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Der Pharmacia Lettre, 2015, 7 (7):329-339
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

A novel stability indicating RP-HPLC method development and validation for simultaneous estimation of phenylephrine, acetaminophen, guaifenesin and dextromethorphan in tablet dosage form

Vijay Kumar Rekulapally[1]* and Vinay U. Rao[2]

[1]Department of Pharmaceutical Sciences, JNTU, Hyderabad, India
[2]Dr. Reddy's Laboratories Ltd., Bachupally, Hyderabad, India

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ABSTRACT

A novel simple, sensitive, accurate and precise RP-High Performance Liquid Chromatography (HPLC) method for the simultaneous estimation of Phenylephrine (PHE), Acetaminophen (ACE), Guaifenesin (GUA) and Dextromethorphan (DEX) combined dosage form has been developed and validated. The components were well separated using Altima, 150 x 4.6 mm, 5µ column with 1ml of Conc. Orthophosphoric acid in a 1000ml of water as Solvent A and Acetonitrile as Solvent B at a flow rate of 1.0 mL/min by using gradient programme. The eluents were detected at 272 nm using UV detector. The retention time of PHE, ACE, GUA and DEX found to be 2.5, 6.1, 8.3 and 9.0 min respectively. The linearity was observed between 2.0-7.0µg/mL,130-455µg/mL, 50-300µg/mL and 25-15 µg/mL for Phenylephrine, Acetaminophen, Guaifenesin and Dextromethorphan respectively. The method was validated for system suitability, specificity, linearity, accuracy, precision, ruggedness and robustness as per ICH guidelines and the results were found to be within the limits. The developed method was used for the stability studies and for the routine quality control testing of PHE, ACE, GUA and DEX combined dosage form.

Keywords: Phenylephrine, Acetaminophen, Guaifenesin & Dextromethorphan respectively, HPLC, Tablet formulation.

INTRODUCTION

Acetaminophen (ACE) chemically 4-hydroxy acetanilide known as paracetamol is an acetanilide derivative analgesic, antipyretic and weak anti-inflammatory action(1,2) and also administered in the management of more severe pains in advanced cancers(3).

Guaifenesin (GUA), chemically (+)-3-(2-methoxyphenoxy)-propane-1,2- diol, is a widely used expectorant, useful for the symptomatic relief of respiratory conditions(4-5).

Dextromethorphan Hydrobromide (DEX) chemically designed as {ent-3-methoxy-9a- methyl morphinan Hydrobromide monohydrate}, it is a cough suppressant(4-5).

Phenylephrine Hydrochloride designed chemically as (R)-1-(3-hydroxyphenyl)-2- methylaminoethanol hydrochloride, it is a decongestant(4-6).

Literature survey revealed that there are no method is available for their simultaneous estimation of ACE, GUA, DEX and PHE in bulk and in pharmaceutical preparations where as several methods were reported for the estimation of these compounds individually as well as in combination with some other drugs. Hence an attempt has been made to develop a novel, simple, precise, accurate and specific RP-HPLC method for the simultaneous determination of ACE, GUA, DEX and PHE in bulk and in pharmaceutical dosage forms because HPLC methods have been widely used for routine quality control assessment of drugs, because of their accuracy, repeatability, selectivity, sensitivity and specificity. This method is validated in accordance with International conference in Harmonization (ICH) guidelines(10-11).

MATERIALS AND METHODS

Material and chemicals

ACE, GUA, DEX and PHE tablets were received from Spectrum laboratories Hyderabad. HPLC grade Acetonitrile, methanol ,water and ortho phosphoric acid from Merck Germany and nylon filter from Millipore Pvt. Ltd, Bangalore, India were used for study.

Instrumentation

A Waters HPLC system with a DAD (2996 detector and 2695 separation module with quaternary gradient) was used for method development and method validation. The output signal was monitored and processed using Waters Empower software. Weighing was performed with a Mettler XS 205 dual range (Mettler-Toledo GmbH, Greifensee, Switzerland).

chromatographic conditions

The mobile phase A prepared by using 1ml Concentrated Orthophosphoric acid in 1000ml of milli-Q water and degas to sonicate finally filtered through nylon 0.45 µm membrane filter Mobile phase B consisted of Acetonitrile. The flow rate was 1.0 ml/min with a timed gradient programme time/A% is 0/88, 3/88, 10/15, 10.5/88, 13/88 , in a C18 column Altima, 150 x 4.6 mm, 5µ column .The effluents were monitored at 272 nm with 10 µL Injection volume.

