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Simultaneous Estimation of Acetaminophen, Phenylephrine HCl, Guaifenesin and Dextromethorphan HBr In Reverse Phase Ultra Performance Liquid Chromatography.

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ABSTRACT

A Novel, rapid, specific reversed phase UPLC method has been developed and validated for simultaneous determination of Acetaminophen, Phenylephrine HCl, Guaifenesin and Dextromethorphan HBr in pharmaceutical dosage form. Chromatographic separation of these four Active pharmaceutical ingredients was carried out on a C18 reversed phase column (100 x 2.1 mm, 1.7µm) using a simple gradient program for 13 minutes with mobile phase-A consisting a buffer (18.3mM phosphoric acid and 4.94mM 1-heptane sulfonic acid sodium salt) and water in ratio 95:5 v/v, mobile phase-B,a buffer (mixture of 18.3mM phosphoric acid and 4.94mM 1-heptane sulfonic acid sodium salt) and water in ratio 20:80, mobile phase flow rate used constantly at 0.3 mL / minute. The chromatography analysis was monitored at 214 nm with column oven temperature at 45° C and injection volume as 2.0 µL. All the components were separated with good resolution. The proposed method has been validated according to ICH guidelines, the method was proven it to be robust, precise, accurate and linear over a range of analysis.

Keywords: Liquid Chromatography, Acetaminophen, Phenylephrine, Guaifenesin and Dextromethorphan HBr, Active Pharmaceutical Ingredients, ICH Guidelines.

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INTRODUCTION

The combinations of analgesic, decongestant and cough preparations are widely used for cough and cold treatments. Acetaminophen [1] chemically named as N–(4-hydroxyphenyl) acetamide as shown in Fig 1,a is a widely used analgesic and antipyretic drug that is used for the relief of fever, headaches, and other minor aches and pains. It is a major ingredient in numerous cold and flu medications and many prescription analgesics. Acetaminophen is used on its own or in combination with Pseudoephedrine, Dextromethophan[4], Chlorpheniramine, Phenylephirine HCl, Doxylamine, Codeine, Guaifenesin, Hydrocodone or Oxycodone. The chemical formula is C8H9NO2 and its molecular weight is 151.16256 g/mol. There were several HPLC methods proposed for estimation of Acetaminophen and its impurities alone or in combination. In literature survey, the methods to estimate Acetaminophen alone [6] and in combination with other compounds including Guaifenesin, Dextromethorphan HBr [5] are developed by using liquid chromatography. Phenylephrine [1] chemically named as 3-[(1R)-1-hydroxy-2-(methylamino)ethyl] phenol as shown in Fig 1, b is a powerful vasoconstrictor, which is used as nasal decongestant and cardiotonic agent. The molecular formula of phenylephrine hydrochloride is C9H13NO2.HCl with the molecular weight 167.205 g/mol. Several HPLC methods are proposed for estimation of Phenylephrine in combination with other compounds. Literature survey shows that Phenylephirine is estimated in combination with other compounds like Guaifenesin, Chlorophenariamine maleate[7], with Paracetamol, Chlorpheniramine Maleate, Dextromethorphan Hydrobromide[8], Caffine and Nimusalide [9], by using liquid chromatography method. Guaifenesin [1], chemically named as 3-(2-methoxyphenoxy) propane-1,2-diol as shown in Figure 1 c is an expectorant. It is used to reduce chest congestion caused by the common cold, infections, or allergies and it helps loosen congestion in chest and throat, making it easier to cough out through the mouth. The molecular formula is C10H14O4. There were several HPLC methods proposed for estimation of Guaifenesin, in combination with other compounds like Propylparabens [12], and with Pseudoephidrine Hydrochloride, and there was a LCMS method proposed in combination with Dextromethorphen [13]. Dextromethorphan [1], chemically named as "(9α,13α,14α)-3-Methoxy-17-methylmorphinan hydrobromide as shown in Fig 1, d suppresses the cough reflex by a direct action on the cough center in the medulla of the brain. It shows high affinity binding to several regions of the brain, including the medullary cough center. The molecular formula is C18H25NO.HBr. There were several HPLC methods reported for estimation of Dextromethorphan. In literature survey Dextromethorphan and its metabolites are estimated by liquid chromatography [10], it is estimated in combination with other compounds like Guaifenesin and Sodium benzoate liquid chromatography [4], and there was a capillary electrophoresis method reported in combination of Diphenlhydramine and Phenylephrine [11]. There were no methods reported in the literature survey for simultaneous estimation of Acetaminophen, Phenylephrine HCl, Guaifenesin and Dextromethorphan HBr using UPLC. Therefore, herein we reported the UPLC method and validated the method according to ICH guidelines [2].

