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Analytical method development and validation of acetaminophen, doxylamine succinate and dextromethorphan hydrobromide in liquicap dosage form by rp-hplc

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ABSTRACT: *A simple, selective, rapid, precise and economical reverse phase hplc method has been developed for the quantitative determination of acetaminophen, doxylamine succinate and dextromethorphan hydrobromide in liquicap formulation. The analysis was performed by using a gradient mobile phase (Sodium salt of butane sulphonic acid buffer solution and acetonitrile) at a flow rate of 1.5 ml/min on a gradient consisiting of Shimadzu LC 2010 HPLC on UV detector using, Inertsil c18 (4.6 mm inner diameter, 250 mm length and 5 µm particle size) column at a wavelength of 272 nm. The retention time were found to be 5 min for acetaminophen, 11 min for doxylamine succinate and 15 min for dextromethorphan hydrobromide. The percent recovery of acetaminophen, doxylamine succinate 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, Doxylamine succinate, Dextromethorphan hydrobromide.*

I. INTRODUCTION

Acetaminophen also called as N-acetyl-p-aminophenol is mild analgesic, commonly used for the relief of headache. It is used in many formulations that treat flu and cold. Doxylamine succinate is an antihistamine; it can be used by itself as a short term sedative and in ccombination with other drugs to provide night time relief from col and allergy. Dextromethorphan HBr is an antitussive or a cough suppressant drug for the temporary relief of cough caused by minor throat and bronchial irritation.Several methods are used to analyze cough and cold remedies. One of these methods requires change in wavelengths in a single run. The use of polyethylene glycol column for determination is also reported in literature. Also few methods do not separate all the active ingredients which are mentioned in the present work. In the present work a reliable gradient reverse phase HPLC method is developed which 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. Also in this method there is no interference from the inert materials or the excipients.

II. EXPERIMENTAL

Materials and methods:

The reference standards of Acetaminophen, Doxylamine succinate and Dextromethorphan HBr 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. Nyquil 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 mettlertoledo pH meter.

Chromatographic conditions: 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 time 0.0 to 6.0 min mobile phase A (100%), 6.0 to 6.5 min mobile phase A (85%), 6.5 to 16.0 min mobile phase A (0%), 16.0 to 16.5 min mobile phase A (100%) and from 16.5 to 20.0 min mobile phase (100%).

Preparation of reference solution: Weighed 325 mg acetaminophen 15 mg dextromethorphan hydrobromide and 6.25 mg doxylamine succinate in a 100 mL volumetric flask, and dissolved in 75 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.

Preparation of sample solution: Transferred 1 intact capsule to 100 mL volumetric flask, added 70 mL of diluent, sonicated 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.

III. RESULTS AND DISCUSSION

Initial method development were 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 doxylamine and dextromethorphan were very low. Also the response of excipient was observed near the peak of dextromethorphan, which resulted in is nonlinear response in the range of 50% to 150% of working concentration. So there was a need to further optimize the method, it was done by making use of an ion pairing agent. The method thus developed was successful in separating all the three components, the peak shape was proper and the tailing factor, resolution and theoretical plates were well within the limits [8]. Each inactivecomponent was analysed in this chromatographic conditions, also the mixture of these inactive ingredients was prepared and analysed. None of the inactive capsule component was detected at this wavelength.

