Quest Journals
Journal of Research in Pharmaceutical Science
Volume 3~ Issue 04 (2016) pp: 05-10
ISSN(Online) : 2347-2995
www.questjournals.org

Research Paper

Determination of Acetaminophen and Caffeine using reverse phase liquid (RP-LC) chromatographic technique

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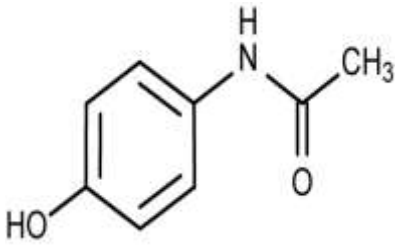
Received; 15 November 2016 Accepted; 06 December 2016; © The author(s) 2016. Published with open access at **www.questjournals.org**

ABSTRACT: *This paper elucidate development and validation of a high-performance liquid chromatographic analytical procedure for simultaneously determination of paracetamol and caffeine in a tablet formulation. Chromatographic determination was performed on a C18 column (4.5mm x 250 mm, 5µm) using a 0.01M KH_2SO_4 and Acetonitrile as mobile phase by (85:15) v/v at a flow rate of 1.0 ml / min with UV detection at 265 nm. The retention times of paracetamol and caffeine were found to be at 3.13 and 3.83 min, respectively. The method was validated for analytical parameters specificity, linearity, precision, accuracy, LOD and LOQ.*

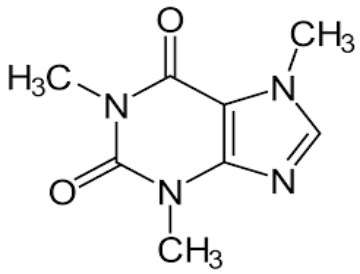
Keywords: *Paracetamol, Caffeine, High performance liquid chromatography, Method development, Validation.*

I. INTRODUCTION

Paracetamol (acetaminophen) is one of the most popular analgesics and antipyretic drugs [1]. Paracetamol is available in different dosage forms tablet, capsules, drops, suspensions and suppositories dosage forms. The usage of paracetamol alone or in combination with other drugs such as caffeine is well-established in the pharmaceutical formulations. Caffeine is used therapeutically in combination with ergotamine in the treatment of migraine or in combination with nonsteroidal anti-inflammatory drugs in analgesic formulations.



Paracetamol (acetaminophen)



Caffeine

Several methods for simultaneous determination of paracetamol and caffeine have been recently reported such as thin layer chromatography, voltammetry, chromatographic [2-6] and spectrophotometric techniques [7-11].

The objective of this study was to develop an analytical procedure for simultaneously determination of paracetamol and caffeine in a tablet and validate a specific, accurate, linearity, precision. It was entrenched that the developed analytical procedure was successfully used for routine analysis of paracetamol and caffeine in model drug dosage form without any interference from involved excipients.

II. MATERIALS AND METHODS

2.1 Reagent and Chemicals

Double distilled water, Paracetamol was used from USP reference standard, caffeine, analytical grade KH_2SO_4, and HPLC grade acetonitrile were obtained from Merck chemicals.

2.2 Instrument and Chromatographic Condition

The method development was performed with a High pressure liquid chromatographic system consisting of a waters model 2695 solvent delivery system, a waters model 2996 photodiode array detector. The system was controlled and data analysis were performed with the empower software. The detector was set at 265nm and peak areas were integrated automatically by using empower software. Separation was carried out at ambient temperature using XDB C18 column (4.5mm x 250 mm, 5µm).

2.3 Preparation of Buffer Solution (Mobile Phase –A)

The accurate weights 1.36 g of Potassium dihydrogen phosphate dissolves in 1000 ml of distilled water and filtered through 0.45µ filter paper.

2.4 Preparation of Standard and Sample Solutions

The standard stock solution of paracetamol (150 mg) and caffeine (7.5 mg) were accurately weighed and transferred to 100 ml standard flask with dissolved in the initial concentration of mobile phase solution and make up to volume with same diluent. Further 1 ml of standard stock solution diluted to 100 ml with diluent to yield a solution with final concentration of 15(µg/ml) and 0.075(µg/ml) of paracetamol and caffeine respectively.

2.5 Specificity

The ability of an analytical method to unequivocally assess the analyte in the presence of other components can be demonstrated by evaluating specificity.

2.6 Linearity

The linearity of the method was determined at six concentration levels ranging from 25 to 150% level for paracetamol and caffeine. The graph constructed by plotting peak areas versus concentration of solution.

2.7 Precision

The precision of the method was evaluated by five times inject of mixture of paracetamol and caffeine standard solution.

2.8 Accuracy

Accuracy was studied by adding known concentration (100 % level) of paracetamol and caffeine to the dilute level of placebo preparation and comparing the actual and measured concentrations.

2.9 Limits of Detection and Quantification

Limits of detection (LOD) were established at s signal to noise ratio (S/N) of 3. Limits of quantification (LOQ) were established at a signal to noise ratio more than 10.

