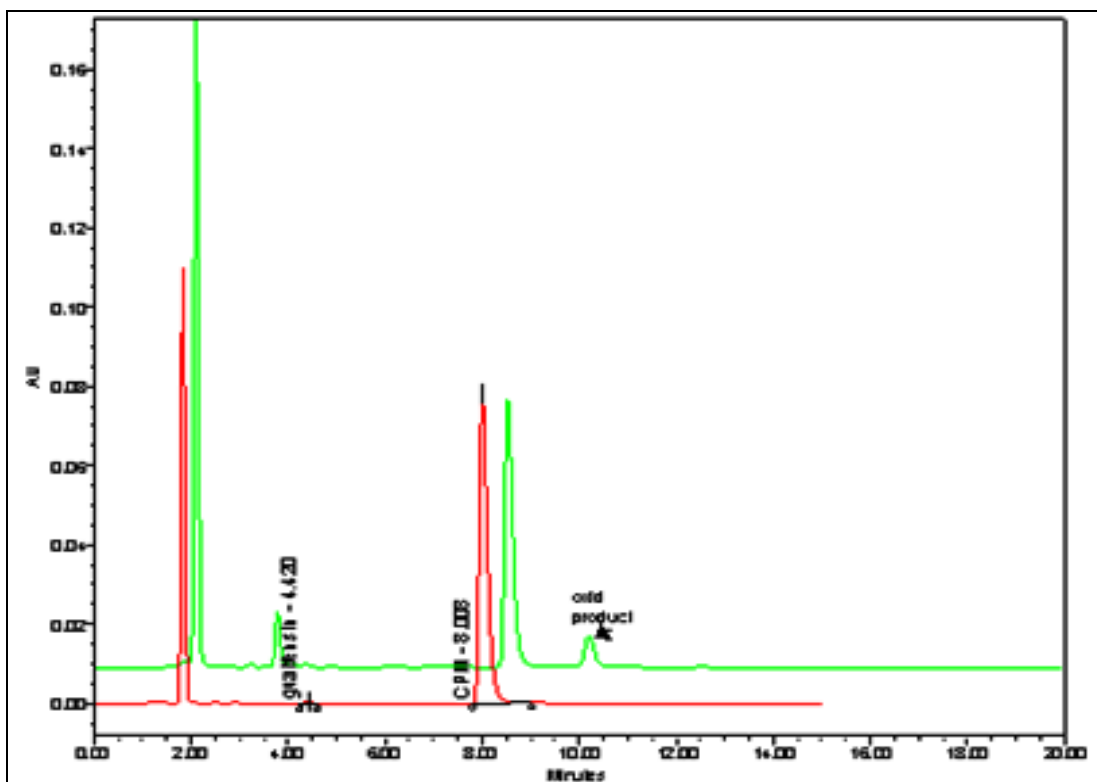


FIGURE 4: A REPRESENTATIVE CHROMATOGRAM OF SEPARATION OF OXIDATION PRODUCTS OF CHLORPHENIRAMINE FROM THE MAIN PEAK

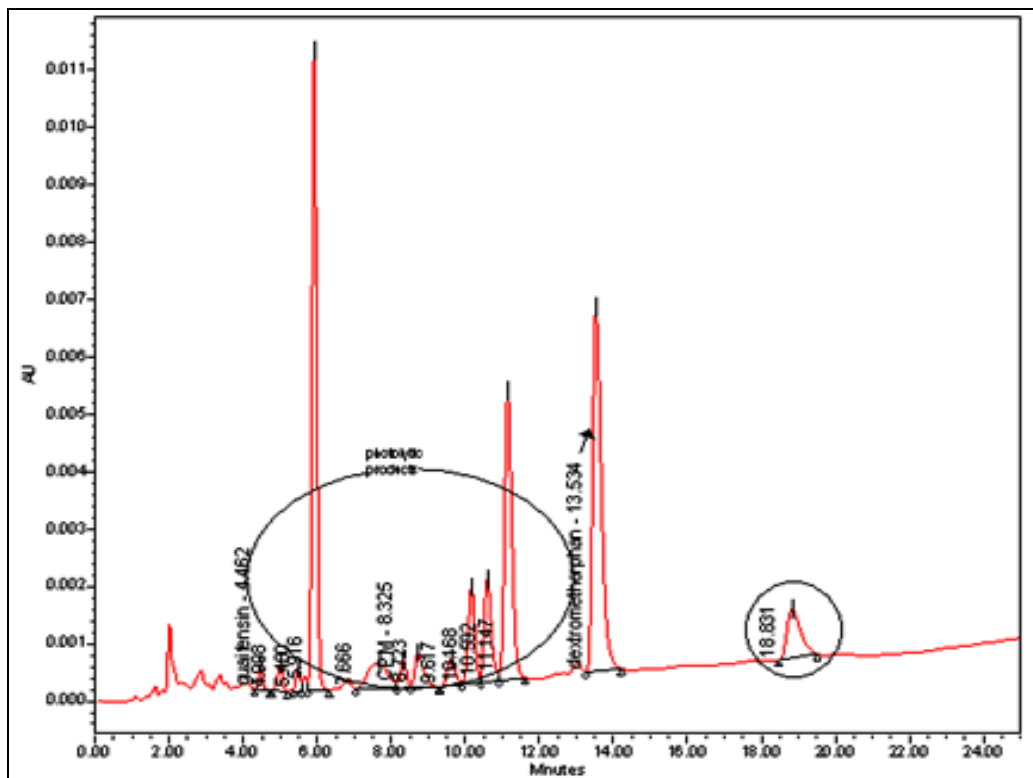


FIGURE 5: A REPRESENTATIVE CHROMATOGRAM OF SEPARATION OF PHOTOLYTIC PRODUCTS OF DEXTROMETHORPHAN HBr FROM THE MAIN PEAK

**System Suitability:** System suitability was performed by analyzing standard solutions. Parameters like Theoretical plates (N), Tailing factor, Resolution (Rs), Capacity factor (K), were determined. Repeatability was performed by 5

replicate injections, and RSD of peak areas of all 3 drugs was below 0.4. A representative chromatogram is shown in **figure 6**. Table showing the values for system suitability parameters is given in **Table 1**.

