RESEARCH ARTICLE

Stability-Indicating Simultaneous Method Development and Validation of Guaifenesin and Dextromethorphan HBr by Reverse-Phase High-Performance Liquid Chromatography

Nathi Rathnakar[1*], Dannana Gowri Shanker[2]

[1]*Research Scholar, A. U. College of Pharmaceutical Sciences, Visakhapatnam, Andhra Pradesh, India*
[2]*Professor, A. U. College of Pharmaceutical Sciences, Visakhapatnam, Andhra Pradesh, India*

Received: 17th March, 2020; Revised: 22nd April, 2020; Accepted: 21st May, 2020; Available Online: 25th June, 2020

ABSTRACT

The developed method was validated according to ICH guidelines with respect to specificity, linearity, limits of detection, quantification, accuracy, precision, and robustness. The stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method is precise; it has been developed for the simultaneous estimation of assay of guaifenesin (GN) and dextromethorphan hydrobromic (HBr) (DN) in drug substance and drug product. The chromatographic separation was done in an isocratic mode using the Syncronus C8 (250 × 4.6 mm, 5 µ particle size) column with mobile phase containing a 10 mM ammonium acetate in water (modulated pH 4.30 with orthophosphoric acid) and acetonitrile in the ratio of 60:40 (% v/v) used for efficient chromatographic separation. The flow rate of the mobile phase was 1 mL/min with ambient column temperature and detection of wavelength at 279 nm; injection volume 10 µL was fixed for achieving good elution of eluents. The retention time for GN was found to 3.46 minutes and DN was found to 7.58 minutes. GN and DN were linear in the concentration range from 357 to 1,428 and 19 to 75 µg/mL, respectively. Regression analysis showed that the r value (correlation coefficient) greater than 0.999 for GN r value was found to be 0.999, GN r value was found to be 0.999, DN r value was found to be 0.999. Limit of detection (LoD) and limit of quantification (LoQ) of GN was found to be 0.151 and 0.904 µg/mL, DN was found to be 0.241 and 0.726 µg/mL. The developed method was validated and found to be accurate, specific, and robust. Both the drugs were subjected to the stress conditions like acidic, basic, oxidative, photolytic, and thermal conditions. The degradation results were found to be satisfactory. In peroxide stress condition, GN was found stable over DN, and DN was found to degrade significantly. The degradation products were well resolved from GN, DN, and their impurities. The peak purity test results confirmed that the GN and DN peak were homogenous and pure in all stress conditions, thus, proving the stability-indicating nature of the method. This method could be applied for the simultaneous estimation of GN and DN in drug substance and drug product.

Keywords: Analytical method development, Analytical method validation, Dextromethorphan HBr (DN), Guaifenesin (GN), Reversed phase-high performance liquid chromatographic (RP-HPLC), Stability indicating.

International Journal of Pharmaceutical Quality Assurance (2020); DOI: 10.25258/ijpqa.11.2.13

How to cite this article: Rathnakar N, Shanker DG. Stability-indicating simultaneous method development and validation of guaifenesin and dextromethorphan HBr by reverse-phase high-performance liquid chromatography. International Journal of Pharmaceutical Quality Assurance. 2020;11(2):262-270.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

The GN, (+)-3-(2-methoxyphenoxy)-propane-1,2diol, is widely used as an expectorant, useful for the symptomatic relief of respiratory conditions. Its molecular formula is $C_{10}H_{14}O_4$ and molecular weight of 198.21 gram/mole. It is a white or slightly grey crystalline substance with a slightly bitter aromatic in taste, soluble in water, and slightly soluble in ethanol and methanol. It is available as extended-release tablets for oral administration.[1-4]

The DN is the dextrorotatory enantiomer of the methyl ether of levorphanol and stereoisomer of levomethorphan. DN is an anti-tussive (cough suppressant) drug used for pain relief and psychological applications.[5-7] Its empirical formula is $C_8H_{25}NOHBr$ and the corresponding molecular weight of the compound 352.32 gram/mole. It is a white powder. It is freely soluble in chloroform and insoluble in water; the hydrobromide salt is water-soluble up to 1.5 g/100 mL at 25°C.

[*]*Author for Correspondence: rathna.nathi@gmail.com*

The combination of GN and DN is used to treat cough and chest congestion caused by the common cold, infections, or allergies. The chemical structures of GN and DN and their impurities are shown in Tables 1 and 2.

GN Impurities

GN Impurity-A (Guaicol)

The guaicol is a process-related impurity, and its molecular formula $C_7H_8O_2$ and molecular weight of the substance is 124.14 gram/mole. It is official in USP for drug substance, and its specification is as per the USP limit is NMT 0.03%.

GN Impurity-B (β-Isomer)/ 2-(2methoxyphenoxy) propane-1, 3-diol

The beta isomer is a process-related impurity; its molecular formula $C_{10}H_{14}O_4$ and molecular weight of the compound is 198.21 gram/mole. It is official in USP and EP for drug substance, and its specification as per EP limit is NMT 1.5%.

