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Analytical method development and validation of simultaneous determination of Diphenhydramine HCL, Guaiphenesin and Bromhexine HCL in liquid dosage form by RP-HPLC technique

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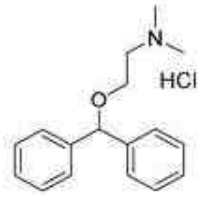
ABSTRACT

The present paper deals with the simultaneous determination of the active ingredients in the multicomponent pharmaceutical product using a RP- HPLC Technique. Diphenhydramine hydrochloride, Guaiphenesin and Bromhexine Hydrochloride were separated and quantitated either in pure form or simultaneously in the other interfering substances such as methyl and propyl parabens which are present in cough syrup. Several trials were carried out to obtain good separation among Diphenhydramine hydrochloride, Guaiphenesin and Bromhexine hydrochloride in mixture. These trials involved the use of different ratios of the mobile phase component, different pH of buffer, different flow rates, and different wavelength. The mobile phase of choice was found to be 0.05M Sodium Acetate in distilled water pH adjusted to 3.0 with acetic acid and solvent (60:40) ratio with the flow rate of 1.3 ml/min on BDS HYPERSIL C18 column with an Internal diameter 250×4.6mm, and 5 micron particle size with UV detection at 258 nm gave a satisfactory Chromatogram with Diphenhydramine Hydrochloride, Bromhexine Hydrochloride and Guaiphenesin of retention time of 10.45min, 16.76 min and 3.5min (Mixed standard) respectively.

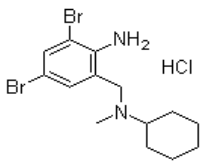
Keywords: Diphenhydramine hydrochloride, Guaiphenesin, Bromhexine Hydrochloride and RP-HPLC.

INTRODUCTION

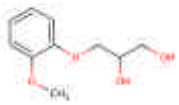
Diphenhydramine works by blocking the effect of histamine at H_1 receptor sites. This results in effect such as the increase of vascular smooth muscle contraction, thus reducing the redness, hyperthermia and edema that occur during an inflammatory reaction. In addition, by blocking the H_1 receptor on peripheral nociceptors. Diphenhydramine decrease their sensitization & consequently reduce itching i.e., associated with an allergic reaction. Bromhexine supports the body's own natural mechanism for clearing mucus from the respiratory tract. It is secretolytic i.e., it increase the production of serous mucus in the respiratory tract and makes the phlegm thinner & less sticky. This contributes to a secretomotor effect. It helps the cilia tiny hairs that line the respiratory tract to transport the phlegm out the lungs. For this reason it is often added to some antitissue (cough syrup).Guaiphenesin is an expectorant which clears chest congestion by loosening and reducing the viscosity of phlegm, increasing the volume of phlegm and making coughs more productive. For the present study a dosage form with combination of Diphenhydramine Hcl, Guaiphenesin, and Bromhexine Hcl in cough syrup was selected. The literature reveled that Diphenhydramine Hcl and Guaiphenesin is official in IP, BP & USP. Bromhexine Hcl is official in IP &USP. From the literature survey conducted, it was found that there are analytical methods reported for Diphenhydramine Hcl[1-3], Guaiphenesin[4-10] & Bromhexine HCl[11-13] separately by HPLC methods. To the best of our knowledge there is no HPLC method for the estimation of Diphenhydramine HCl, Guaiphenesin & Bromhexine HCl in cough syrup.



Diphenhydramine HCl



Bromhexine HCl



Guaiphenesin

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MATERIALS AND METHODS:

Single pan balance(Electronic Digital), Vacuum pump with filtration kit, HPLC-Agilent 1100 series auto sampler, Agilent UV detector, Chromatographic data software-Agilent chemstation, Column-HYPERSIL BDS C_{18} (250 x4.6 mm) ID column with 5µm particle size (Agilent), Sonicator, pH meter

Reagents and Chemicals

HPLC water, Sodium acetate (analytical grade) – Rankem, Solvent (methanol) – Rankem, Acetic acid (analytical grade) - Rankem.

SYRUP BRAND NAME:

T-MUCOLE SYRUP Each 5ml contains Diphenhydramine Hydrochloride: 8mg, Guaiphenesin : 50 mg, Bromhexine Hydrochloride : 4 mg

METHODOLOGY

Chromatographic conditions:

Instrument: Agilent 1100 series
Column : HYPERSIL BDS C_{18} (250 X 4.6mm) Id with5µm particle size.
Mobile phase : Buffer: methanol (60:40) 0.05Msodium acetate adjusted to pH 3.0
Flow rate : 1.3 ml/minute
*Wavelength :*258nm
Injection volume: 50µl
Run time: 25 minutes
Temperature : Ambient
Mode of operation : Isocratic elution

Preparation of 0.05M sodium acetate buffer pH 3.0:

Weigh accurately 6.8508 gm of sodium acetate (AR) in to 1000ml standard flask and made up to volume with HPLC grade water. Adjust the pH to 3.0 using acetic acid.