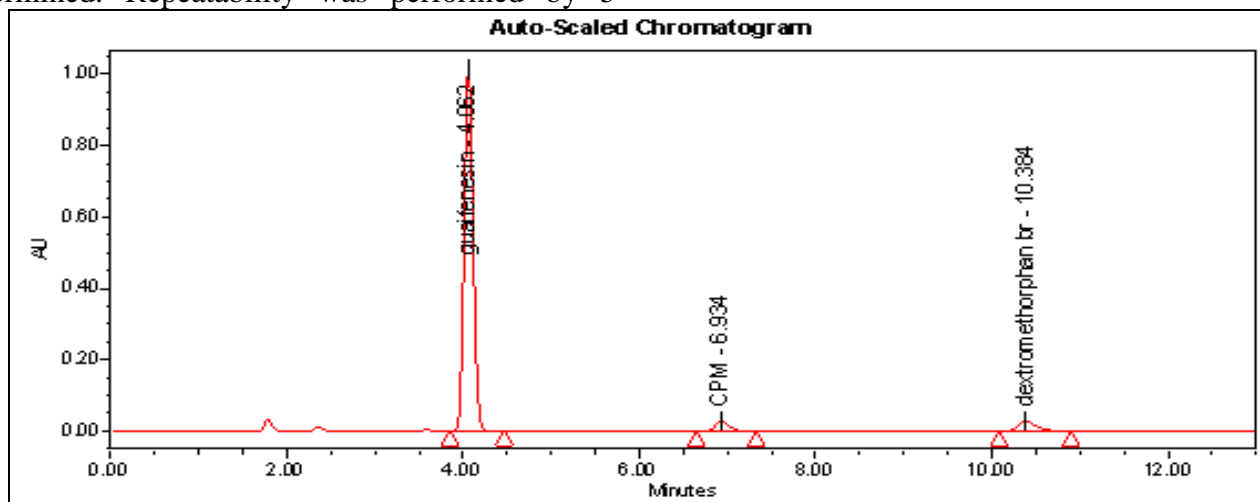


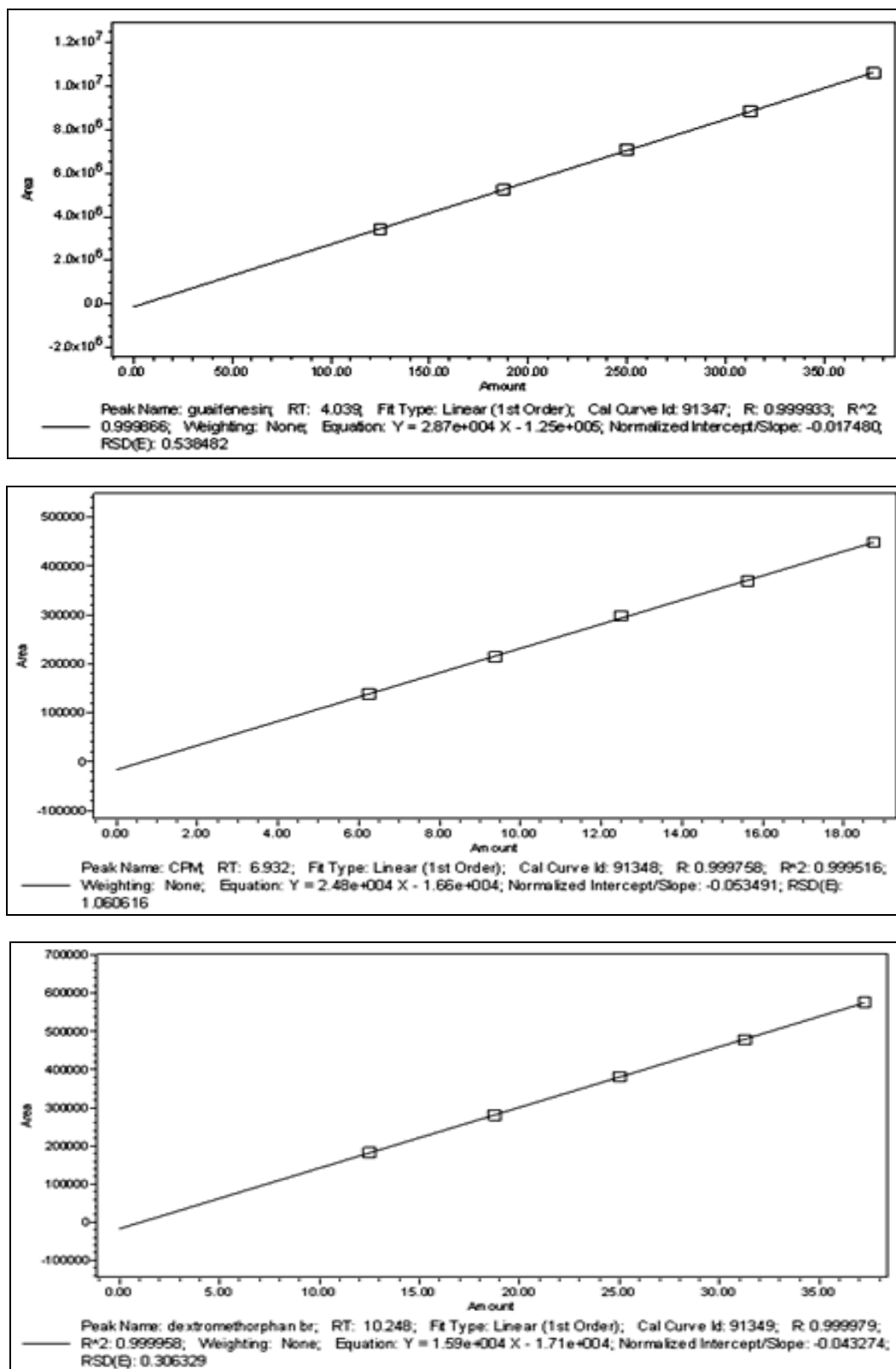
FIGURE 6: A REPRESENTATIVE CHROMATOGRAM OF GUAIFENESIN, CHLORPHENERAMINE, AND DEXTROMETHORPHAN HBR IN RAW MATERIALS

TABLE 1: SHOWING SYSTEM SUITABILITY PARAMETERS FOR GUAIFENESIN, CHLORPHENERAMINE AND DEXTROMETHORPHAN HBR

Drug	RT	N	USP Tailing	%RSD (n=5)	K prime	Resolution
Guaiphenesin	4.063	7557	1.08	0.2	3.06	---
Chlorpheniramine Maleate	6.932	9939	1.25	0.2	5.93	12.5
Dextromethorphan HBr	10.38	11788	1.36	0.2	9.39	10.69

**Linearity:** Linearity curves were obtained with 5 concentration levels and regression value (R<sup>2</sup>) was above 0.999. Individual linearity range established was 125-375ppm for Guaiphenesin, 6.25-18.75ppm for Chlorpheniramine Maleate, and 12.5-37.5ppm

for Dextromethorphan HBr. The individual values of Correlation coefficient, slope are given in **Table 2** and graph showing the linearity for Guaiphenesin, Chlorpheniramine, and Dextromethorphan HBr is given in **Figure 7**.



**FIGURE 7, SHOWING THE LINEARITY PLOT OF GUAIPHENESIN, CHLORPHENERAMINE, AND DEXTROMETHORPHAN HBR**

**Limit of Detection (LOD) & Limit of Quantitation (LOQ):** LOD was established at S/N ratio of 3.3 & LOQ at S/N ratio of 10. LOD & LOQ were verified by injecting standard samples

of their respective concentrations. These value shows that analytical concentration of drug is much above the LOQ limits. Individual values for each drug are provided in **Table 2**.

