Research Article

Simultaneous Determination of Acetaminophen, Phenylephrine Hydrochloride and Dextromethorphan Hydrobromide in Liquicap Dosage form by RP-HPLC

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ABSTRACT

A new reverse phase high performance liquid chromatographic method for the simultaneous determination of acetaminophen, phenylephrine hydrochloride and dextromethorphan hydrobromide in liquicap pharmaceutical formulation has been developed and validated. Acetaminophen, phenylephrine hydrochloride and dextromethorphan hydrobromide are frequently associated with cough and cold remedies. The separation was carried out by using a mobile phase which consists of an ion pairing agent (sodium salt of butane sulphonic acid) as a buffer solution and acetonitrile as an organic modifier at a flow rate of 1.5 ml/min on a gradient. The study was carried out on Shimadzu LC 2010 HPLC having UV detector at the wavelength of 272 nm. The stationary phase used was Inertsil c-18 column having 4.6 mm inner diameter, 250 mm length and particle size of 5 µm, the column temperature was maintained at 30° C. The retention time were found to be 5 minutes for acetaminophen, 3 minutes for phenylephrine hydrochloride and 15 minutes for dextromethorphan hydrobromide. The peaks were well resolved and no interference from the excipients was observed, the percent recovery of acetaminophen, phenylephrine hydrochloride and dextromethorphan hydrobromide were within limit of 98.0 % to 102.0 %. The developed method was accurate, reproducible and therefore suitable for routine analysis.

Keywords: Acetaminophen, dextromethorphan hydrobromide, ion pairing agent, phenylephrine hydrochloride

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INTRODUCTION

Acetaminophen or Paracetamol is classified as a mild analgesic. It is commonly used for the relief of headaches and other minor aches and pains and is a major ingredient in numerous cold and flu remedies. Phenylephrine is used as a decongestant sold as an oral medicine, as a nasal spray, also as an eye drops. It is the most common over-the-counter decongestant. The primary use of dextromethorphan is as a cough suppressant, for the temporary relief of cough caused by minor throat and bronchial irritation (such as commonly accompanies the flu and common cold), as well as those resulting from inhaled particle irritants.

Several methods are used to analyze cough and cold remedies [1-3]. One of these methods requires change in wavelengths in a single run [4]. The use of polyethylene glycol column and the use of ion pairing agents for determination are also reported in literature [5, 6].

The aim of this study is to develop a simple a reliable gradient reverse phase HPLC method to quantify the analytes without having any interference from its excipients.

The present method makes use of ion pairing agents; this method can determine three active ingredients wherein the label claim of acetaminophen is significantly higher than those of other active ingredients.

MATERIALS AND METHODS

The reference standards of Acetaminophen, Phenylephrine hydrochloride and Dextromethorphan hydrobromide were obtained from ACME formulations private limited. Excipients like glycerin, polyethylene glycol, propylene glycol,

sorbitol, titanium dioxide and povidone were obtained from Merck. Acetonitrile, 1-butane-sulphonic acid sodium salt, triethylamine, and formic acid were used of hplc grade. Dayquil capsules were purchased from www.ebay.in

The HPLC analysis was carried out on Shimadzu LC-2010 the pH measurements of mobile phase were carried out on a mettler toledo pH meter.

The newly optimized method used a gradient; the aqueous component of the mobile phase was a 0.4 % formic acid and 0.13 % 1-butane sulphonic acid sodium salt, pH adjusted to 3.0 with triethylamine. The composition of mobile phase A (90:10) buffer: acetonitrile and mobile phase B (50:50) buffer: acetonitrile pH adjusted to 3.0. The flow rate was maintained at 1.5 ml/min and column temperature pre-set at 30°C injection volume of 20 µl having detection at wavelength 272 nm. The gradient was programmed as shown in (**Table 1**).

Table 1: Gradient Program used for Analysis

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	100	0
6.0	100	0
6.5	85	15
16.0	0	100
16.5	100	0
20.0	100	0

Standard preparation: weighed 650 mg acetaminophen 20 mg dextromethorphan hydrobromide and 10 mg phenylephrine hydrochloride in a 200 mL volumetric flask, and dissolved in 150 mL of mobile phase A and made upto volume with the same, further transferred 10.0 mL of the above solution to 100 ml and make up to the mark with mobile phase A.