Preparation of Standard Solutions

Accurately Weighed and transferred 5mg, 32.5mg, 20mg& 5mg of Phenylephrine, Acetaminophen, Guaifenesin & Dextromethorphan working Standards into a 50 ml, 10ml, 10ml and 50ml clean dry volumetric flasks, add 25ml diluent in 50 ml volumetric flask and 7ml of diluent in 10 ml volumetric flask respectively, sonicate for 30 minutes and make up to the final volume with diluents. (5µg/ml Phenylephrine, 325µg/ml Acetaminophen, 200µg/ml Guaifenesin &10µg/ml Dextromethorphan) From the above stock solution 0.5 ml of Phenylephrine, 1ml of Acetaminophen, Guaifenesin and Dextromethorphan was pippeted out in to a 10ml volumetric flask and then make up to the final volume with diluent.

Figure 1: Overlaid chromatogram with different column

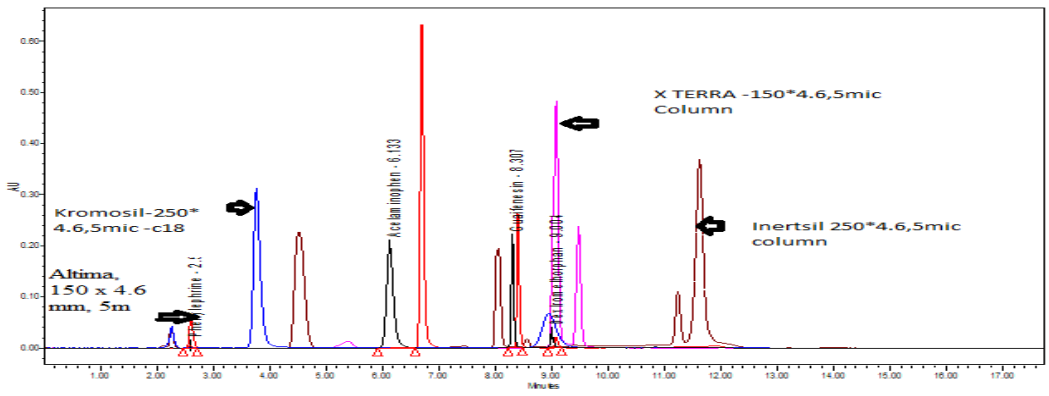


Figure2: Standard chromatogram

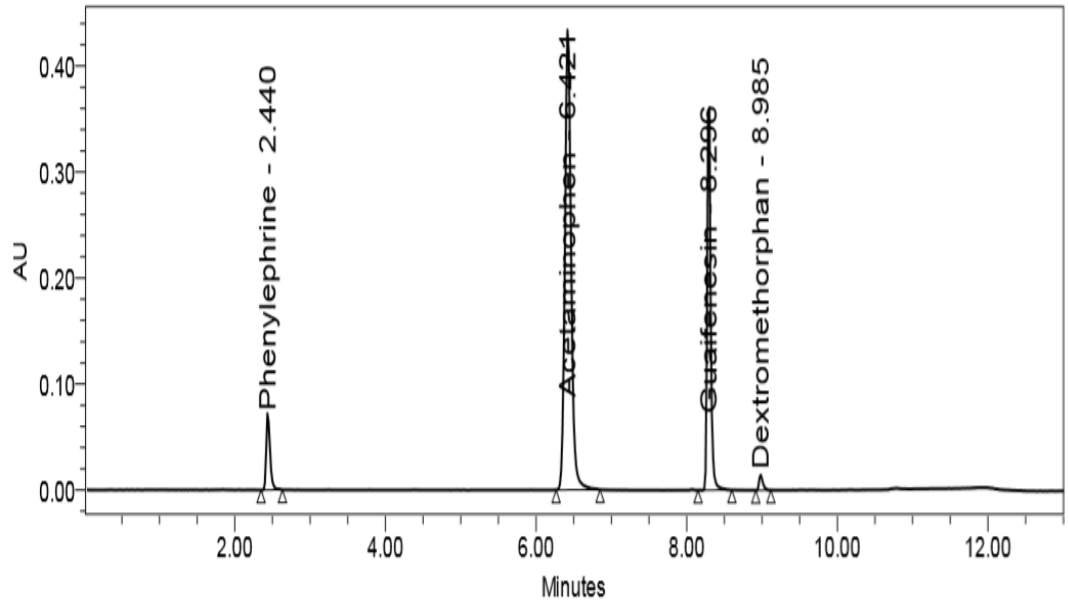
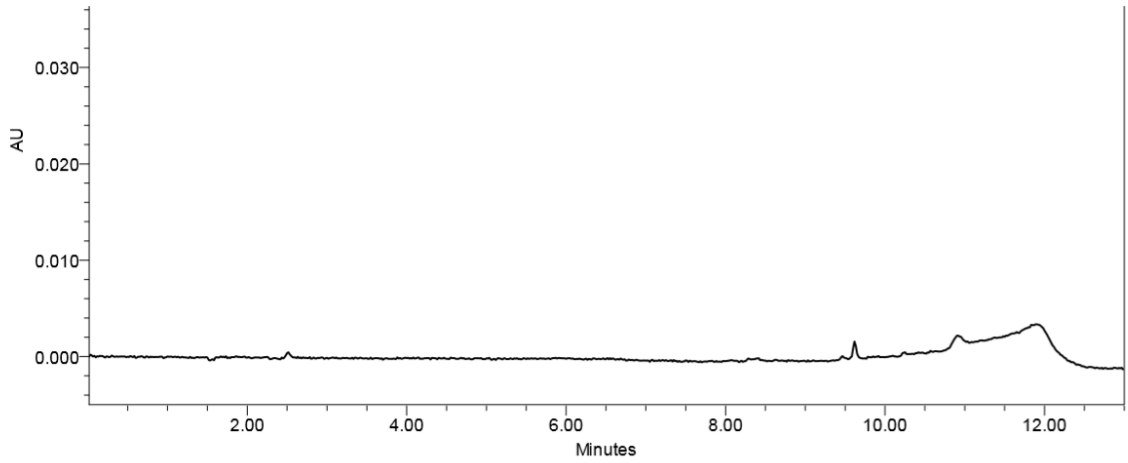