Preparation of mobile phase:

Buffer solution 600ml is mixed with 400ml of solvent (HPLC Grade) then filtered through 0.5micron filter and degassed.

Standard stock solution:

Weigh accurately 40mg of Diphenhydramine hydrochloride working reference standard and 20 mg of Bromhexine hydrochloride working reference standard into 100 ml volumetric flask add 50 ml of mobile phase and sonicate for 10 minutes. Cool and make up the 100 ml with mobile phase.

Table.1. Validation and system suitability parameters

Parameters	Diphenhydramine Hcl	Guaiphenesin	Bromhexine Hcl
Linearity range µg/ml	64-96µg / ml	400-600 µg / ml	32-48 µg / ml
Correlation Coefficient (r^2) ± S.D	0.9904	0.9893	0.9915
Retention time (min) ± S.D	10.45	3.5	16.76
Resolution	14.89		6.97
Tailing factor	1.28	1.14	1.43
Theoretical Plate	3266	5118	3925
Limit of detection (µg/ml)	4.1 µg / ml	27.10 µg / ml	1.98 µg / ml
Limit of Quantification (µg/ml)	12.43 µg / ml	82.12 µg / ml	5.84 µg / ml
Precision (RSD %) intraday (n=6)	0.53%	0.11%	0.66%

Fig.1. HPLC Chromatogram of Diphenhydramine Hydrochloride, Guaiphenesin and Bromhexine hydrochloride

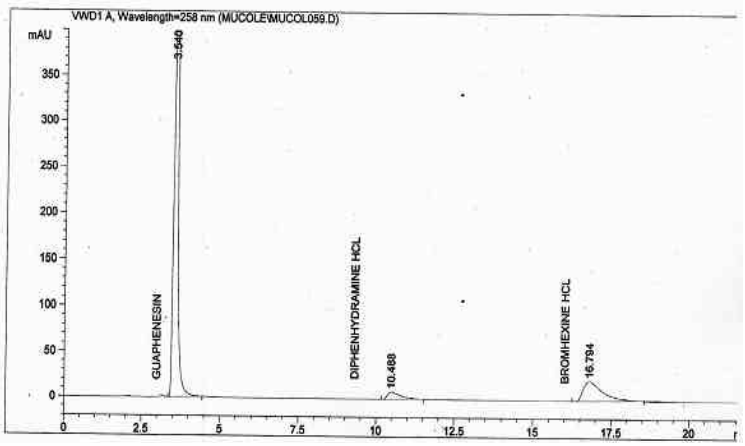
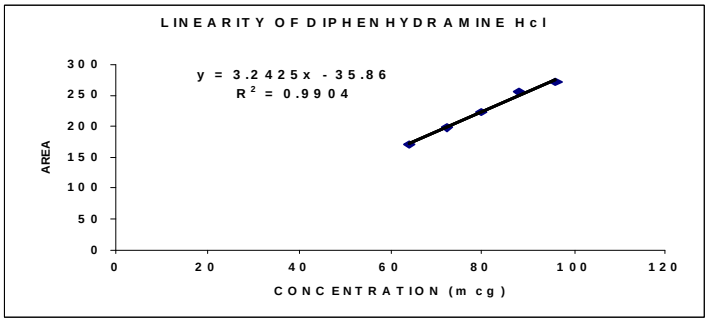
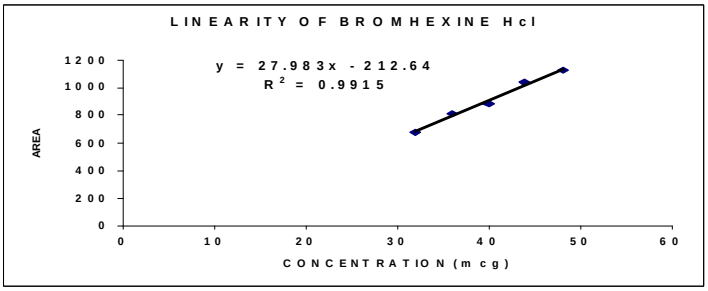
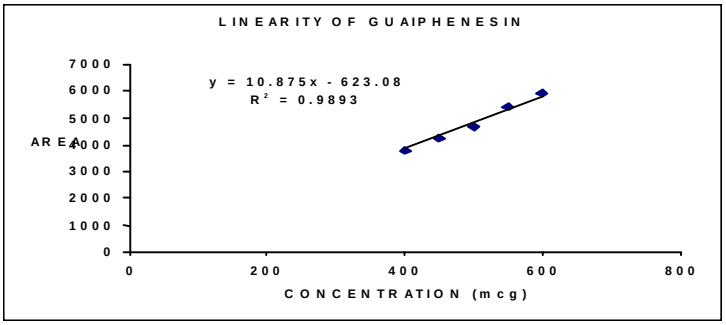


Fig.2. Linearty curve for Diphenhydramine Hydrochloride, Guaiphenesin and Bromhexine hydrochloride







Standard preparation:
Weigh accurately 25mg of Guaiphenesin working reference standard into 50 ml volumetric flask. Add 10 ml of mobile phase and 10 ml of standard stock solution sonicate for 10 minutes. Cool and make up the volume with mobile phase.