Sample preparation: transferred 1 intact capsule to 100 mL volumetric flask, added 70 mL of diluent, sonicate for 30 minutes with intermittent shaking ensure complete dissolution and make up to the mark with diluent transferred 5.0 mL of this solution to 50 mL volumetric flask make up to the mark with mobile phase A.

RESULTS AND DISCUSSION

Initially method development was made on an isocratic system using 0.05 percent orthophosphoric acid and methanol in a proportion of 45:55 using inertsil C-18 column 250 mm length, 4.5 mm inner diameter and 5 µm particle size using a wavelength of 225 nm and 20 µl injection volume and column temperature at 25°C, using these chromatographic conditions separations were obtained, but the responses of phenylephrine and dextromethorphan were very low. Also the response of excipient was observed near the peak of dextromethorphan, due to which linearity parameter was not found to be meeting the acceptance criteria. Hence, the method was further optimized by making use of an ion pairing agent.

The use of ion pairing agent thus developed a method which was successful in separating the three analytes of interest. The peak shape, tailing factor, resolution and theoretical plates were found to meet the acceptance criteria [7]. Each excipient was prepared and analyzed in this chromatographic conditions, also the mixture of these inactive ingredients was prepared and analyzed. None of the inactive capsule component was detected at this wavelength.

The method was further subjected to validation.

Specificity refers to the extent to which a method can determine particular analyte in mixtures or matrices without interferences from other components. In this assay, each individual excipient solution was analyzed as well as the mixture of placebo was prepared and analyzed there is no peak in the retention times corresponding to the analytes. The mixture of standard was injected and the peak of three analytes was well resolved.

Linearity and Range were carried out over a range of 50 to 150 percent of working level concentration. The linearity regression correlation coefficient, % Y-intercept and % RSD for peak area response and retention time for lower and higher range were calculated. The linearity regression correlation coefficient for the component was found within limit (Not less than 0.999). The % Y-intercept for the component was found within the limit (Not more than +2.0).

Accuracy was determined by spiking the placebo preparation with 50, 80, 100, 120 & 150 percent of working level concentration of analyte mixture, prepared in triplicate for each level in six replicates for 100 % level and the percentage recovery were calculated for each level separately. The percentage recoveries observed for the levels were found well within the limit set for the accuracy study (Not less than 98.0% and not more than 102.0%).

For precision six injections of standard solution and six sample preparations were injected into the chromatographic system and the assay were calculated. For intermediate precision same sequence of precision was injected using new standard and sample preparation on the next day by another analyst. The difference in assay results of precision and intermediate precision was between ± 2.0 %.

Table 2: Statistical Evaluation of Linearity Data

Component	Correlation coefficient	Slope	Intercept
Acetaminophen	0.9999	0.00007	-1.9
Dextromethorphan	0.9998	0.00058	-0.3
Phenylephrine	0.9999	0.00013	0.2

Table 3: Statistical Evaluation of Accuracy Data

Recovery level	Acetaminophen	Dextromethorphan	Phenylephrine
50	99.69	98.83	100.02
80	99.02	99.27	98.81
100	98.58	99.76	99.24
120	98.61	99.63	99.60
150	98.64	98.53	99.70

Table 4: Statistical evaluation of precision data

Component	Analyst I	Analyst II	Difference
Acetaminophen	99.6	99.3	0.3
Dextromethorphan	98.8	99.1	-0.3
Phenylephrine	99.9	99.2	0.7