Figure 3: Blank chromatogram



Preparation of Sample Solutions

Four individual APIs and 100mg each placebo(Information taken from innovator pill) was weighed and transferred into a 500 mL volumetric flask, 260mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pippeted out into a 10 ml volumetric flask and made up to 10ml with diluent.

RESULTS

The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures.

System suitability testing

System suitability is used to verify that the system is adequate for the analysis to be performed. This method shows all the values for the system suitability parameters are within limits .The column efficiency is about 7050, 6800, 5600 and 5000 theoretical plates for Phenylephrine, Acetaminophen, Guaifenesin & Dextromethorphan (Figure 2)

respectively. The tailing factors are about 1.4, 1.1, 1.2 and 1.1 for Phenylephrine, Acetaminophen, Guaifenesin & Dextromethorphan respectively.

Precision

The precision (repeatability) of an analytical method refers to the use of the analytical procedure within a laboratory over a short period of time using the same analyst with the same equipment and is expressed as the %RSD. The precision study (Table IX) showed that method has a good reproducibility which was approved by the analysis of five replicate injections of the working standard solution having Phenylephrine, Acetaminophen, Guaifenesin & Dextromethorphan.

Table IX: Precision of Standard Data

Injection No.	PHE	ACE	GUA	DEX
I	274955	2752328	1156015	81489
II	274197	2763007	1151653	81296
III	277326	2732746	1156751	81535
IV	274129	2751860	1146106	81654
V	275408	2688046	1135963	80628
Average	275473	2737597.4	1149297.6	81320
SD	1339.49	29769.6	8576.4	407.9
RSD	0.48	1.08	0.74	0.5

Accuracy

Accuracy was determined by recovery studies of Phenylephrine, Acetaminophen, Guaifenesin & Dextromethorphan known amount of standard was added to the pre analysed sample and subjected to the proposed HPLC analysis. Results of recovery study are shown in Table V, VI, VII&VIII. The study was done at three different concentration levels 50%, 100% and 150%.

Table V: Accuracy of Drug Product Data for Phenylephrine

Conc. %	Conc. PHE (µg/ml) Added	Conc. PHE (µg/ml) Found	% Accuracy (Recovery)	Average
50%				
50%	3	3.03	100.1	
50%	3	2.98	99.5	100.0
50%	3	2.99	99.3	
100%				
100%	5	5.09	101.7	
100%	5	4.99	99.8	100.8
100%	5	5.04	100.8	
150%				
150%	7	7.07	100.9	
150%	7	7.01	100.1	100.4
150%	7	7.02	100.2	

Table VI: Accuracy of Drug Product Data for Acetaminophen

Conc. %	Conc. ACE (µg/ml) Added	Conc. ACE (µg/ml) Found	% Accuracy (Recovery)	Average
50%				
50%	195	196.4	100.7	
50%	195	196.5	100.8	100.6
50%	195	195.7	100.4	
100%				
100%	325	329.1	101.3	
100%	325	324.1	99.7	100.5
100%	325	326.3	100.4	
150%				
150%	455	453.9	99.8	
150%	455	460.7	101.3	100.2
150%	455	452.8	99.5	