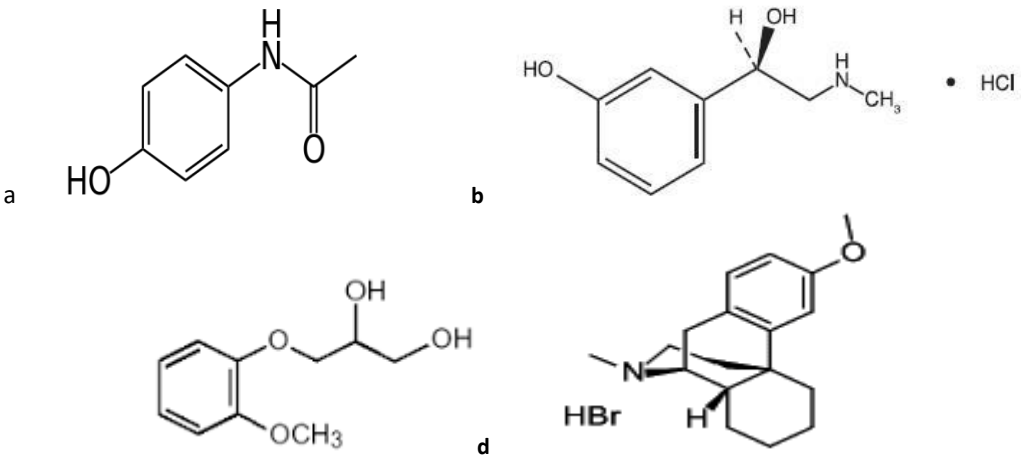

Figure 1 Chemical structures of compounds; a) Acetaminophen b) Phenylephrine HCl c) Guaifenesin d) Dextromethorphan HBr

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EXPERIMENTAL

Chemicals and Reagents: HPLC grade of Acetonitrile is from Acros (New Jersey, USA), 1-Heptane-sulfonic acid is from Sigma Aldrich and phosphoric acid is from Labchem (Pittsburgh, PA, USA). DI water was used for the mobile phase preparation. Drug product Acetaminophen, Phenylephrine HCl, Guaifenesin and Dextromethorphan HBr was procured from pharmacies.

Samples and Solutions

Working Standard Solution Preparation: Standard solutions at a concentration of about 50 µg / mL of Acetaminophen, 33 µg / mL of Phenylephrine HCl, 32 µg / mL of Guaifenesin and 64 µg / mL of Dextromethorphan were prepared by dissolving the appropriate amount of standard in the water.

Sample Stock Solutions: Sample stock solutions were prepared by taking representative sample equivalent to 520 mg, 320 mg, 8 mg and 16 mg of Acetaminophen, Gauifenesin, Phenylephrine HCl and Detromethorphan HBr respectively (prepared from Maximum Strength Day time Cold Relief Liquid product) in 100 mL volumetric flask, dissolved and diluted to volume with Diluent (consist of 95:5 V/V ratio of DI water and Acetonitrile).

Working Sample Preparation for Acetaminophen and Guaifenesin: Working samples were prepared by taking 1 mL of sample stock and diluted with diluent to 100mL to get required concentration according to standard.

Working Sample Preparation for Phenyephirine HCl and Dextromethorphan HBr: Working samples were prepared by taking 10 mL of sample stock and diluted with diluent to 25mL.

Equipments and experimental conditions: The UPLC system was Waters Acquity (Milford, USA) equipped with binary solvent mangers, Ultra fast auto-sampler and a UV visible Detector used to make sample injections. A reversed phase C18 column (100 x 2.1 mm dimension, 1.7 µm particle size) was used for analysis with column temperature at 45ºC. Mobile phase –A consist a buffer (mixture of 18.3 mM phosphoric acid and 4.94 mM 1-heptane sulfonic acid sodium salt) and water in ratio 95:5 v/v and mobile phase-B consist a buffer and water in ratio 20:80 and delivered at 0.3 mL/Min. The Sample injection volume used as 2.0 µL and monitored at Detector wavelength 214nm.