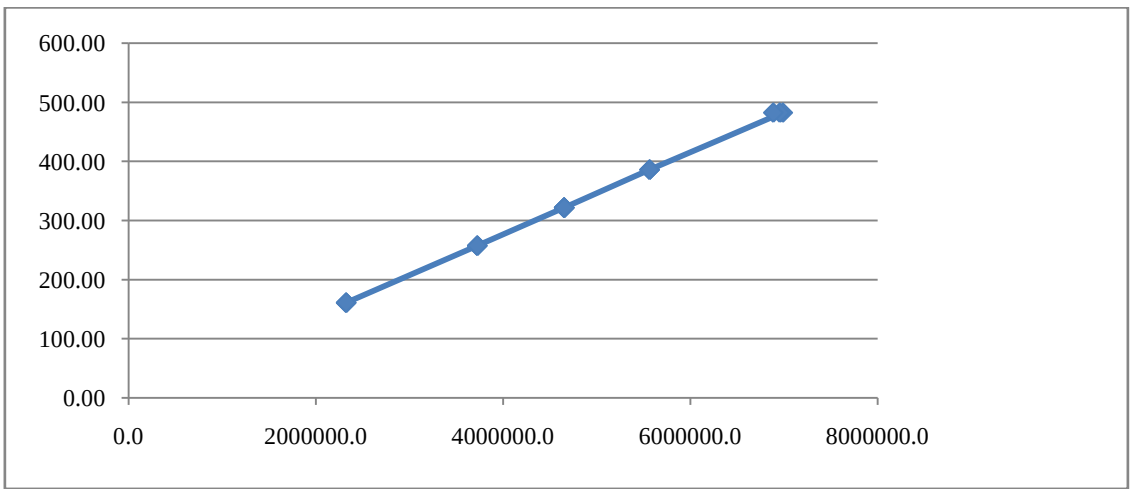

Figure 1: Concentration in ppm versus Detector Response for Acetaminophen

The robustness of method was carried out by changing the different chromatographic conditions (one at a time) such as:

1. Change in flow rate from 1.5 to 1.4 ml/min
2. Change in flow rate from 1.5 to 1.6 ml/min
3. Change in column temperature from 30°C to 25°C
4. Change in column temperature from 30°C to 35°C

The % RSD of standards and the assay values was found to be within limit for each change in parameter.

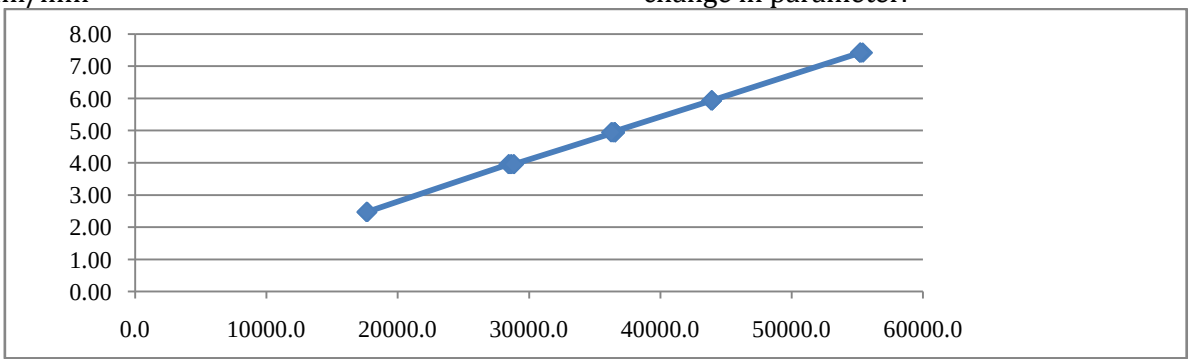

Figure 2: Concentration in ppm versus Detector Response for Phenylephrine Hydrochloride