Table VII: Accuracy of Drug Product Data for Guaifenesin

Conc. %	Conc. GUA (µg/ml) Added	Conc. PHE (µg/ml) Found	% Accuracy (Recovery)	Average
50%				
50%	100	100.4	100.4	
50%	100	100.5	100.5	100.5
50%	100	99.8	99.8	
100%				
100%	200	200.8	100.4	
100%	200	198.0	99.0	99.0
100%	200	200.3	100.1	
150%				
150%	300	302.2	100.7	
150%	300	301.0	100.3	100.3
150%	300	299.7	99.9	

Table VIII: Accuracy of Drug Product Data for Dextromethorphan

Conc. %	Conc. DEX (µg/ml) Added	Conc. DEX (µg/ml) Found	% Accuracy (Recovery)	Average
60%				
60%	3	2.9	99.8	
60%	3	3.0	100.2	100.0
60%	3	3.0	100.0	
100%				
100%	5	5.0	100.0	
100%	5	5.1	102.0	100.1
100%	5	5.0	100..0	
140%				
140%	7	7.1	100.2	
140%	7	7.0	100.0	100.1
140%	7	7.0	100.0	

Linearity and Range

The calibration curve was plotted over the concentration range of 2.0-7.0µg/mL,130-455µg/mL, 50-300µg/mL and 2.5-15 µg/mL for Phenylephrine, Acetaminophen, Guaifenesin & Dextromethorphan respectively. Dilutions and final concentration were shown in table I,II,III&IV. Each of this drug solution (10 µL) was injected under the operating chromatographic conditions as described above. The correlation coefficient was found to be 0.9999 indicating functional linear relationship.