GN Impurity-C (bisether)/ 1, -Oxybis [3-(2-methoxyphenoxy) propane-2-ol]

The GN impurity-C is a process-related impurity; its molecular formula $C_{20}H_{26}N_4O_7$ and molecular weight of the compound is 378.42 gram/mole. It is official in USP for drug substance, and its specification as per the USP limit is NMT 0.5%.

Guaifenesin (GN) Impurity-D/ 1, 3-bis(2-methophenoxy) propane-2-ol

The GN impurity-D is a process-related impurity; its molecular formula $C_{17}H_{20}O_5$ and molecular weight of the compound is 304.33 gram/mole. It is official in USP for drug substance, and its specification as per the USP limit is NMT 0.5%.

DN Impurities

Dextromethorphan Related Compound-A: (+)3-methoxy morphine

The DN impurity-A is a process-related impurity; its molecular

Table 1: Chemical structure of the Guaifenesin and Dextromethorphan HBr

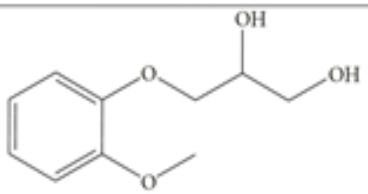
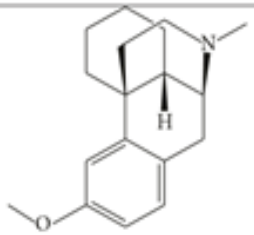
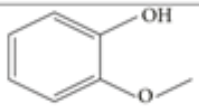
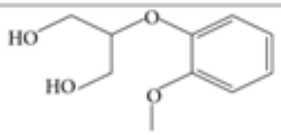
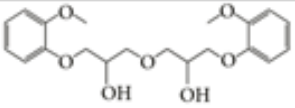
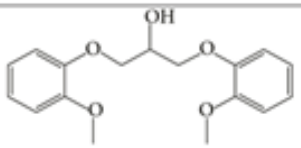
Molecule name	Chemical name	Chemical structure
Guaifenesin	(+)-3-(2-Methoxyphenoxy)-propane-1,2-diol	
Dextromethorphan	Ent-3-methoxy-17-methylmorphinan	

Table 2: Structure of impurities a) Guaifenesin a) Dextromethorphan HBr

(a)

Impurity name	Chemical name	Chemical structure
Impurity A (degradant)	2-Methoxyphenol	
Impurity B (degradant)	2-(2-Methoxyphenoxy)propane-1,3-diol	
Impurity C (degradant)	1,1′-Oxybis[3-(2-methoxyphenoxy)propan-2-ol]	
Impurity D (process related impurity)	1,3-Bis(2-methoxyphenoxy)propan-2-ol	

formula $C_{17}H_{23}NO$ and molecular weight of the compound is 257.37 gram/mole, and the log P value of the compound is 3.86.

Dextromethorphan Related Compound-B: Ent-17-methyl morphinan-3-ol

The DN impurity-B is a process-related impurity; the molecular formula $C_{17}H_{23}NO$ and molecular weight of the compound is 257.37 gram/mole, and the log P value of the compound is 4.11.

Dextromethorphan Related Compound-C: Ent-3-methoxy-17methyl morphinan-10-one

The DN impurity-C is a process-related impurity; the molecular formula $C_{17}H_{25}NO$ and molecular weight of the compound is 271.19 gram/mole, and the log P value of the compound is 4.11.

Dextromethorphan Related Compound: Ent-3-methoxy N-Formyl Morphinan

The DN N-formyl morphinan impurity is a process-related impurity; its molecular formula $C_{10}H_{23}NO$ and molecular weight of the compound is 288.39 gram/mole, and the log P value of the compound is 2.9.

Dextromethorphan Related Compound: N-Formyl Octabase

The DN N-formyl octabase impurity is an isomer and a process-related impurity; its molecular formula is $C_{10}H_{23}NO_2$, and the molecular weight of the compound is 288.19 gram/mole, and its log P value is 2.9.

Analytical method development and validation of bulk material and finished formulations are one of the greatest challenging tasks for scientists.[8] The presence of unsolicited or unknown chemicals, even in slight amounts, may impact not only the therapeutic efficacy, but also the safety of the pharmaceutical dosages.[9] For these reasons, both the formulated active pharmaceutical ingredients (APIs) and bulk compounds established limits by international agencies and pharmacopeias. As per the requirements of various regulatory authorities, the assay profile study of drug substances and drug products must be carried out using a suitable analytical method in the final product.[10,11]

The GN and DN drug substances are official in the United States Pharmacopeia and European Pharmacopeia, but its combination is not official in any of the pharmacopeias. In the literature survey, there were several Liquid chromatography (LC) assay approaches that have been reported for the determination of GN and DN in pharmaceutical preparation either individually or in combination with other drugs[12-21] and Liquid chromatography-Mass Spectrometry (LC-MS) in human plasma.[22] Few procedures were available for the determination of GN and DN.[21-23]

There is no single method reported in terms of assay evaluation for the simultaneous determination in pharmaceuticals formulations of GN and DN. It has shown aspiration to develop a stability-indicating method for the simultaneous determination of GN and DN in pharmaceutical formulation.

