

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF CODEINE IN SYRUP DOSAGE FORM USING UPLC TECHNOLOGY

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ABSTRACT

A specific, precise, accurate ultra-pressure liquid chromatography (UPLC) method is developed for estimation of Codeine in bulk drug and syrup dosage form. The method employed, with Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 μm) in a gradient mode, with mobile phase of Acetonitrile, methanol, and 1% triethylamine and Buffer solution in the ratio 70: 15: 15 %v/v/v. The flow rate was 1.5 ml/min and effluent was monitored at 210 nm. Retention time was found to be 3.416±0.020 min. The method was validated in terms of linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) etc. in accordance with ICH guidelines. Linear regression analysis data for the calibration plot showed that there was good linear relationship between response and concentration in the range of 20- 100 μg/ml respectively. The LOD and LOQ values for were found to be 2.093(μg/ml) and 6.3437(μg/ml) respectively. No chromatographic interference from syrup excipients and degradants were found. The proposed method was successfully used for estimation of Codeine in syrup dosage form.

KEYWORDS: Codeine, syrup dosage form, UPLC method estimation.

1. INTRODUCTION

Codeine is an opiate used to treat pain, as a cough medicine. Chemically it is (5α,6α)-7,8-didehydro-4,5-epoxy-3-methoxy-17-methylmorphinan-6-ol. Codeine is use for acute cough suppression in children or adults. [1-5]

Codeine was discovered in 1832 by Pierre Jean Robiquet. In 2013 about 361,000 kilograms of codeine were produced while 249,000 kilograms were used. This makes it the most commonly taken opiate. [6-8] It is on the World Health Organization's List of Essential Medicines, the most effective and safe medicines needed in a health system. The wholesale cost in the developing world is between 0.04 and 0.29 USD per dose as of 2014. In the United States it costs about one dollar a dose. Codeine occurs naturally and makes up about 2% of opium. [9-10]

Analytical methods are commonly used for the quantitative and qualitative analysis of raw materials, drug substances, drug products, and compounds in biological samples in pharmaceutical industry. [11-15] The validation of a specific method must be demonstrated through laboratory experiments by routinely analysing samples. [16-17]

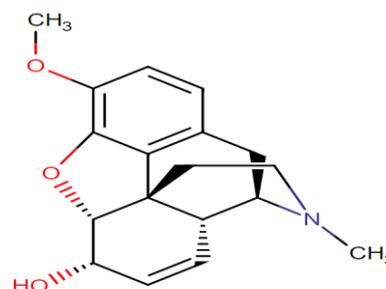


Fig.1: Molecular Structure of Codeine, (5α,6α)-7,8-didehydro-4,5-epoxy-3-methoxy-17- ethylmorphinan-6-ol.

2. EXPERIMENTAL MATERIALS

Codeine (99.60 % purity) used as analytical standard was procured from Active Pharma Labs (Hyderabad).

HPLC grade methanol, Acetonitrile (HPLC grade) was purchased from Qualigens fine chemicals, Mumbai, India. Distilled, 0.45 μm filtered water used for UPLC quantification and preparation of buffer. Buffers and all other chemicals were analytical grade.

The syrup - dosage (Codeine Phosphate Syrup) labelled to contain 5 mg per 10mL in 100 mL of container for Codeine. All chemicals used were of pharmaceutical or special analytical grade.

Instrumentation

Acquity, Waters UPLC system consisting of a Water 2695 binary gradient pump, an inbuilt auto sampler, a column oven and Water 2996 wavelength absorbance detector (PDA) was employed throughout the analysis.

The data was collected using Empower 2 software. The column used was Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 μ m). A Band line sonerex sonicator was used for enhancing dissolution of the compounds.

Chromatographic Conditions

Table 1: Chromatographic Conditions of the validating method.

Parameter	Value
Column	Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 μ m)
Mobile Phase	Acetonitrile, methanol, and 1% triethylamine and Buffer solution in the ratio 70 : 15 : 15 %v/v/v
Flow rate	1.5mL/min
Run time	8 Min.
Column Temperature	Maintained at 25°C
Injection volume	20 μ L
Detection wavelength	210 nm
Diluent	Mobile Phase

Preparation of Standard Stock Solution

Preparation of Diluent

In order to achieve the separation under the optimized conditions after experimental trials that can be summarized. Stationary phase like Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 μ m) column was most suitable one, since it produced symmetrical peaks with high resolution and a very good sensitivity and with good resolution. The flow rate was maintained 1.5 mL min⁻¹ shows good resolution. The PDA detector response of Codeine was studied and the best wavelength was found to be 210 nm showing highest sensitivity.

The mixture of three solutions Acetonitrile, methanol, and 1% triethylamine and Buffer solution in the ratio 70:15:15 %v/v/v. The buffer used is 2.35 g of monobasic potassium phosphate were dissolved into 1000 ml of water, and adjusted pH to 2.5 with o-phosphoric acid. with gradient programming was used as mobile phase at 1.5 mL/min was found to be an appropriate mobile phase for separation of Codeine. The column was maintained at 25°C temperature.