Figure 4: Linearity Curve of Phenylephrine

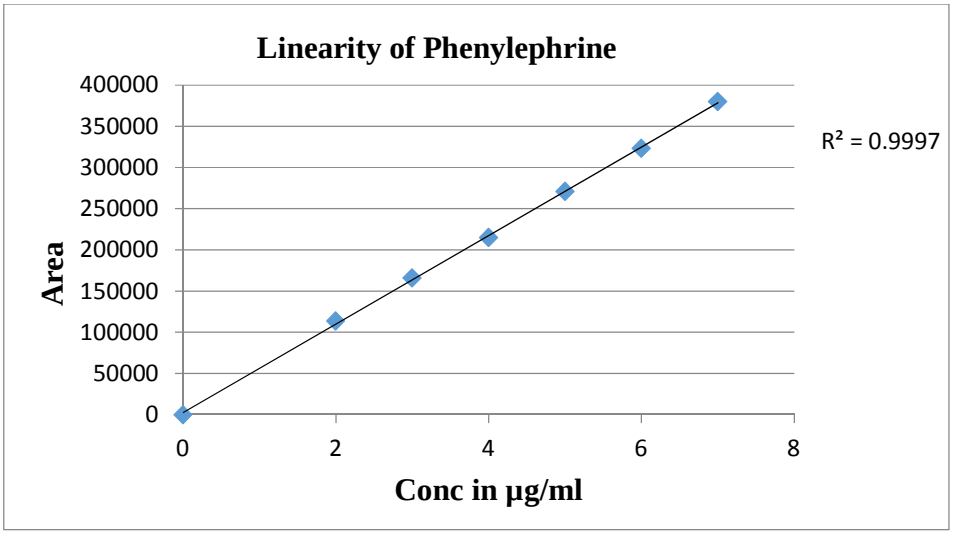


Figure 5: Linearity Curve of Acetaminophen

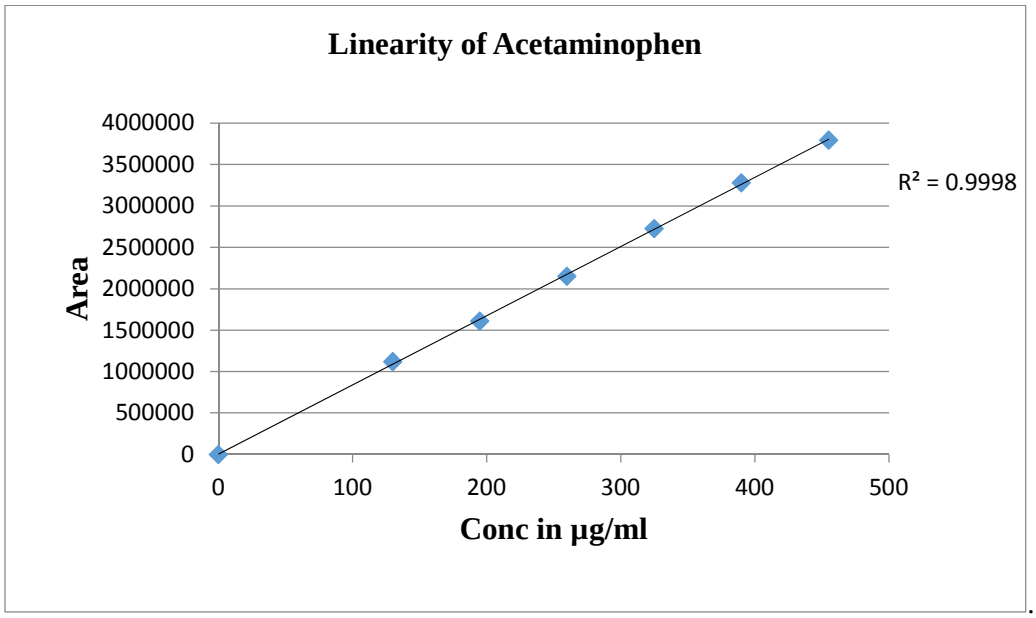


Figure 6: Linearity Curve of Guaifenesin

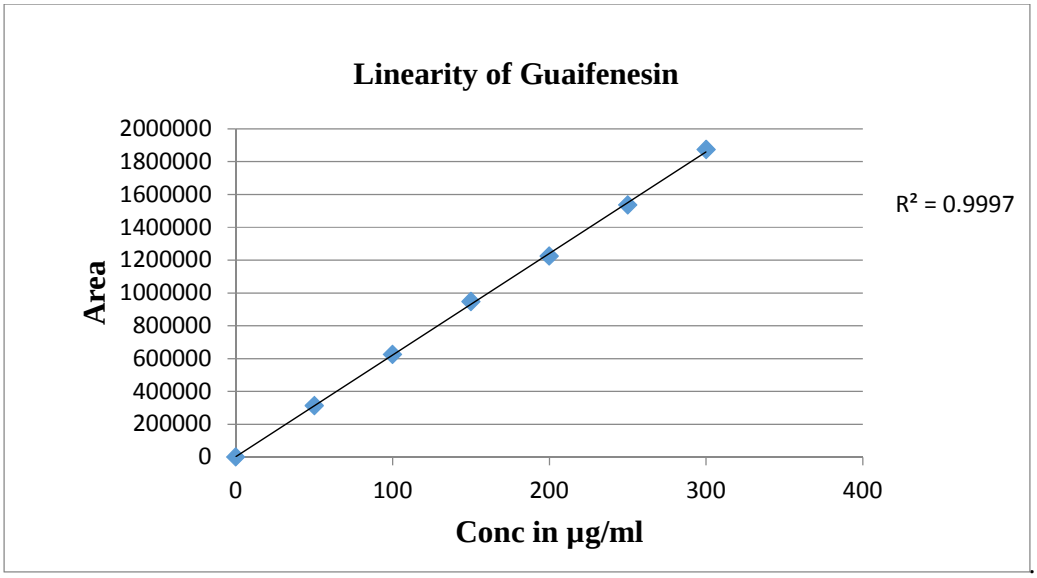


Figure 7: Linearity Curve of Dextromethorphan

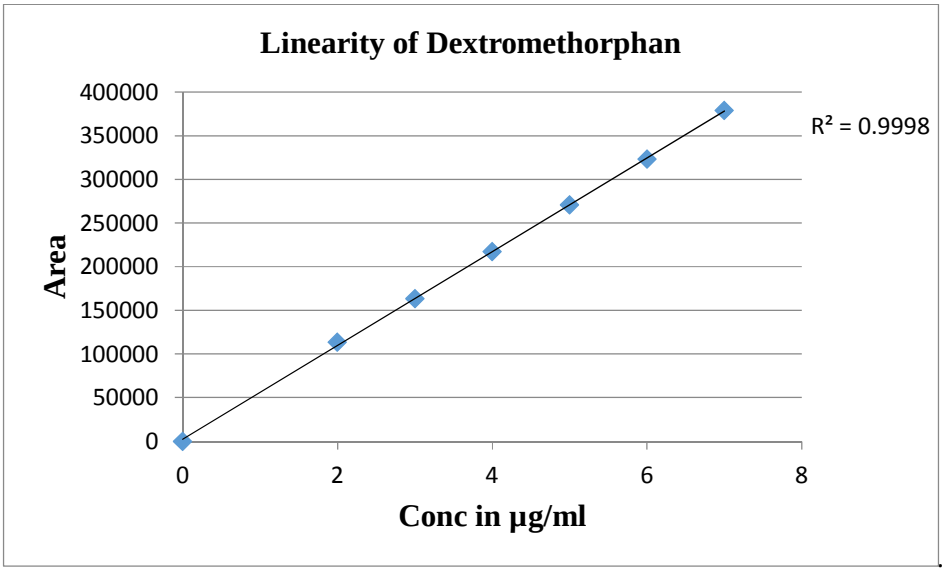


Table I: Linearity values of Phenylephrine by RP- HPLC method

Concentration (µg/ml)	Peak area
2	113805
3	165870
4	215091
5	271147
6	323450
7	380160