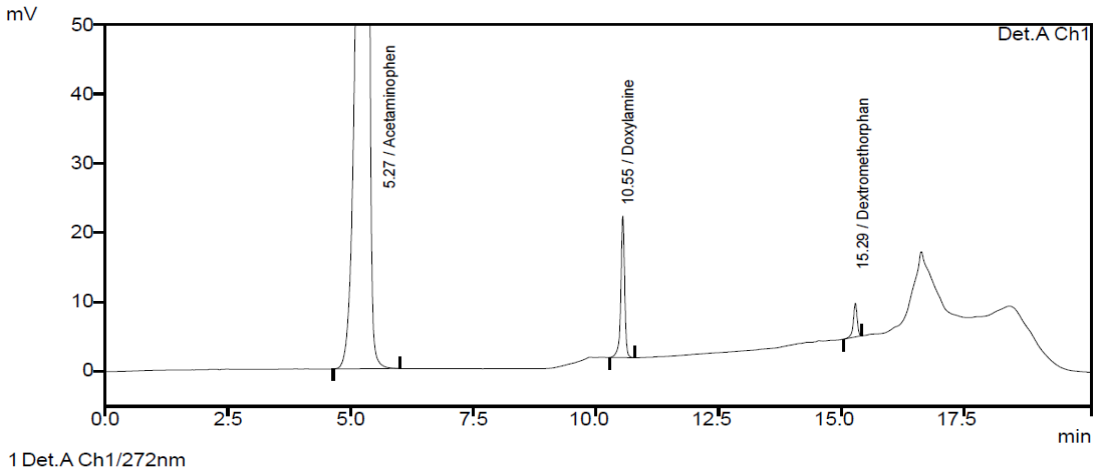


Figure 1: A typical chromatogram of reference solution

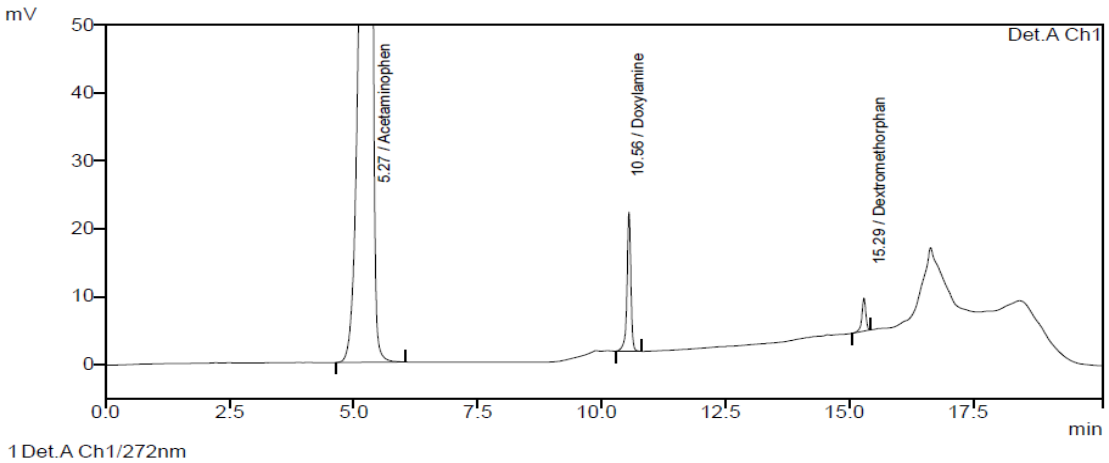
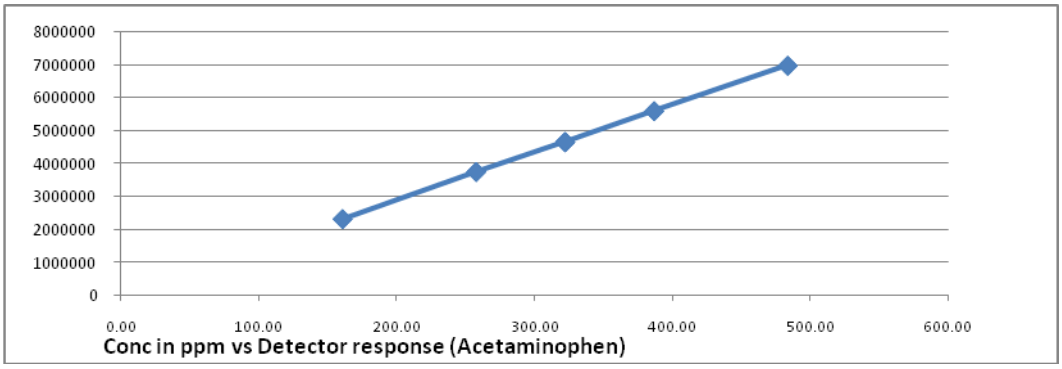