Hence, an attempt has been made to develop an accurate, rapid, specific, and reproducible method for the simultaneous determination of GN and DN assay in pharmaceutical dosage forms, along with method validation as per ICH norms.[10] As per ICH norms, the stability tests were also performed on both drug substances and drug products.[11]

MATERIALS AND METHOD

Chemicals and Reagents

The GN, DN reference standards, and tablets were gifted from the formulation research and development laboratory of Pellets Pharma Laboratories Ltd., Hyderabad, India. GN API and its impurities were procured from Synthochem Lab., India. DN API and impurities were procured from Divis Laboratories Ltd., India. HPLC grade acetonitrile, methanol, and glacial acetic acid were purchased from Merck, Germany, Regis Technologies Inc, USA, and highly pure water was prepared by using Millipore MilliQ plus purification system.

Equipment

The LC system was used for method development and method validation. Detection was carried by Waters with a diode array detector (model: 2996 detector 2487 separation module). The output signal was supervised and processed using Waters Empower Software. LC GC Ragward Dual Range balance was used to perform weighing. Photostability studies were carried out in a photostability chamber. Thermal stability studies were performed in at thermostat dry air.

Chromatographic Conditions

The RP-HPLC measurements were carried out using a reversed-phase Syncronus C8 (250 × 4.6 mm, 5 µ particle size) column with mobile phase containing a 10 mM ammonium acetate in water (adjusted pH 4.30 with OPA) and acetonitrile in the ratio of 60:40 (% v/v) used isocratic chromatographic separation. The flow rate of the mobile phase was 1 mL/min with ambient column temperature and wavelength detection at 279 nm, and injection volume 10 µL was fixed for achieving good elution of eluents.

Diluent: 0.1 N HCl used as diluent

Preparation of Standard Solution and System Suitability Solution

Accurately prepared individual stock solutions of GN, DN, working standard, and their impurities of each 500 µg/mL (48 µg/mL of GN and 2.4 µg/mL of DN impurity stock solution). A mixture of all impurities (48 µg/mL of GN impurities and 2.4 µg/mL of DN impurities) and these solutions were used for specificity.

From the above 500 µg/mL stock working standard solution 70 µg/mL and 37 µg/mL system-suitability standard solutions were prepared. Stock solutions were used for method development and intermittent method validation.

Preparation of Control Solution

Accurately weighed the equivalent of 4,800 mg of GN and 240 mg of DN into a 250 mL volumetric flask to it added about 180 mL of diluent, and it is sonicated for 60 minutes with intermediate shaking. The solution was diluted to 250 mL and centrifuged at 3,000 rpm for 10 minutes. The supernatant

(24,000 µg/mL of GN and 1,200 µg/mL of DN) was collected and filtered through a 0.45 µm pore size nylon membrane filter (make: Rankem). The filtrate was used as a sample solution.

Preparation of Test Solution

Twenty tablets (1,200 mg of GN + 60 mg of DN) were weighed, and the average weight was calculated. The tablets were crushed into a fine powder, and the powder equivalent of 4800 mg of GN (or equivalent to 240 mg of DN) was transferred into a 250 mL volumetric flask. Approximately, 180 mL of diluent was added, shake to disperse the material, and sonicated for 60 minutes with intermediate shaking. The solution was diluted to 250 mL and centrifuged at 3,000 rpm for 10 minutes. The supernatant (24,000 µg/mL of GN and 1,200 µg/mL of DN) was collected and filtered through a 0.45 µm pore size nylon membrane filter (make: Rankem). The filtrate was used as a sample solution.

METHOD VALIDATION

The proposed method was validated as per ICH guidelines.[8]

System Suitability

System suitability parameters were evaluated to verify system performance. System precision was determined by six replicate injections of standard preparations. All the important characteristics, including the relative standard deviation, peak tailing, and theoretical plate number, were measured. The resolution between impurities was measured by injecting the system suitability solution. All these system suitability parameters covered the system, method, and column performance.

Specificity

Stress studies were performed at an initial concentration of 24,000 µg/mL of GN and 1,200 µg/mL of DN in APIs and formulated samples to provide the stability-indicating property and specificity of the proposed method. Intentional degradation was attempted by the stress conditions of exposure to photolytic stress (1.2 million lux hours followed by 200 Watt hours), heat (exposed at 105°C for 15 hours), acid (1 N HCl for 2 hours at 60°C), base (1 N NaOH for 2 hours at 60°C), oxidation (10% peroxide for 30 minutes at 60°C), water (refluxed for 12 hours at 60°C), and humidity (exposed to 85% RH for 72 hours).

Precision

The precision of the GN and DN was checked by injecting six individual test preparations of (24,000 µg/mL of GN and 1,200 µg/mL of DN) test preparation and calculated % relative standard deviation (RSD) of each compound. The intermediate precision of the method was also assessed using different analysts and a different instrument in the same laboratory.