Table II: Linearity values of Acetaminophen by RP- HPLC method

Concentration (µg/ml)	Peak area
0	0
130	1118634
195	1610860
260	2151341
325	2725851
390	3279440
455	3797449

Table III: Linearity values of Guaifenesin by RP-HPLC method

Concentration (µg/ml)	Peak area
0	0
50	314121
100	624808
150	947232
200	1224539
250	1535601
300	1873045

Table IV: Linearity values of Dextromethorphan by RP- HPLC method

Concentration (µg/ml)	Peak area
2	113805
3	165870
4	215091
5	271147
6	323450
7	380160

Detection and quantification limit (LOD &LOQ)

The detection limit or LOD is the lowest amount of analyte in a sample that can be detected It may be expressed as a concentration that gives a signal to noise ratio of approximately 3:1. While the Quantification limit or LOQ is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy with a signal to noise ratio of approximately 10:1 can be taken as LOQ of the. Our method showed the (LOD) for Phenylephrine, Acetaminophen, Guaifenesin & Dextromethorphan were found to be 0.027µg/ml, 0.197µg/ml 0.475µg/ml and0.029 µg/ml respectively and The LOQ values for Phenylephrine, Acetaminophen, Guaifenesin & Dextromethorphan were found 0.083 µg/ml, 0.599µg/ml ,1.4 33µg/ml and 0.089µg/ml respectively.

Figure 8: Acid Degradation Chromatogram

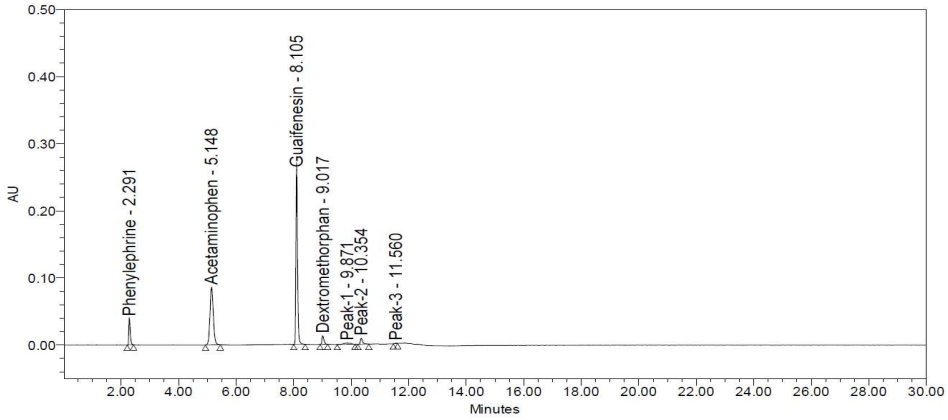
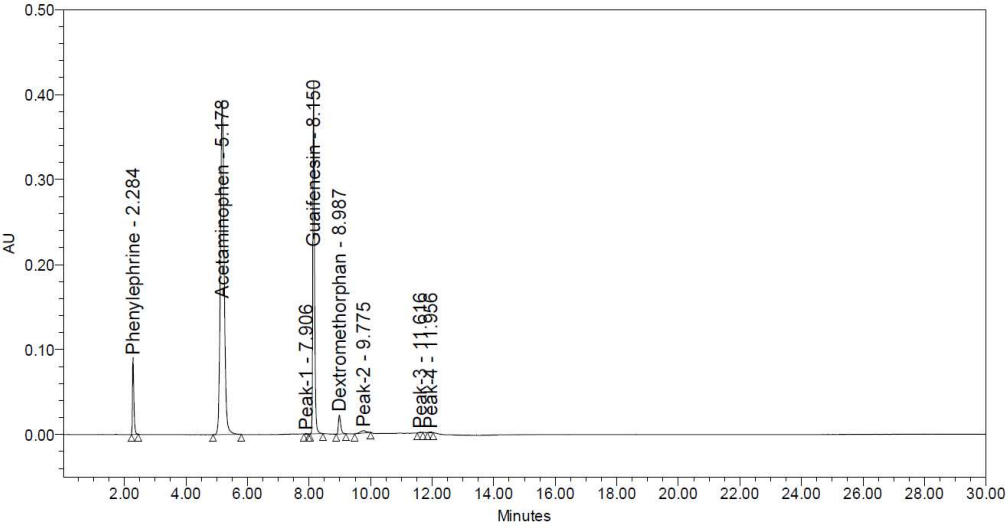


Figure 9: Base Degradation Chromatogram



Robustness

The robustness of the proposed method was evaluated by slight modification in the organic composition and pH values of aqueous phase of the mobile phase and flow rate. During these studies it was found that there was not much change retention time, area and symmetry of peak. The developed method was used for the assay of commercially available tablets. The interference of excipients was studied by comparing the chromatography of standards and excipients. The shape and retention times of peaks showed that there was no interference from excipients.