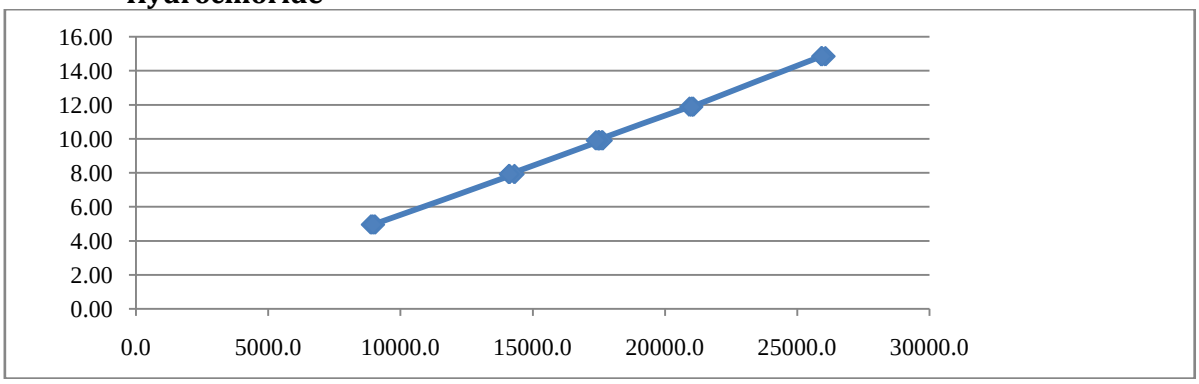

Figure 3: Concentration in ppm versus Detector Response for Dextromethorphan Hydrobromide

Acquired by	: Parag B
Sample Name	: DayQuil
Sample ID	: Blank-(Diluent)
Vail #	: 1
Injection Volume	: 20 µL
Data File Name	: Blank-(Diluent).lcd
Method File Name	: Day_Ny.lcm
Data file	: E:\Data\Dayquil\Precision\Blank-(Diluent).lcd
Batch File Name	: Precision.lcb
Report File Name	: Assay.lcr
Data Acquired	: 23-Apr-14 09:18:52 AM
Data Processed	: 24-Apr-14 09:15:29 AM

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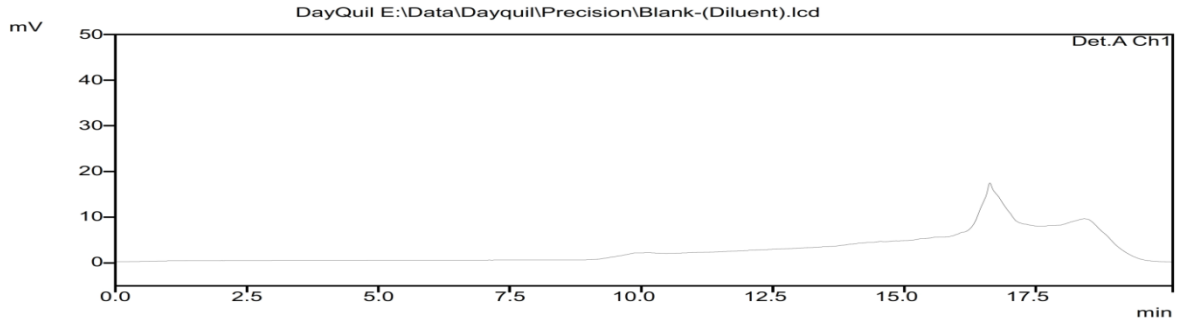

1 Det.A Ch1/272nm

<Results>

Detector A Ch1 272nm

Figure 4: Chromatogram of Diluent

Acquired by : Parag B
Sample Name : DayQuil
Sample ID : Std-Day-1
Vail # : 2
Injection Volume : 20 µL
Data File Name : Std-Day-1.lcd
Method File Name : Day_Ny.lcm
Data file : E:\Data\Dayquil\Precision\Std-Day-1.lcd
Batch File Name : Precision.lcb
Report File Name : Assay.lcr
Data Acquired : 23-Apr-14 09:39:56 AM
Data Processed : 24-Apr-14 09:17:35 AM