LoD and LoQ

The LoD and LoQ of GN and DN were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations. The precision study was also carried out at the LoQ level, and the result was calculated.

Linearity

Linearity examination was prepared by diluting the stock solution to the required concentrations. The solutions were prepared at six concentrations. The peak area *vs.* concentration (in µg/mL) data was subjected to the method of least squares linear regression analysis.

Accuracy

Accuracy of the method was evaluated by using concentration levels of 50, 100, and 150% GN, DN tablets. Standard addition and recovery experiments were conducted on a real sample to determine the accuracy method. The percentages of recoveries GN and DN were calculated.

Robustness

To examine the robustness of the developed method, experimental conditions were deliberately changed, and the resolution between GN, DN tailing factor, and theoretical plates of GN and DN peaks were evaluated. To study the outcome of the flow rate on the developed method, it was changed ± 0.2 mL/minute. The effect of column temperature on the developed method was studied at ±5°C (instead of 25°C). The effect of pH was studied by varying ±0.2 pH units (i.e., 2.8 and 3.2), and the mobile phase composition was changed ±10% from the initial composition of the organic phase. In all the above varied conditions, the aqueous component of the mobile phase was held constant.

RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions

The main criteria for developing RP-HPLC method for the simultaneous determination of assay in GN and DN pharmaceutical dosage form in a single run, with an importance on the method being accurate, reproducible, robust, stability-indicating, linear, free of interference from other formulation excipients, and convenient enough for routine use in quality control laboratories.

Conducted different choreographic trails on Individual stock solutions of GN, DN, and their impurities, spectra of each component were checked, and it is shown in (Figure 1). From the spectral data GN, DN were having absorbance maximum at about 273 and 279 nm. With a respective low concentration of DN, 279 nm was selected for the estimation of GN and DN. A spiked solution of impurities (48 μgmL^{-1} of GN impurities and 2.4 μgmL^{-1} of DN impurities), GN + DN (24,000 μgmL^{-1} + 1,200 μgmL^{-1}), and placebo peaks were subjected to separation by RP-HPLC. Initially, the separation was tried with the existing methods (USP, EP, and the literature method).[24,25] In the USP method, data was observed that placebo peaks and GN impurity peaks were merging with each other, and two DN known impurities (NFM and NFO) were not eluting from DN. In USP, EP GN API method, DN impurities were not separated from DN peak, and two DN known impurities were eluting at longer retention time, and asymmetry peak was observed.

Method development was introduced by changing different gradient programmers, different pH values of the mobile phase