Specificity

Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method. Intentional degradation was carried out by exposing of samples to stability condition 0.1 N HCl at 60

^{0}C(Figure 8), 0.1 N NaOH at 60 ^{0}C (Figure 9) , Heat at 60 ^{0}C 30min(Figure 10) water at 60°C for 30min(Figure 11), and Photolysis (Figure 12)by using photolytic chamber.

Figure 10: Thermal Degradation Chromatogram

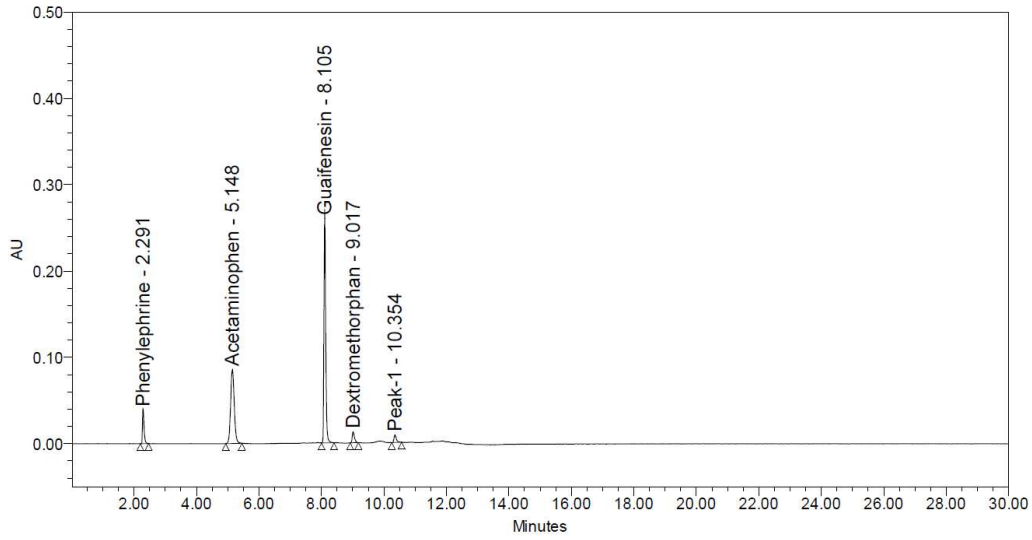


Figure 11: Water Degradation Chromatogram

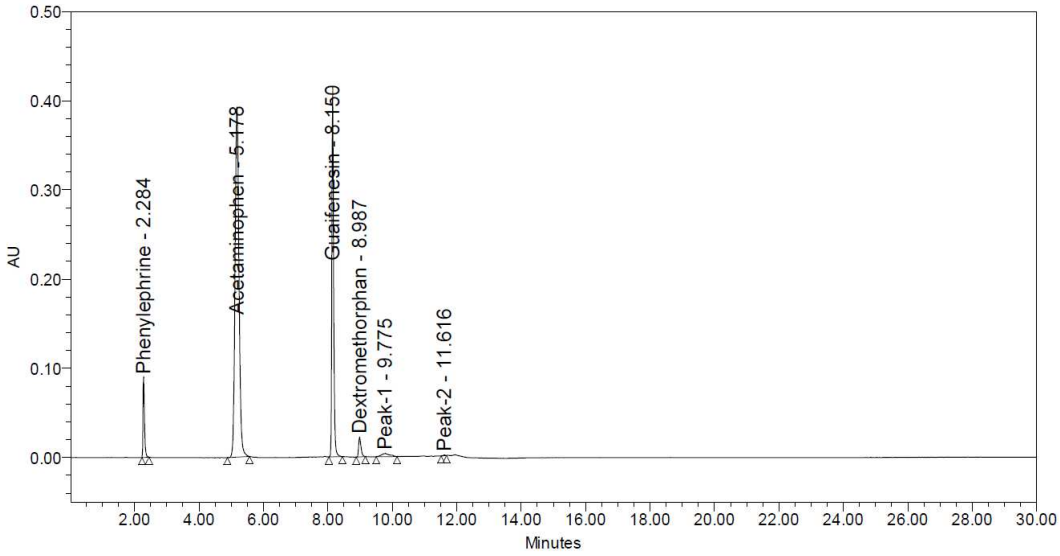


Figure 12: Photolytic Degradation Chromatogram

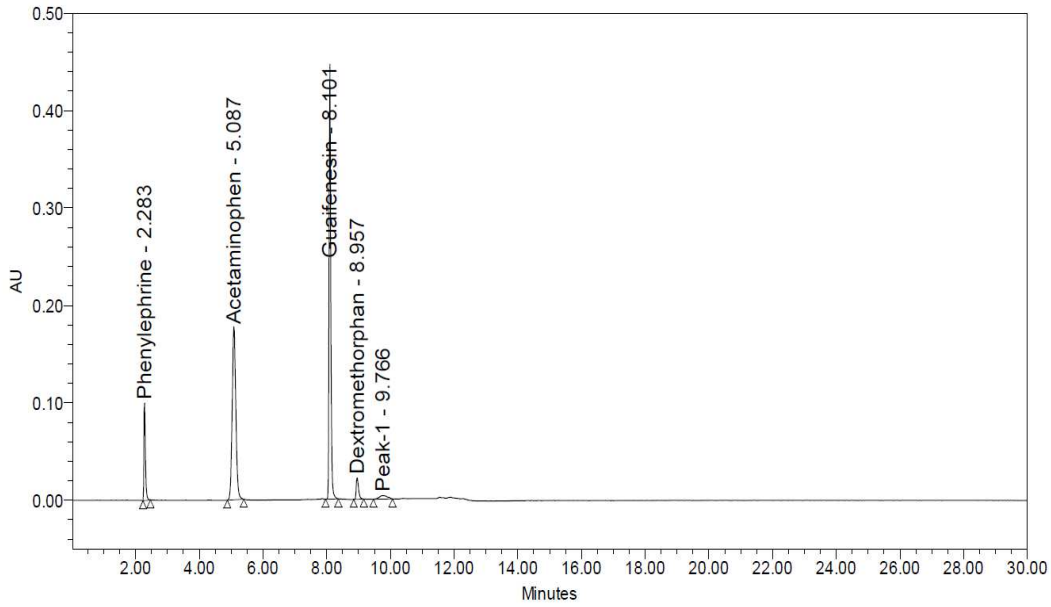


Table X: Specificity and stability indicating study

	PHE	ACE	GUA	DEX
Stress Condition	**% Degradation**	**% Degradation**	**% Degradation**	**% Degradation**
0.1 N HCL at 60°C for 30 Min	5.2	4.2	8.9	3.4
0.1 N NaOH at 60°C for 30 Min	4.5	3.1	7.9	2.1
3% H2O2 at 60°C for 30 Min	3.4	2.8	6.7	2.0
Heat at 60°C for 30 Min	4.6	2.3	5.7	3.2
Water at 60°C for 30 Min	2.4	1.3	4.3	2.2
Photolysis	2.2	2.1	3.3	2.5

To evaluate the linearity of the method, six different preparations were made to achieve in the range of 2.0-7.0µg/mL, 130-455µg/mL, 50-300µg/mL and 2.5-15 µg/mL for Phenylephrine, Acetaminophen, Guaifenesin & Dextromethorphan respectively.

In order to determine the accuracy of the method, three different concentrations (50%, 100% and 150%) levels were used and their recovery was calculated. Regarding the determination of the precision (repeatability) five replicate injections of the working standard Phenylephrine, Acetaminophen, Guaifenesin & Dextromethorphan were injected and the relative standard deviation (RSD) of the peak areas was calculated for the replicate injections. To