Sample preparation:
Weigh accurately 5.945g of the sample into a 100ml volumetric flask. Add 50 ml of mobile phase and sonicate for 10 minutes. Cool and make up the volume with mobile phase. The amount of Diphenhydramine Hydrochloride, Guaiphenesin and Bromhexine Hydrochloride in each syrup formulation was calculated by comparing the peak area of the standard

Procedure
Separately inject standard preparation and the sample preparation into the High Performance liquid chromatograph system and record the peak areas for major peaks.

RESULTS AND DISCUSSION
The simultaneous determination of the active ingredients in the multicomponent pharmaceutical product using a RP- HPLC technique. Diphenhydramine hydrochloride, Guaiphenesin and Bromhexine Hydrochloride were separated and quantitated either in pure form or simultaneously in the other interfering substances such as methyl and propyl parabens which are present in cough syrup. Several trials were carried out to obtain good separation among Diphenhydramine hydrochloride, Guaiphenesin and Bromhexine hydrochloride in mixture. These trials involved the use of different ratios of the mobile phase component, different pH of buffer, different flow rates, and different wavelength. The mobile phase of choice was found to be 0.05M Sodium Acetate in distilled water pH adjusted to 3.0 with acetic acid and solvent (60:40) ratio with the flow rate of 1.3 ml/min on BDS HYPERSIL C18 column with an Internal diameter 250×4.6mm, and 5 micron particle size with UV detection at 258 nm gave a satisfactory Chromatogram with Diphenhydramine Hydrochloride, Bromhexine Hydrochloride and Guaiphenesin of retention time of 10.45min, 16.76 min and 3.5min (Mixed standard) respectively.

Estimation
A RP-HPLC method was developed for the simultaneous estimation of Diphenhydramine Hydrochloride, Guaiphenesin and Bromhexine Hydrochloride in combined dosage forms, which can be conveniently employed for routine quality control in pharmaceutical dosage forms. The chromatographic conditions were optimized in order to provide a good performance of the assay. The standard and sample solutions were prepared and chromatograms were recorded.The peak area ratios of standard and sample solutions were calculated. The assay procedure was repeated for 6 times and mean peak area, mean peak area ratio, mean weight of standard drugs, mean weight of sample taken for assay were calculated. The percentages of individual drugs found in formulations, mean and relative standard deviation in formulations were calculated. The result of analysis shows that the amount of drugs present in the formulation has a very good correlation with the label claim of the formulation.

Validation of the method
The accuracy of the method was determined by recovery experiments. A known quantity of the pure drug was added to the pre-analyzed sample formulation at 80%, 100% and 120% levels. The recovery studies were carried out 3 times of each level and the percentage recovery and mean of the percentage recovery were calculated. From the data obtained, it was observed that the recoveries of standard drugs were found to be accurate and within the specified limits.The precision of the method was determined by studying repeatability and reproducibility. The area of drug peaks and percentage relative standard deviation were calculated .The results revealed that the developed method was found to be reproducible in nature.The standard drug solutions in varying concentrations ranging from 80 to 120 % of the targeted level of the assay concentration were examined by the assay procedure. Diphenhydramine Hydrochloride, Guaiphenesin and Bromhexine hydrochloride were found to be linear in the range of 64 to 96 µg/ml, 400 to 600 µg/ml and 32 to 48 µg/ml respectively. The slope, intercept and correlation coefficient values were also calculated. The correlation co-efficient of Diphenhydramine Hydrochloride, Guaiphenesin and Bromhexine hydrochloride were found to be 0.9904, 0.9893 and 0.9915 respectively.The calibration curves were plotted as peak area Vs concentration of the standard solutions (Fig.2). The calibration graph shows that linear response was obtained over the range of concentrations used in the assay procedure. These data demonstrates that the methods have adequate sensitivity of the concentration of the analytes. The range demonstrates that the method is linear outside the limits of expected use.The additional

peaks were observed in the chromatogram of the formulation which may be due to excipients present in the formulation. These peaks do not interfere with the standard peaks, which clearly confirm the assay method was found to be highly specific (Fig.1) The LOD and LOQ of the developed method were determined by analyzing progressively low concentration of the standard solutions using the developed methods. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3.3). LOD of Diphenhydramine Hydrochloride, Guaiphenesin and Bromhexine hydrochloride were found to be 4.10mcg/ml, 27.10mcg/ml, 1.98mcg /ml respectively. The LOQ is the smallest concentration of the anlayte, which gives response