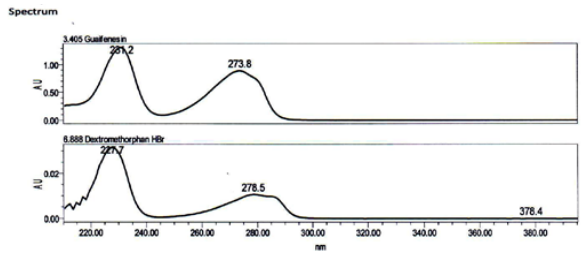


Fig 1: Optimized spectrums of Guaifensin and Dextromethorphan HBr

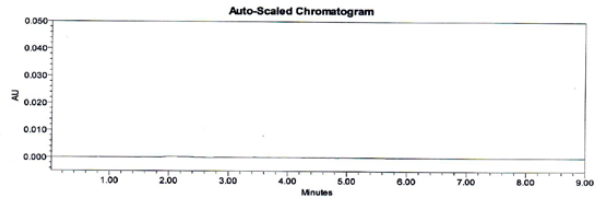


Fig 2: Blank Chromatogram

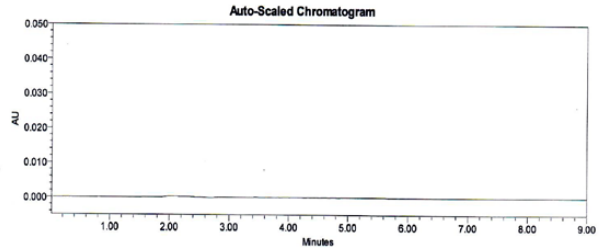


Fig 3: Placebo Chromatogram

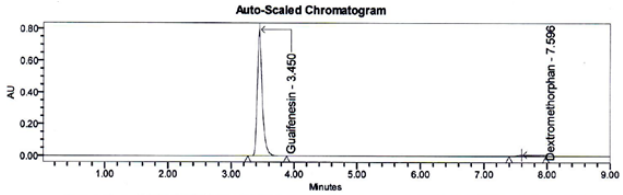


Fig 4: Optimized chromatogram of Guaifenesin and Dextromethorphan standard

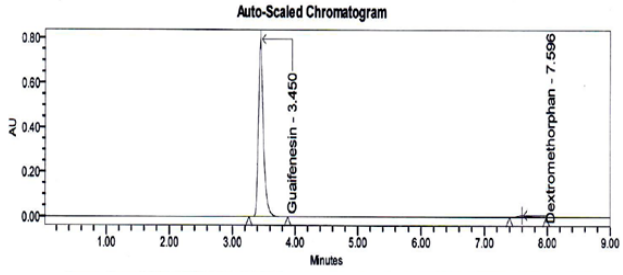


Fig 5: Optimized chromatogram of Guaifenesin and Dextromethorphan sample

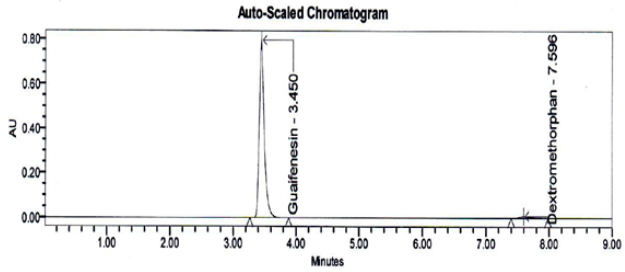


Fig 6: Chromatogram of Spiked solution of Impurities

buffer, and different columns within the literature method. Sharp peak shapes were observed with the Syncronus (C8, 250 × 4.6 mm, 5 µ particle size) column with mobile phase containing a 10 mM ammonium acetate (adjusted pH 4.30 with OPA) and acetonitrile in the ratio of 60:40 (% v/v) used for resolute isocratic chromatographic separation. The flow rate of the mobile phase was 1 mL/min with column ambient temperature, and detection was carried at 279 nm, and injection volume 10 µL was fixed for achieving good elution of eluents. Sharp peak shapes were found based on column end-capping properties of the syncronus column (high mass loading capability, excellent low pH stability, superior peak shapes, and high efficiency). Since we were using a very high concentration of GN (24,000 μgmL^{-1}), hence finalize to use syncronus column for further analytical method development and validation. The optimized chromatograms of a placebo, blank, and standard are shown in Figures 2–5.

Method Validation

To optimize the developed method, and it is subjected to method validation as per ICH guidelines. The method was validated to demonstrate that it is suitable for its intended purpose by the standard procedure to evaluate adequate validation characteristics (system suitability, specificity, accuracy, precision, linearity, robustness, ruggedness, solution stability, LoD, LoQ, and stability-indicating capability).

System Suitability

The percentage area of RSD from six replicate injections was found below 2% (diluted standard solution, 70 µg/ml of GN and 37 µg/ml of DN). Low values of RSD of replicated injections indicate that the system is precise. The results of other system suitability parameters, such as, resolution, peak tailing, and theoretical plates are presented in Table 3a. As seen from this data, the acceptable system suitability parameters would be as follows: the relative standard deviation of replicate injections is not more than 2%, the resolution between impurities 2, the tailing factor for GN and DN is not more than 1.5, and the theoretical plates are not less than 2,000. All system-suitable parameters were found to be satisfactory.

Specificity and Forced Degradation

Blank, placebo, and degradation samples were analyzed with the above mentioned HPLC conditions using a PDA detector to monitor the homogeneity and purity of the GN, DN, and their related impurities. Blank, placebo, individual impurities of GN, DN were verified and proved to be non-interfering with each other, thus, proving the specificity of the method. Figures 6 and 7 show that there is no interference at the retention time of GN, DN, and all known impurities from the other excipients. Degradation was not observed in photolytic stress, humidity, acid, base, water hydrolysis, and thermal stress studies. Significant degradation was observed in oxidative conditions. It was interesting to note that all the peaks due to degradation were well resolved from the peaks of GN, DN, and their impurities. Further, the peak purity of GN, DN, and their impurities was found to be homogeneous based

Table 3a: System suitability of Guaifenesin and Dextromethorphan HBr

No of Injections	*GN-RT*	*DN-RT*	*GN-Area*	*DN-Area*	*GN-TF*	*DN-TF*	*GN-NTP*	*DN-NTP*
1	3.452	7.596	7903452	267187	1.21	1.21	7546	9126
2	3.497	7.580	7902545	265625	1.20	1.22	7555	9123
3	3.456	7.590	7992448	265254	1.22	1.20	7576	9152
4	3.421	7.548	7901270	266462	1.22	1.22	7544	9142
5	3.480	7.561	7992548	266632	1.22	1.21	7536	9126
6	3.481	7.580	7907421	267615	1.21	1.21	7555	9135
Stdv	0.03	0.02	45915.63	899.31	0.01	0.01	13.78	11.30
Avg	3.46	7.58	7933280	266462	1.21	1.21	7552.00	9134
% RSD	0.78	0.24	0.58	0.34	0.67	0.62	0.18	0.12