determine the LOD and LOQ, serial dilutions of the combination were made from the standard stock solution the signal from the samples was compared with those of blank samples. LOD and LOQ values were identified as signal-to-noise ratio (S/N) of 3:1 and 10:1 respectively.

DISCUSSION

The chromatographic conditions were optimised by different means i.e. using different buffers, Organic modifiers, different flow rate, different columns, different wave lengths and different diluents. The proposed method found to be linear in the concentration range of 2.0-7.0µg/mL,130-455µg/mL, 50-300µg/mL and 2.5-15 µg/mL for Phenylephrine, Acetaminophen, Guaifenesin & Dextromethorphan respectively. The method was specific since degradants are not interfering in the estimation of above four compounds. Accuracy of the method indicated by recovery values from 99% to 100.8% for Phenylephrine, Acetaminophen, Guaifenesin & Dextromethorphan.

Precision is reflected by %RSD values less than 2.The LOQ values for Phenylephrine, Acetaminophen, Guaifenesin & Dextromethorphan were found 0.083 µg/ml, 0.599µg/ml, 1.4 33µg/ml and 0.089µg/ml respectively. These low values suggest sensitivity of the developed method.

CONCLUSION

In conclusion, the presented HPLC method is novel, simple, selective, cost-effective, and reproducible and can be reliably used by almost every drug laboratory. The method enables simultaneous determination of ACE, GUA, DEX and PHE in pharmaceutical preparations. In the process of developing the method, forced degradation and validation studies were carried out. Finally, the method was applied to the analysis for four drug formulations.

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