**TABLE 2: SHOWING VALUES OF LOD, LOQ, SLOPE, AND REGRESSION**

Name of Drug	Linearity range	R <sup>2</sup>	Slope	LOQ	LOD
Guaiphenesin	125-375ppm	0.99993	-0.01748	0.3ppm	0.1ppm
Chlorpheniramine Maleate	6.25-18.75ppm	0.9995	-0.05349	3.5ppm	1.1ppm
Dextromethorphan HBr	12.5-37.5ppm	0.99998	-0.04327	3ppm	1ppm

**Accuracy:** Accuracy is the *closeness of agreement* between a measured value and the true value. Accuracy was determined on the product by injecting 3 replicates of 3 different known concentrations by adding known amounts of drugs to the matrix (80%, 100%, and 120% of nominal values).

The results obtained correspond to the amount of drug present in the sample. % Recovery for individual drugs is mentioned in **Table 3**, RSD values obtained were below 0.3%.

**TABLE 4: SHOWING VALUES FOR PRECISION**

	Guaiphenesin (peak area)			Chlorpheniramine Maleate (peak area)			Dextromethorphan HBr (peak area)		
	Inj	80%	100%	120%	80%	100%	120%	80%	100%
1	10798806	13267121	15579299	467552	579044	705623	609400	763307	923075
2	10809203	13286677	15499428	467401	582256	703237	610082	765859	920603
3	10870680	13306193	15525455	469902	581505	704438	612346	765546	918632
4	10841775	13300412	15519261	469715	582411	705873	612432	764640	920739
5	10874902	13302789	15538207	471476	583171	706131	614596	765551	920980
RSD	0.3	0.1	0.2	0.4	0.3	0.2	0.3	0.1	0.2

**Intermediate precision:** was also established by injecting 3 replicates of 3 different concentrations, the next day and RSD was found below, 0.5%

**Stability of Analytical solution:** Stability of both standard & test solutions were determined by measuring the peak responses at 0hr & 8 hrs, there was no significant difference after 8hrs.

**Method Application:** The validated LC method was successfully applied to determination of cough syrup containing Guaiphenesin-50mg, Chlorpheniramine Maleate 2.5mg, Dextromethorphan HBr 5mg and Ammonium Chloride 60mg per 5ml, 3 batches of the product were analyzed & assay results were accurate & corresponds to the drug

**Precision:** Precision is the measure of method variability. It was performed on standard solutions of 3 different concentrations 80%, 100%, 120% of the established concentration, for all the 3 drugs. Individual values for SD, RSD are given in **Table 4**.

**TABLE 3: SHOWING MEAN RECOVERY**

Name of Drug	Mean % Recovery	RSD% (n=9)
Guaiphenesin	99.3-100.37%	0.2
Chlorpheniramine Maleate	99.26-100.4%	0.3
Dextromethorphan HBr	100.27-100.7%	0.1

added. A representative chromatogram is shown in **Figure 8**.

**CONCLUSION:** The developed HPLC method with UV-Visible detection offers simplicity, selectivity, precision, accuracy and linearity in the established range.

It produces symmetric peaks with less tailing, good resolution and reasonable time for estimation. It can be used for simultaneous estimation of Guaiphenesin, Chlorpheniramine Maleate, and Dextromethorphan HBr in cough syrup formulation and other dosage forms for routine analysis.