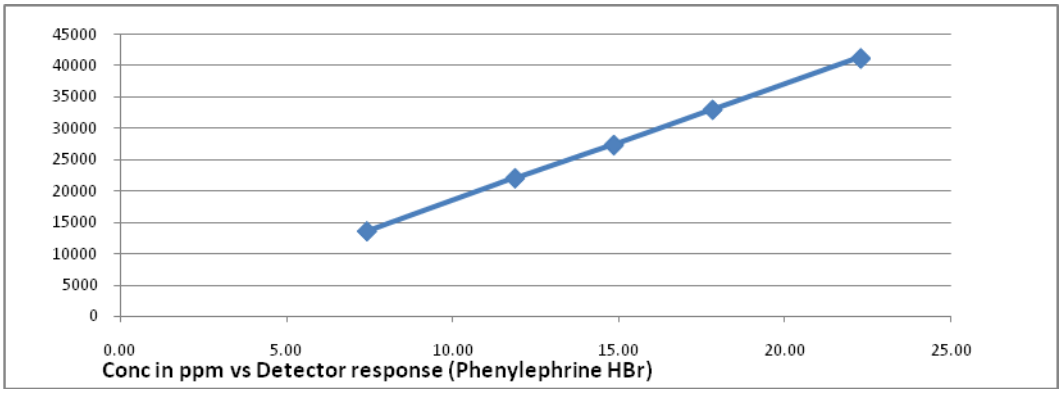
Figure 2: A typical chromatogram of Sample solution

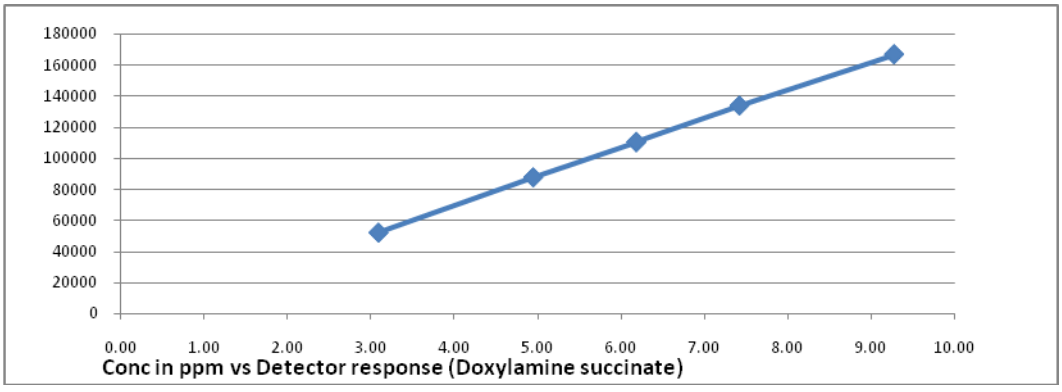
IV. METHOD VALIDATION

Specificity: 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 analysed as well as the mixture of placebo was prepared and analysed 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: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).







	Corelation coefficient	Slope	Intercept
Acetaminophen	0.99997	0.00007	-1.99
Dextromethorphan HBr	0.99994	0.00054	0.04
Doxylamine succinate	0.99988	0.00005	0.24

Table 1.Statistical evaluation of linearity data

Accuracy: 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%).

Recovery level	Acceptance criteria	Acetaminophen	Dextromethorphan	Doylamine
50	98 % to 102 %	99.9	99.5	99.9
80		100.0	99.1	99.0
100		99.8	100.9	100.0
120		99.0	98.5	99.7
150		98.3	100.4	98.9

Table 2. Statistical evaluation of accuracy data

Precision: 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 %.

Analysis done by	Mean Assay (%)		
	Acetaminophen	Dextromethorphan HBr	Doxylamine succinate
Analyst - I day one	99.4	99.5	99.1
Analyst - II day two	99.6	99.3	99.2
Difference	-0.2	0.2	-0.1

Table 3. Statistical evaluation of Precision data

Robustness: 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

V. CONCLUSION

The method thus optimized and validated simple, sensitive, precise and accurate and hence can be used for the routine analysis of acetaminophen, doxylamine succinate and dextromethorphan hydro bromide in liquicap pharmaceutical preparation. The sample recoveries from all formulations were in good agreement with their respective label claims, and sensitivity of this method is within the range.

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