Time	Mobile Phase-A	Mobile Phase-B	Curve
0.5	90	10	0
5.0	90	10	0
6.0	20	80	1
2.0	90	10	0

Linearity: Linearity solutions were prepared from Standard stock solution at five different concentrations levels ranging from 50-150% of assay sample solution. The responses were measured as peak areas and plotted against concentration.

Specificity: Forced degradation studies were performed to demonstrate selectivity and stability-indicating capability of the proposed method. The sample and Placebo were exposed to acid (0.5N HCl, 60 min at 60ºC), base (0.5N NaOH, 60 min at 60ºC), strong oxidation (5% H_2O_2 for 50 min at room temperature), thermal (105ºC, 7days), humidity (90% RH, 25ºC, 7 days) and photolytic (1.2million lux h, 200 wh/m^2, 7 days) degradation conditions. Samples were withdrawn at appropriate times and subjected to HPLC analysis after dilution equal to sample solution concentration to evaluate the ability of the proposed method to separate analytes from its impurities and placebo. Photo diode array detector was employed to check and ensure the homogeneity and purity of each analyte peak in all the stressed sample solutions.

Precision:

System Precision: For evaluation of System Precision the working standard solution was used by injecting 6 injections.

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Method Precision: Method precision was performed by injecting six independent assay preparations of sample against working standard and calculated the % RSD.

Intermediate Precision (Ruggedness): Intermediate Precision (Ruggedness) was performed by a different Chemist by injecting six independent assay preparation of sample against working standard on different day, different UPLC system, different column.

Accuracy: The recovery experiments were carried out by preparing the solutions at 50, 100 and 150% to the test concentration, i.e., equivalent to 520 mg, 320mg, 8mg and 16mg of Acetaminophen, Gauifenesin, Phenylephrine HCl and Dextromethorphan HBr, respectively.

Robustness: The robustness of the method was determined as a measure of the analytical method capability to be unaffected by small deliberate variations in method parameters. The different variations such as variation in flow rate by ± 0.02 mL/Minute; variation in wavelength by ± 2nm, variation in column temperature by ± 5 ºC and variation in composition of mobile phase by ± 5 % absolute (in terms of Organic component). The system suitability was evaluated in each varied condition.

Stability of the Solution: Sample solution stability was established by storage of sample solution at ambient temperature (25ºC) for 48 hours.

Limit of Detection (LOD) and Limit of Quantification (LOQ): The LOD and LOQ of Analytes were established by using signal to Noise ratio approach as defined in ICH guidelines [2]

RESULTS AND DISCUSSION

Method Development and Optimization: During literature survey there is no stability indicating UPLC assay method proposed for simultaneous estimation of Acetaminophen (ACE), Phenylephrine Hydrochloride (PHE), Guaifenesin (GUA) and Dextromethorphan Hydrobromide (DEX). The present work was aimed to develop a stability indicating method by UPLC for determination of targeted analytes. After trying different columns the final choice for the stationary phase that provided satisfactory resolution and run time was the reverse phase BEH C18 (100*2.1mm 1.7µm) column. Dextromethorphen peak was retaining more in column, hence to elute this peak within 10 minutes of run time, different acetonitrile ratios were tested in mobile phases. The target achieved with good separation was with mobile phase-A consisting 95:5 of Buffer : Acetonitrile and with mobile phase-B consisting 20:80 of Buffer : Acetonitrile. A detection wavelength 214nm was selected where all components give a similar response. A typical chromatogram of standard illustrating the separation is shown in Figure 2 and the system suitability data were presented in Table 1.