Table 3b: Robustness of Guaifenesin and Dextromethorphan HBr

	% RSD of RT		*%RSD of Area*		*% RSD of Tailing*		*% RSD of NTP*	
Parameter	GN	DN	GN	DN	GN	DN	GN	DN
Optimized Method	0.78	0.24	0.58	0.34	0.67	0.62	0.18	0.12
Flow rate 0.8mL/min	0.76	0.25	0.54	0.32	0.32	0.65	0.19	0.13
Flow rate 1.2mL/min	0.74	0.27	0.52	0.36	0.65	0.67	0.16	0.12
Temperature 20°C	0.72	0.26	0.51	0.38	0.69	0.63	0.20	0.15
Temperature 30°C	0.79	0.24	0.56	0.34	0.66	0.62	0.21	0.14
Buffer pH at 3.87	0.77	0.29	0.53	0.38	0.70	0.65	0.18	0.15
Buffer pH at 4.73	0.72	0.23	0.54	0.39	0.65	0.61	0.17	0.12
Organic phase +10%	0.71	0.26	0.59	0.37	0.64	0.61	0.19	0.16
Organic phase -10%	0.74	0.25	0.51	0.32	0.63	0.65	0.17	0.13

	Peak Name	Retention Time (min)	Area	% Area	Height	USP Plate Coun	USP Tailing	Purity1 Flag	Purity1 Angle	Purity1 Threshold	USP Resolution
1	Guaifensin-IMP-B	3.126	2702	0.07	411	6249	0.9	Yes	32.887	25.848	
2	Guaifensin	3.528	3500936	93.58	86736	8671	1.2	No	0.097	0.278	2.5
3	Guaifensin-IMP-A	5.618	55951	1.50	7139	11993		No	1.139	1.511	11.3
4	Dextro-IMP-A	5.873	7787	0.21	1088	9964		No	5.955	7.542	
5	Dextromethorphan HBr	6.807	101352	2.71	8900	8729	1.5	No	1.663	1.954	
6	Guaifensin-IMP-C	9.237	16183	0.43	1284	13040	1.1	No	6.269	7.497	7.7
7	Dextro-IMP-C	11.913	6549	0.18	328	8400	1.1	No	11.185	13.305	6.2
8	Guaifensin-IMP-D	14.991	29618	0.79	1465	13876	1.2	No	6.654	6.895	5.8
9	3-methoxy N-fromyl m	21.837	6442	0.17	221	18726	0.7	No	3.861	3.983	9.5
10	N-formyl Octabas1	30.427	4705	0.13	190	50201	1.1	No	37.609	50.231	11.4
11	N-formyl Octabase-2	31.411	8818	0.24	251	10028	1.0	No	29.914	34.658	1.1

Fig 7: Chromatogram of purity level

on the evaluation parameters such as purity angle and purity threshold using Waters Empower Networking software. The verification of peak purity indicates that there is no interference from degradants, facilitating error-free quantification of GN and DN impurities. Hence, the method is considered to be "stability-indicating." The specificity results were shown in Tables 4a and 4b.

Precision

The six homogeneous test solutions of % RSD data of GN, DN was within 2%. The results obtained in the intermediated precision study was found to be 0.5% RSD high precision of the method. The results are shown in Table 5.

Accuracy

Recovery of GN and DN was found to be 98 to 102%. The summary of % recovery for individual data was mentioned in Tables 6a and 6b.

Linearity

Linear calibration plots are tested at different concentration levels. The correlation coefficient obtained was greater than 0.997 for all the components. The slope and y-intercept values were also provided in Tables 7a and 7b, which confirmed good linearity between peak areas and concentration. The linearity graphs were shown in Figures 8a and 8b.

LoD and LoQ

The LoD and LoQ of GN and DN were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively. The determined limit of detection, the limit of quantification, from precision data at LoQ level of GN, DN was obtained, and the result was shown in Table 8.

Robustness

No significant effect was observed on system suitability parameters deliberate change such as resolution, RSD, tailing factor, or the theoretical plates of GN, DN. The results were presented in Table 3b, along with the system suitability parameters of normal conditions. Thus, the method was found to be robust with respect to variability in applied conditions.

CONCLUSIONS

The HPLC method developed and validated for the simultaneous determination of GN and DN in pharmaceutical dosage form

Table 4a: Specificity and Forced degradation data for Guaifenesin

Degradation condition	*% Assay*	*% Degraded*
Control sample	100.0	--
Sample	99.1	--
Acid stress (1N HCl 2Hrs-60°C)	99.0	1
Base stress (1N NaOH 2Hrs-60°C)	100.5	-0.5
Peroxide stress (10% H2O2 2Hrs-60°C)	96.5	3.5
Humidity (85% RH 72Hrs)	102.4	-2.4
Refluxed water (12 hours at 60°C)	101.3	-1.3
Thermal stress (60°C-72 Hrs)	98.2	1.8
U.V stress (200-400 nm -72 Hrs)	98.6	1.4
Fluorescent (200 Watts /hr and 1.2 million lux/hr)	101.5	-1.5

Table 4b: Specificity and Forced degradation data Dextromethorphan HBr

Degradation condition	*% Assay*	*% Degraded*
Control sample	98.7	--
Sample	97.1	--
Acid stress (1N HCl 2Hrs-60°C)	96.9	1.8
Base stress (1N NaOH 2Hrs-60°C)	100.1	-1.4
Peroxide stress (10% H2O2 2Hrs-60°C)	95.7	3
Humidity (85% RH 72Hrs)	103.5	-4.8
Refluxed water	99.9	-1.2
Thermal stress (60°C-72 Hrs)	103.9	-5.2
U.V stress (200 Whats /hr)	97.9	0.8
Fluorescent (1.2 million lux/hr)	98.0	0.7
Photo stress	99.0	-0.3