That can be accurately quantified (signal to noise ratio of 10). The LOQ of Diphenhydramine Hydrochloride, Guaiphenesin and Bromhexine hydrochloride were found to be 12.43mcg/ml; 82.12mcg/ml and 5.84mcg/ml respectively.The system suitability studies were performed for the standard solutions shows the resolution values of the separated peak with other chromatographic parameters. The calculated resolution values for Guaiphenesin peak relative to Diphenhydramine hydrochloride is 14.86, while the selectivity factor is 2.96 and peak relative to Bromhexine hydrochloride is 6.97, while the selectivity is 1.60.the tailing factor for Diphenhydramine hydrochloride is 1.28, Guaiphenesin is 1.14 and Bromhexine HCl is 1.43. The method was validated for statistical parameters i.e. precision, accuracy, specificity, linearity, and robustness criteria. Results of the method validation experiments are given in Table.1. From the above finding it can be suggested that the developed method for the simultaneous estimation of Diphenhydramine Hydrochloride, Guaiphenesin and Bromhexine hydrochloride in combined dosage forms was found to be accurate, precise, linear, specific, simple and rapid in analysis and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories.

REFERENCES

1. Meatherall RC, Guay DR. Isothermal gas chromatographic analysis of diphenhydramine after direct injection onto a fused – silica capillary column. Journal of chromatography. 1984 may 11.
2. Trivedi P, and sachan A.; 'simultaneous spectrophotometric analysis of liquid oral formulation containing Bromhexine Hcl, Diphenhydramine Hcl and Guaiphenesin,; Indian drugs, 36(12) 1999. 735-738.
3. Maria R.', Lorena Sombra, Roberto A. Olsina, Luis D.Martinez and Maria F. Silva; codeine, diphenhydramine, ephedrine and noscapine in development and validation of a capillary electrophoresis method for the determination of pharmaceuticals.22 December 2004.
4. Chen, T M: Pacifico, J R: Daly, R E· High – pressure liquid chromatography assay if dextromethorphan hydro bromide, Guaiphenesin, and sodium benzoate in an expectorant syrup. Journal of chromatography science 26(12). 1988.
5. Patil K.M; Bhoir I.C; Raman B; Sunderesan M; Simultaneous Derivative Spectrophotometeric Determination of Terbutaline sulfate and Guaiphenesin. Indian journal of pharmaceutical science. 60(6): 1998 407-9.
6. Gupta A; Garg R; Sharma AK simultaneous determination of terbutaline sulphate, bromhexine hydrochloride and Guaiphenesin in three component tablet formulation. By UV spectroscopy. Indian journal of pharmaceutical science. 61(2): 1999: 128-3
7. Trivedi P, and sachan A.; 'simultaneous spectrophotometric analysis of liquid oral formulation containing Bromhexine Hcl, Diphenhydramine Hcl and Guaiphenesin,; Indian drugs,.36(12) 1999 735-738.
8. Giorgio Graosa, Erika Del Grosso, Roberta Russo and Gianna Allegrone; simultaneous, stability indicating, HPLC-DAD determination of Guaiphenesin and methyl and propyl –parabens in cough syrup. February 2006. 23
9. Artem U. Kulikov and Aleksey G. Verushkin; simultaneous Determination of Paracetamol, caffeine, Guaiphenesin and Preservative in syrups by Micellar LC. Journal of chromatography; 67: 2008:, 5-6.
10. Nerissa L. Deola; Noel S. Quiming; Yoshihira Saito; Alicia P. Catabay; Kiyokatsu Jinno; sensitive micellar electrokinetic chromatographic determination of salbutamol, Guaiphenesin, and Dyphyillne in oral formulation. Journal of liquid Chromatography & Related Technologies, 32, 10 June 2009, 1407-1422
11. M I Santoro, M Santos, J F Magalhaes.Spectrophotometric determination of Bromhexine hydrochloride in pharmaceutical preparation. Journal – association of official Analytical Chemists.; 67(3): 1984 May –June 532-4.
12. E. Venkata Rao, G. Ramana Rao, S. Raghuveer and P. Khadgapathi; 1987; Gas-liquid chromatographic and ion-pair high-performance liquid chromatographic determination of pseudoephedrine hydrochloride and bromhexine hydrochloride in pharmaceuticals; Analyst, , 112, 1987 871 – 874.
13. V.Gupta et.al., simulataneous spectrophotometric estimatiom of bromhexine hydrochloride and pseudoephedrine hydrochloride in tablet dosage form; Asian Journal Of Chemistry;, 21 2009 1633-1635

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