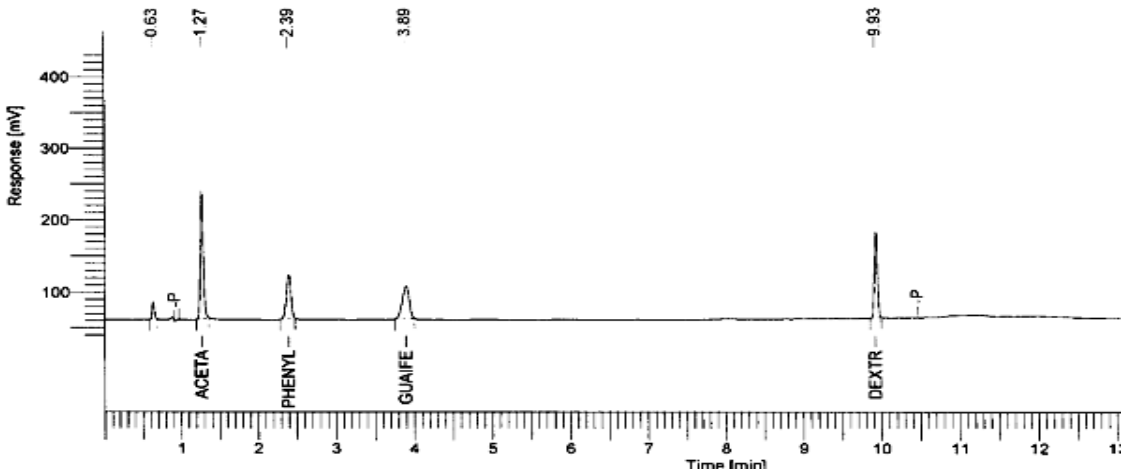

Figure-2 Typical Chromatogram for Standard

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Table 1 chromatographic System Suitability Data

Compound Name Replicates	t_R Retention time (Min)	Tailing factor	% RSD for 6
ACE	**1.3**	**1.2**	**0.4**
PHE	**2.4**	**1.0**	**0.8**
GUA	**3.9**	**1.0**	**1.0**
DEX	**9.9**	**1.1**	**0.7**

Analytical Parameters and Validation: After satisfactory method development, the product was subjected to method validation according to ICH guidelines [2]. The method was validated to demonstrate its suitability for intended purpose using the standard procedure and the validation characteristics including specificity, accuracy, precision, ruggedness, robustness, LOD, LOQ, Linearity and stability of solution were evaluated.

System Suitability: The main purpose to perform system suitability was to check the percentage Relative Standard Deviation for an average area of six replicate injections of working standard. The % RSD was found below 1.0 and the Tailing factor for all the analyte peaks was found not more than 2.0.

Specificity: There was no interference from the blank (Fig-3) observed in the chromatogram. The forced degradation data revealed that there was no interference from degradation impurities at retention time of targeted peaks. The Purity data for each analyte peak shows the peak is pure and there were no co-eluting peaks. Hence method proved to be specific.

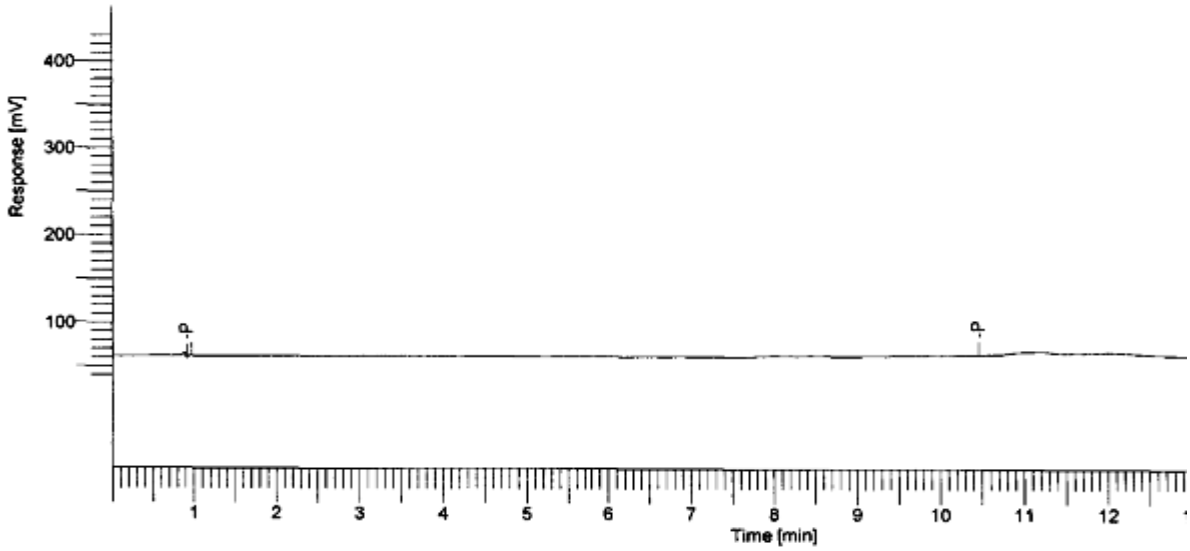

Figure 3 Typical Chromatogram for Blank

LOD and LOQ: The concentration with Signal to Noise ratio of at least 3 was taken as LOD and Concentration with Signal to Noise ratio of at least 10 was taken as LOQ, which meets the criteria defined by ICH guidelines [2] Q2 (R1). The LOD and LOQ results for each analyte peak were presented in Table 2.