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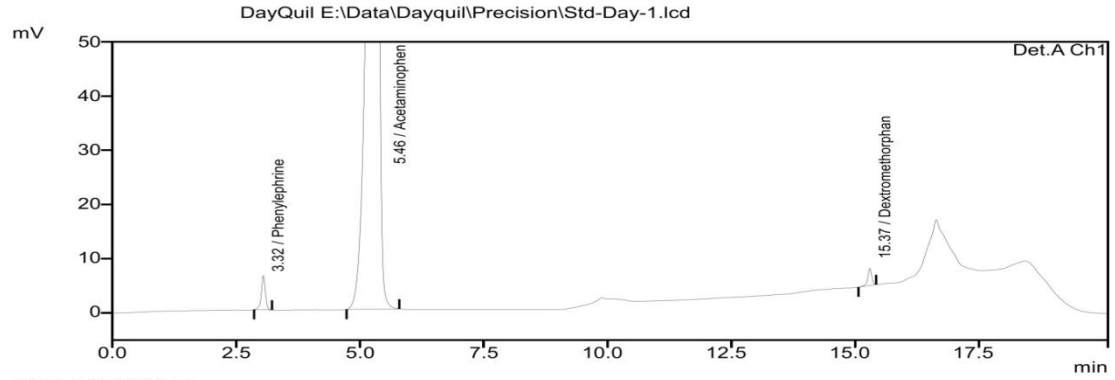


1 Det.A Ch1/272nm

<Results>

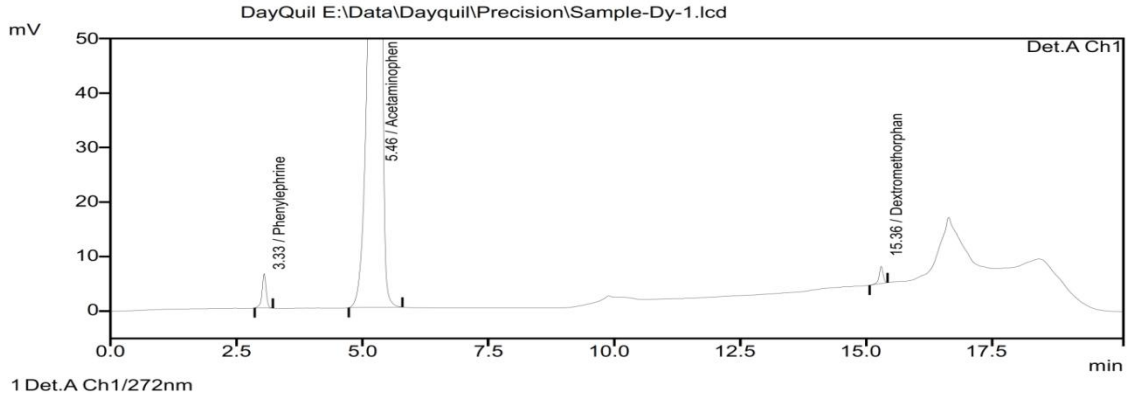
Detector A Ch1 272nm

Peak #	Name	Ret. Time	Area	Area %	Theoretical Plate#	Tailing Factor
1	Phenylephrine	3.32	37514	0.80	6853	0.92
2	Acetaminophen	5.46	4651587	98.83	7611	0.91
3	Dextromethorphan	15.37	17506	0.37	155457	0.90
Total			4706607	100.00		

Figure 5: Chromatogram of Standard Solution

Acquired by : Parag B
Sample Name : DayQuil
Sample ID : Sample-Dy-1
Vail # : 3
Injection Volume : 20 µL
Data File Name : Sample-Dy-1.lcd
Method File Name : Day_Ny.lcm
Data file : E:\Data\Dayquil\Precision\Sample-Dy-1.lcd
Batch File Name : Precision.lcb
Report File Name : Assay.lcr
Data Acquired : 23-Apr-14 11:46:20 AM
Data Processed : 24-Apr-14 09:28:12 AM

<Chromatogram>



1 Det.A Ch1/272nm

<Results>

Detector A Ch1 272nm

Peak #	Name	Ret. Time	Area	Area %	Theoretical Plate#	Tailing Factor
1	Phenylephrine	3.33	35221	0.74	6883	0.98
2	Acetaminophen	5.46	4676360	98.89	7552	0.94
3	Dextromethorphan	15.36	17423	0.37	154894	0.91
Total			4729004	100.00		

Figure 6: Chromatogram of Sample Solution

CONCLUSION

The method developed in this work is simple, sensitive, precise and accurate and hence can be used for the routine analysis of acetaminophen, phenylephrine hydrochloride and dextromethorphan hydrobromide in liquicap pharmaceutical preparation.

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