Preparation of internal standard solution

Weighed accurately about 10 mg of paracetamol into a clean and dry 100 mL volumetric flask, dissolved with sufficient volume of mobile phase. The volume was then made up to 100 mL with mobile phase to get the concentration of 100 μ g/mL of stock solution of working standard. Then it was ultrasonicated for 10 minutes and filtered through 0.20 μ membrane filter.

Preparation of Codeine standard solution

Transfer accurately about 10 mg of Codeine into 100 ml volumetric flask, add 50 ml of mobile phase and sonicate to dissolve it completely dissolved with sufficient volume of mobile phase. The volume was then made up to 100 mL with mobile phase to get the concentration of 100 μ g/mL of standard stock solution of working standard. Then it was ultrasonicated for 10 minutes and filtered through 0.20 μ membrane filter. Linearity was determined in the range of 20-100 μ g mL⁻¹.

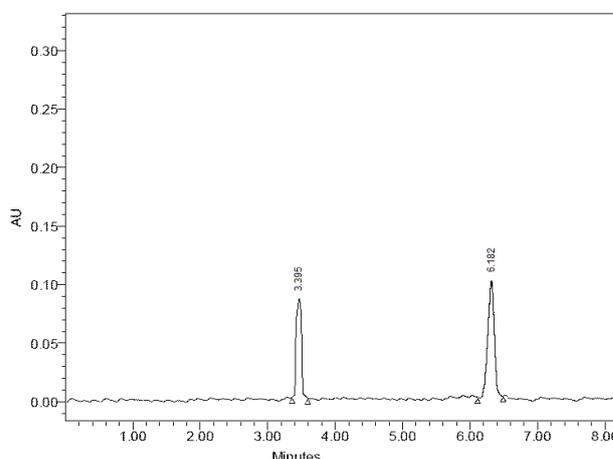


Fig. 2: Optimized chromatogram of Codeine and internal standard using mobile phase of Acetonitrile, methanol, and 1% triethylamine and Buffer solution in the ratio 70: 15: 15 %v/v/v.

3. RESULTS AND DISCUSSIONS

Validation

The analytical method was validated with respect to parameters such as linearity, precision, specificity and accuracy, limit of detection (LOD), limit of quantitation (LOQ) and robustness in compliance with ICH guidelines.

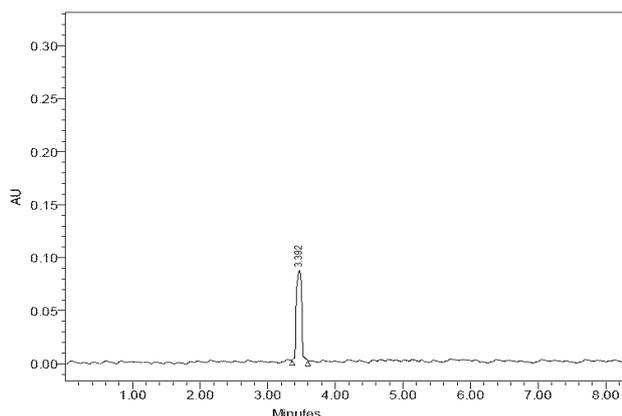


Fig. 3: Standard Chromatogram of Codeine and internal standard using mobile phase of Acetonitrile, methanol, and 1% triethylamine and Buffer solution in the ratio 70 : 15 : 15 %v/v/v.

Linearity and Range

The linearity of an analytical procedure is the ability to obtain test results that are directly proportional to the concentration of an analyte in the sample.

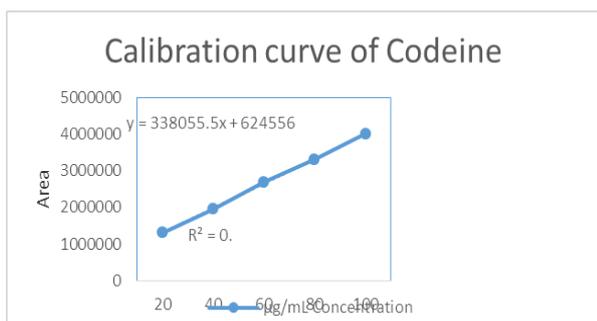
The calibration curve showed good linearity in the range of 20-100 µg/mL, for Codeine (API) with correlation coefficient of 0.9997. A typical calibration curve has the regression equation of $y = 338055.5x + 624556$ for Codeine. Results are given in Table 2.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ of Codeine were calculated by mathematical equation. $LOD = 3.3 \times \text{standard deviation} \div \text{slope}$ and $LOQ = 10 \times \text{standard deviation} \div \text{slope}$. The LOD of Codeine was found to be 2.093(µg/ml) and the LOQ of Codeine was found to be 6.3437(µg/ml). Results are given in Table 2.

Table 2: Summary of validation parameters for the proposed method.