Linearity: The linearity study was executed by injecting analytes at 5 different concentrations levels, i.e., 50, 75, 100, 125 and 150% to the target concentration, i.e., 50 µg/mL for Acetaminophen, 32µg/mL for Phenylephirine HCl, 33µg/mL for Guaifenesin and 64µg/mL for Dextromethorphen HBr. The correlated coefficient was calculated and found greater than 0.999 indicating magnificent correlation between the analyte concentration and peak area. The slope, Y intersept, and regression coefficient (R^2) results were presented in Table 2.

Accuracy: The accuracy results were expressed in terms of mean percentage of Assay. The percentage recoveries obtained from triplicate sample assay were found in a range of 98.6 to 101.3. The results were presented in Table 2.

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Table 2 Summary of Validation Parameters

Parameter	ACE	PHE	GUA	DEX
Linearity range				
Slope	0.00224	0.00406	0.00369	0.00327
Y Intersept	-1.599	1.1838	-0.2023	2.1143
R^2	0.9998	0.9999	0.9997	0.9999
Accuracy (% Recovery)				
50%	100.1	99.7	99.4	99.9
100%	101.3	100.0	101.0	100.0
150%	98.6	101.4	100.3	99.9
LOD µg /mL	2.3	3.3	3.5	4.9
LOQ µg /mL	6.2	11.1	11.9	14.7

Precision and Intermediate Precison (Ruggedness): The values of the relative standard deviation for six replicate injections of the standard solution containing all the analytes of interest are well within the limits i.e % RSD < 2.0, indicating the system is precise. The values of the % relative standard deviation for sample repeatability also are well within 2.0 indicating the sample repeatability of the method. Also the values of the relative standard deviation for second chemist are well within 2.0 indicating the method is rugged. The results are presented in Table 3. The typical chromatograms for samples are presented in Figure 4 and 5.