Table 5: Inter day and Intra day Precision

	Inter day		*Intra day*	
S.NO	*GN*	*DN*	*GN*	*DN*
1	101.0	100.1	101.0	100.1
2	101.5	100.2	101.5	100.2
3	101.6	99.9	101.6	99.9
4	100.8	100.8	100.8	100.8
5	100.5	100.7	100.5	100.7
6	99.9	100.6	99.9	100.6
Stdv	0.6	0.4	0.6	0.4
Avg	100.9	100.4	100.9	100.4
% RSD	0.6	0.4	0.6	0.4

Table 6a: Guaifenesin Accuracy and Recovery:

Spiked concentration level	*Placebo added in mg*	*API added in mg*	*% Assay*	*% RSD*
50	540	1200	51.0	0.66
10	540	2400	101.8	0.55
150	540	3600	150.2	1.00

Note * recovery study was carried by triplicate sample analysis

Table 6b: Dextromethorphan Hydrobromide Accuracy and Recovery:

Spiked concentration level	*Placebo added in mg*	*API added in mg*	*% Assay*	*% RSD*
50	540	60	50.1	0.9
10	540	120	100.2	0.5
150	540	180	151.0	1.1

Note * recovery study was carried by triplicate sample analysis

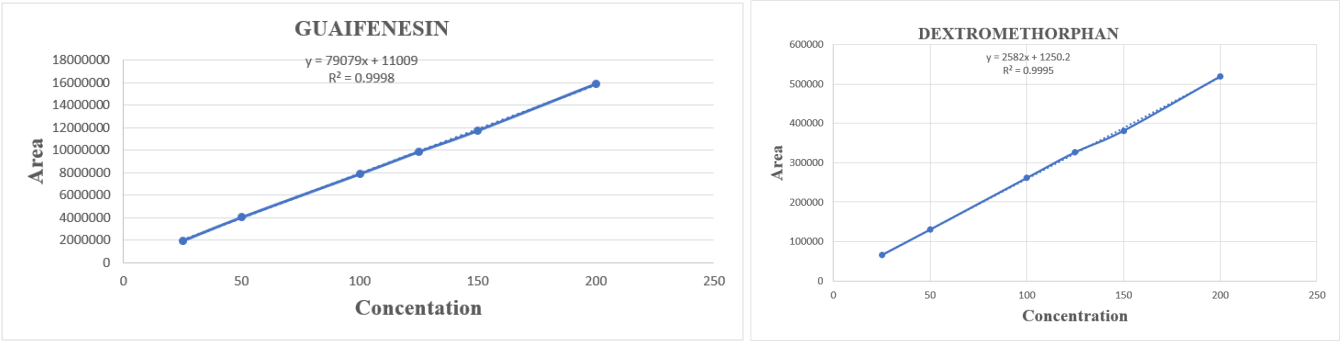


Fig 8: Linearity graph of Guaifenesin and Dextromethorphan HBr

Table 7a: Linearity data of Guaifenesin and Dextromethorphan Hydrobromide

Parameter	*GN*	*DN*
Calibration range (µg mL^{-1})	25-150	25-150
Linearity concentration range ppm	357-1428	19-75
Intercept	y = 2582x + 1250.2	y = 2582x + 1250.2
Correlation coefficient	0.9998	0.9995

Table 7b: Linearity concentration levels data of Guaifenesin of Dextromethorphan Hydrobromide

S.No.	*GN Concentration Level*	*Concentration PPM*	*Area response*
1	25	357	1962754
2	50	535	4051272
3	100	714	7903448
4	125	892	9878912
5	150	1071	11758241
6	200	1428	15912471
S.No.	*DN Concentration Level*	*Concentration PPM*	*Area response*
1	25	19	65321
2	50	28	130250
3	100	38	261688
4	125	47	326954
5	150	56	381632
6	200	75	519965

Table 8: LoD and LoQ result

Parameter	*GN*	*DN*
Detection limit (µg mL^{-1})	0.01	0.506
Quantitation limit (µg mL^{-1})	0.03	1.534

was precise, accurate, and specific. The method is validated as per ICH guidelines, and found to be specific, precise, linear, accurate, rugged, and robust. The developed method can be used for the regular analysis and stability analysis of GN and DN, either individually or in their combination dosage forms.

ACKNOWLEDGMENTS

The authors wish to thank the management of Pellets Pharma Ltd., and CSIR IICT, Hyderabad group for supporting this work. The authors wish to acknowledge the formulation development group for providing the samples for their research. They would also like to thank colleagues in bulk manufacturers for providing chemicals and impurity standards for research work.