Parameter	Codeine
Linearity	20- 100µg/ml
Intercept (c)	624556
Slope (m)	338055.5
Correlation coefficient	0.9997
LOD	2.093 (µg/ml)
LOQ	6.3437 (µg/ml)



Precision

The Precision of the method was studied in terms of intraday and interday precision of sample injections (20.44 µg/ml). Intraday precision was investigated by injecting six replicate samples of each of the sample on the same day. The % RSD was found to be 0.03%. Interday precision was assessed by analysis of the 6 solutions on three consecutive days. The % RSD obtained was found to be 0.03%. Low % RSD values indicate that the method is precise. The results are given in table 3.

Table 3: Results of Precision Studies.

Replicate	Codeine		
S.No.	Concentration Taken (µg/ml)	Area	%LC
1	20.44	1301862	99.99%
2		1302076	99.98%
3		1302242	99.97%
4		1302498	99.95%
5		1302654	99.93%
6		1302839	99.92%
Average			99.95%
Std.Dev			0.0280
% RSD			0.03%
Standard weight			20.44mcg
Standard potency			99.60 %

Robustness

Small deliberate changes in chromatographic conditions such as change in temperature (± 2°C), flow rate (± 0.1ml/min) and wavelength of detection (± 2nm) were studied to determine the robustness of the method. The results were in favour of (% RSD < 2%) the developed UPLC method for the analysis of Codeine. The results are given in table 4.

Table 4: Results of Robustness Studies.

Robustness Studies			
Parameter	Value	Peak Area	% RSD
Flow Rate	Low	1303979	0.02%
	Actual	1304134	
	Plus	1304382	
Temperature	Low	1303268	0.01%
	Actual	1303447	
	Plus	1303462	
Wavelength	Low	1303732	0.02%
	Actual	1303958	
	Plus	1304123	

Accuracy

To study the accuracy of method, recovery studies were carried out by spiking of standard drug solution to pre-analyzed sample at three different levels i.e., at 50, 100, and 150%. The resultant solutions were then reanalyzed by the proposed method. At each level of the amount, six

determinations were performed. From the data obtained, the method was found to be accurate. The % recovery

and %RSD were calculated and presented in Table 5.

Table 5: Results of accuracy study.

Codeine						
Level %	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery	Mean recovery (%)	Std.Dev	% RSD
50	10.29	10.25	99.61	99.66	0.0737	0.07%
100	20.58	20.53	99.75			
150	30.87	30.76	99.64			

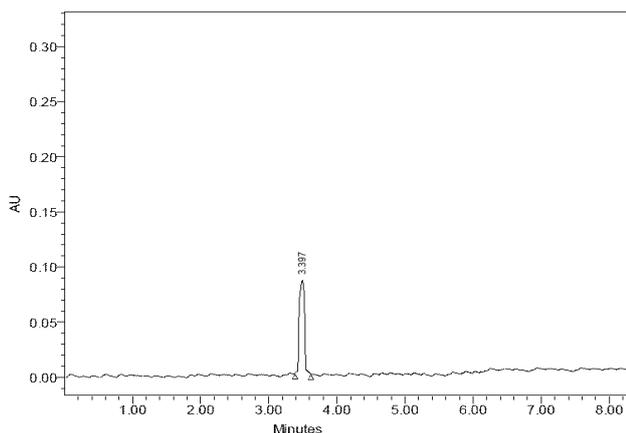


Fig. 4: Chromatogram Showing accuracy results.

Analysis of Formulation

Assay studies for the analysis of spray- dosage formulation of Codeine. Fixed chromatographic conditions were made use for the analysis of formulation and was found to be 100.34%.

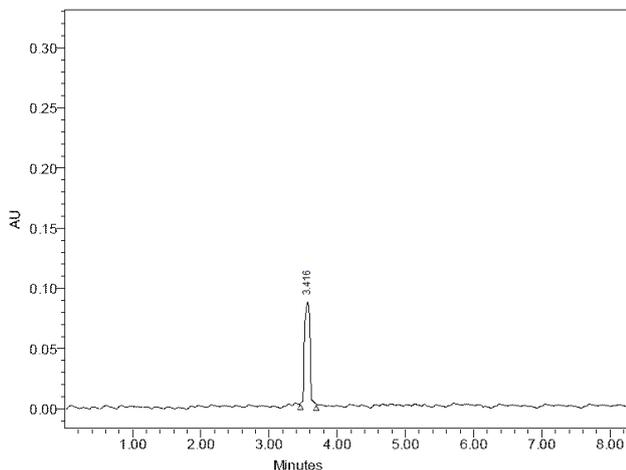


Fig. 5: Chromatogram of Assay Studies

4. CONCLUSION

The method provides selective quantification of Codeine without interference from blank affirming precise method. The proposed method is highly sensitive, reproducible, specific and rapid. The method was completely validated showing satisfactory data for all the method validation parameters.

The developed method was robust in the separation and quantification of Codeine in syrup dose. This method can be used for the routine analysis of production samples. The information presented herein could be very useful for quality monitoring of bulk samples and as well employed to check the quality during stability studies. The current method is validated for the assay study of the formulation and was found to be beneficial.

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