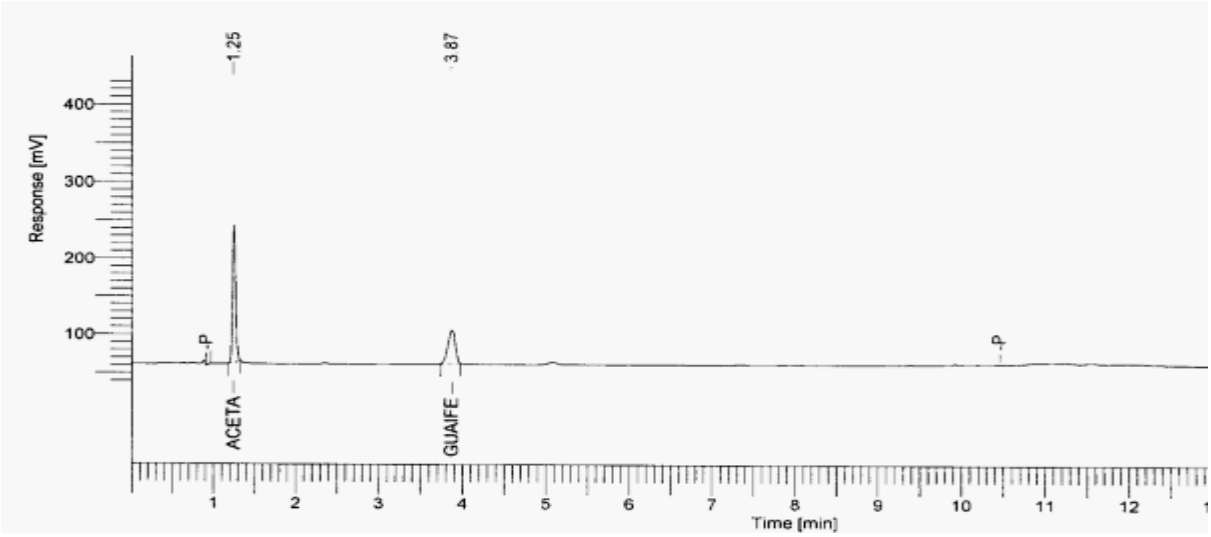

Figure 4 Typical Chromatogram for Acetamonphen and Guaifenesin Sample

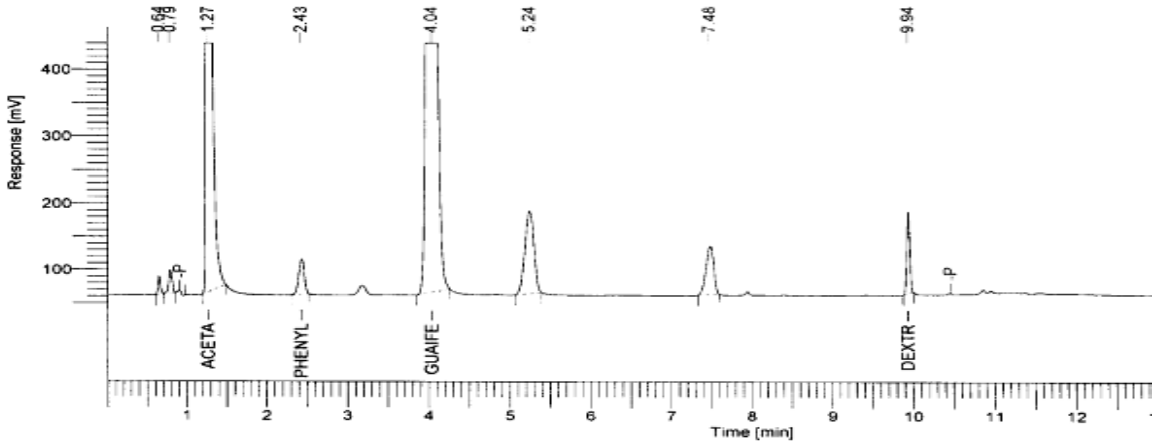

Figure 5 Typical chromatogram for Phenylephrine and Dextromethorphan Sample

Table 3 Compiled Data of Method Precision and Ruggedness.

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S.No	% Assay of							
	ACE		PHE		GUA		DEX	
	I	II	I	II	I	II	I	II
1	100.53	98.83	98.14	98.09	99.96	99.26	96.28	101.88
2	98.88	99.29	97.16	98.52	98.60	99.90	99.83	100.51
3	99.22	99.08	96.99	98.32	98.76	99.45	99.17	100.91
4	98.09	99.77	97.38	96.84	98.44	100.53	100.76	98.61
5	98.29	98.60	96.24	98.09	97.98	99.13	100.54	99.79
6	99.95	96.87	98.24	99.50	99.53	97.42	99.96	101.34
Mean	99.2	98.7	97.4	98.2	98.87	99.28	99.42	100.51
& RSD	0.9	1.0	0.8	0.9	0.7	1.1	1.7	1.2

I : First Chemist, II: Second Chemist.

Robustness: In all robustness conditions the relative standard deviation for replicate standard injections was found less than 2.0%. The peak shapes are found very well within tailing factor of 2.0. So, the method was found to be robust with respect to variability in all-robust conditions.

Stability of Sample Solution: The results of solution stability for standard and samples were found to be stable up to 48 hours at room temperature 25 ± 2 ºC.

Method application: The UPLC method is novel, rapid and sensitive for the quantitative determination of Acetaminophen, Phenylephrine HCl, Guaifenesin and Dextromethorphan HBr Cough Oral solution.

CONCLUSIONS

A rapid specific gradient UPLC method has been developed for the simultaneous determination of Acetaminophen, Phenylephrine HCl, Guaifenesin and Dextromethorphan HBr using UV-VIS detector. The method was validated for specificity, accuracy, precision, linearity, robustness and solution stability according to ICH guidelines. The method uses a simple mobile phase composition, easy to prepare. The method has short run time of 13 minutes and relatively low flow rate (0.3 mL/Minute) allowing the analysis of large number of samples with less mobile phase that proving to be cost effective. Hence this UPLC method can be used for the routine samples.

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