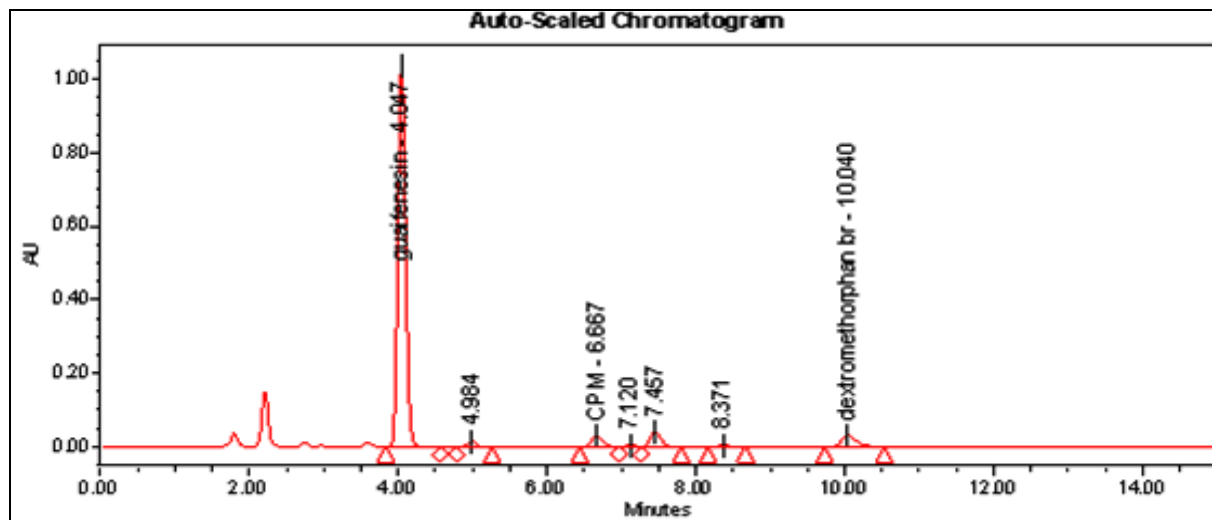


FIGURE 8: A REPRESENTATIVE CHROMATOGRAM OF GUAIFENESIN, CHLORPHENERAMINE, AND DEXTROMETHORPHAN HBR IN COUGH SYRUP FORMULATION

**ACKNOWLEDGEMENTS:** The author is grateful to Managing Director, Overseas Health Care Pvt. Ltd., for providing necessary facilities and encouragement. Author is also thankful to all the staff of Quality Control Department for their generous help during research study.

#### REFERENCES:

1. Indian Pharmacopoeia 2010, Indian Pharmacopoeia Commission Sector-23, Raj Nagar Ghaziabad, India.
2. United States Pharmacopoeia 35/National Formulary, NF-30, Published 2011; official May 2012
3. URL: <http://www.drugbank.ca/drugs>(assessed June 2013)
4. Sakshi Sawant and Nitin Borkar. Review of simultaneous determination of analytes by High Performance liquid chromatography in multicomponent cough and cold oral drug products. *Int J Adv Pharm Biol Sci* Vol. 2, Issue 1, 56-63;
5. URL:<http://www.labome.org/topics/chemicals/organic/ethers/methyl/guaiacol/guaifenesin-10097.html>.(assessed June 2013)
6. URL:[http://www.ncbi.nlm.nih.gov/pubmed?cmd=Link&LinkName=pubmed\\_pubmed&from\\_uid=11022915](http://www.ncbi.nlm.nih.gov/pubmed?cmd=Link&LinkName=pubmed_pubmed&from_uid=11022915). (assessed June 2013)
7. URL: <http://lib.bioinfo.pl/meid:89726/pmid>. (assessed June 2013)
8. Louhaichi MR, Jebali S, Loueslati MH, Adhoum N, Monser L; Simultaneous determination of pseudoephedrine, pheniramine, Guaiphenesin, pyrilamine, chlorpheniramine and dextromethorphan in cough and cold medicines by high performance liquid chromatography.
9. Validation of Analytical Procedures, ICH, Q2 (R1): Text and Methodology, November 2005.
10. Karen M. Alsante, Linda Martin, and Steven W. Baertschi; A Stress Testing Benchmarking Study.
11. Sweetman, Sean; Martindale: The Complete Drug Reference, (PhP) Pharmaceutical Press, London, UK, 37th edition, 2011.
12. Dr. P.D. Sethi; Quantitative Analysis of Pharmaceutical Formulations.
13. L. R. Snyder and J. J. Kirkland, John W. Dolan, Introduction to Modern Liquid Chromatography, 3rd edition., Wiley-Online Library, 2010.
14. Anthony C Moffat, M David Osselton, Brian Widdop; Clarke's Analysis of Drugs and Poisons, 4th edition 2011.
15. Allen, LoydV., Jr.; Remington: the Science and Practice of Pharmacy 22nd edition, 2012.
16. Tripathi KD: Essentials of Medical Pharmacology, 7th edition, 2010.
17. Rang HP, Dale MM, Ritter JM, Flower RJ, Henderson G. Rang and Dale's Pharmacology. 7th edition, London (UK): Churchill Livingstone; 2011.

#### How to cite this article:

Goyal T: A novel stability indicating hplc method for simultaneous estimation of Guaiphenesin, Chlorpheneramine maleate & Dextromethorphan HBr. *Int J Pharm Sci Res* 2013; 4(11): 4435-41. doi: 10.13040/IJPSR. 0975-8232.4(11).4435-41

All © 2013 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **ANDROID OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)