REFERENCES

1. Available at https://www.drugbank.ca/drugs/DB00874.
2. Gupta VD, Ghanekar AG. Quantitative Determinations of Codeine Phosphate, Guaifenesin, Pheniramine Maleate, Phenylpropanolamine Hydrochloride, and Pyrilamine Maleate in an Expectorant by High-Pressure Liquid Chromatography. J of pharm sci 1977;66(6):895-7.
3. Heidemann DR. Rapid, stability-indicating, High-Pressure Liquid Chromatographic determination of theophylline, guaifenesin, and benzoic acid in liquid and solid pharmaceutical dosage forms. J of pharm sci 1979; 68(4):530-2.
4. Muhammad N, Bodnar JA. Quantitative determination of guaifenesin, phenylprolamine hydrochloride sodium benzoate codeine phosphate in cough syrups by High-Pressure Liquid Chromatography. J of Liq Chrom 1980;3(1):113-22.
5. Available at https://www.drugbank.ca/salts/DBSALT000376.
6. Mahesh P, Swapnalee K, Aruna M, Anilchandra B, Prashanti S. Analytical method development and validation of acetaminophen, caffeine, phenylephrine hydrochloride and dextromethorphan hydrobromide in tablet dosage form by RP-HPLC. Inventi J of Pharm Sci Invention 2013; 2:9-15.
7. Kukanich B, Papich MG. Plasma profile and pharmacokinetics of dextromethorphan after intravenous and oral administration in healthy dogs. J of veterinary pharmacol and therapeutics 200;27(5):337-41.
8. Green JM. Peer reviewed: a practical guide to analytical method validation. Analytical chemistry 1996 ;68(9):305A-9A.
9. Bari SB, Kadam BR, Jaiswal YS, Shirkhedkar A. Impurity profile: significance in active pharmaceutical ingredient. Eurasian journal of analytical chemistry 2007;2(1):32-53.
10. Guideline ICH. Validation of analytical procedures: text and methodology Q2 (R1). In International conference on harmonization; Geneva Switzerland: 2005:11-12).
11. ICH DR. Stability testing of new drug substances and products Q1A (R2). In Proceedings of the International Conference on Harmonization; Geneva: 2003.
12. Grosa G, Del Grosso E, Russo R, Allegrone G. Simultaneous, stability indicating, HPLC-DAD determination of guaifenesin and methyl and propyl-parabens in cough syrup. J of pharm and biomed anal 2006 ;41(3):798-803.

13. Reddy SP, Babu KS, Kumar N, Sekhar YS. Development and validation of stability indicating the RP-HPLC method for the estimation of related compounds of guaifenesin in pharmaceutical dosage forms. Pharm methods 2011;2(4):229-34.
14. Senthilraja.M and Giriraj. P, Reverse Phase HPLC method for the simultaneous estimation of terbutanile sulphate, bromhexine HCl and guaifenesin in cough syrup, Asian Journal of Pharmaceutical and Clinical Research 2011;4(2):13–15.
15. Wilcox ML, Stewart JT. HPLC determination of guaifenesin with selected medications on underivatized silica with an aqueous-organic mobile phase. J of pharm and biomed anal 2000;23(5):909-16.
16. Ozdemir A, Aksoy H, Dinç E, Baleanu D, Dermis S. Determination of guaifenesin and dextromethorphan in a cough syrup by HPLC with fluorometric detection. Revue Roumaine de Chimie 2006;51(2):117.
17. Demian I. High Performance liquid chromatography (HPLC) chiral separations of guaifenesin, methocarbamol, and racemorphan. Chirality 1993;5(4):238-40.
18. Suzen S, Akay C, Cevheroğlu S. Simultaneous determination of guaiphenesin and codeine phosphate in tablets by high-performance liquid chromatography. Il Farmaco 1999;10(54): 705-709.
19. Amer SM, Abbas SS, Shehata MA, Ali NM. Simultaneous determination of phenylephrine hydrochloride, guaifenesin, and chlorpheniramine maleate in cough syrup by gradient liquid chromatography. J of AOAC International 2008;91(2):276-84.
20. GalliV, Barbas C. High-performance liquid chromatographic analysis of dextromethorphan, guaifenesin and benzoate in a cough syrup for stability testing. J of Chrom A 2004;1048(2):207-11.
21. Zhang G, Terry Jr AV, Bartlett MG. Sensitive liquid chromatography/tandem mass spectrometry method for the simultaneous determination of olanzapine, risperidone, 9-hydroxyrisperidone, clozapine, haloperidol and ziprasidone in rat brain tissue. Journal of Chromatography B. 2007;858(1-2):276-81.
22. Gudipati RM, Wallace JE, Stavchansky SA. High performance liquid chromatography determination of guaifenesin in dog plasma. Analytical letters 1991;24(2):265-74.
23. Ge QH, Zhou Z, Zhi XJ, Ma LL, Ding CG. Simultaneous determination of guaifenesin, bromhexine and ambroxol in human plasma by LC-MS/MS and its pharmacokinetic studies. Chinese Pharm J 2009;44(13):1025-8.
24. The United States Pharmacopeia, USP35-NF30:3829–3837.
25. European Pharmacopeia,7.0:1821-1822&2128-2129.