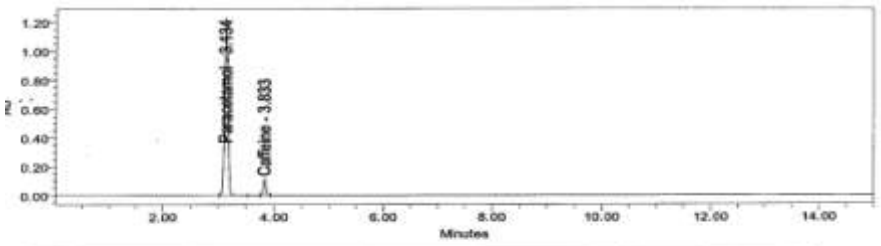
III. RESULTS AND DISCUSSION

3.1 Specificity and System Suitability

From the chromatogram shown in Fig.1, it is event, that under the proposed chromatographic conditions, paracetamol and caffeine were completely separated. The chromatographic parameters such as resolution, selectivity and peak asymmetry were satisfactory for these compounds (Table-1). The calculated resolution values between each peak were not less than 7.0 and tailing factor was not more than 1.50.

Table.1: System suitability parameters for paracetamol and caffeine

Compound name	System suitability parameters			
	Retention time (min)	Resolution	Plate Count	Tailing factor
Paracetamol	3.13	-	15268	1.09
Caffeine	3.83	7.2	31532	1.08



	RT	Area	% Area	USP Plate Count	USP Tailing	USP Resolution	RT Ratio	Name
1	3.13	4669188	92.85	15268	1.09			Paracetamol
2	3.83	359442	7.15	31532	1.08	7.44	1.22	Caffeine

Fig.1: System suitability chromatogram of paracetamol and caffeine

3.2 Linearity

Linearity of the curves was validated by the value of correlation coefficients (r^2= 0.99). The calibration value shown in Table-2 and chromatogram of different concentration (Fig -2 to Fig-8).

Table.2: Linearity solution of paracetamol and caffeine

Run No.	Paracetamol		Caffeine	
	Concentration (%)	Area	Concentration (%)	Area
1	25	48989	25	3406
2	50	2375171	50	180612
3	75	3521630	75	268991
4	100	4732205	100	362368
5	120	5768608	120	446339
6	150	7102902	150	554823
correlation coefficients (r^2)		**0.990469869**		**0.99201014**

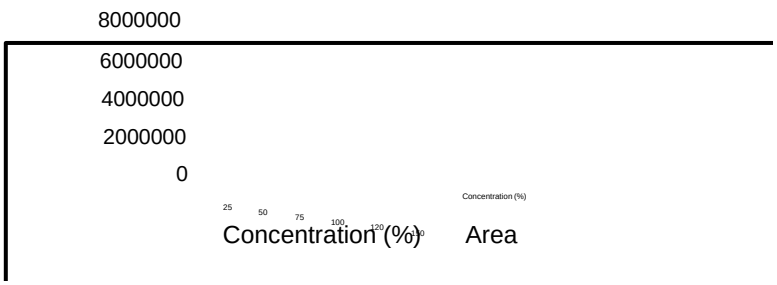
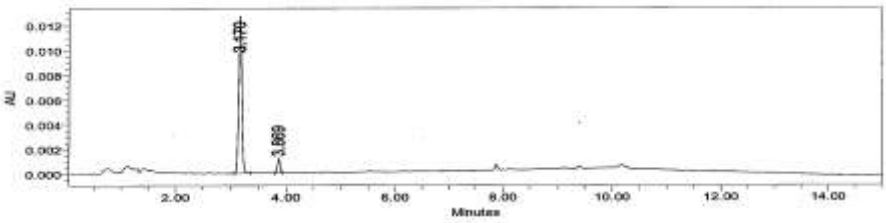
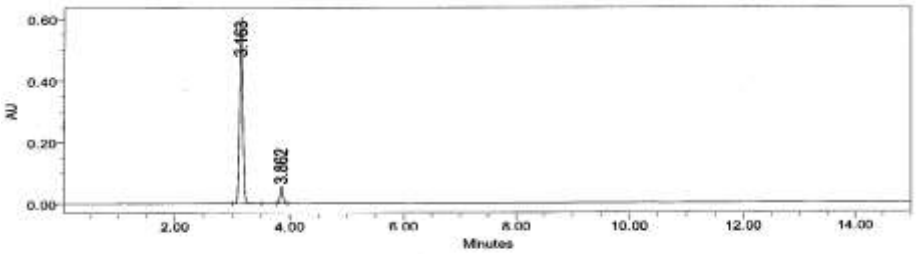


Fig.2: Linearity solution of paracetamol and caffeine



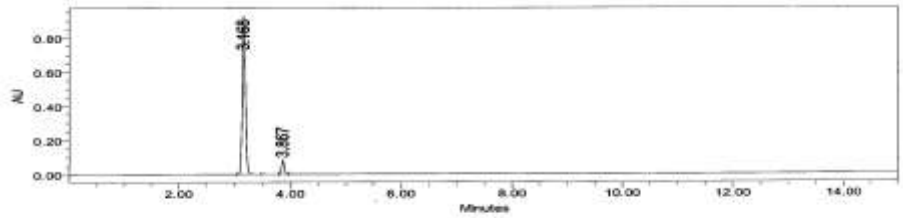
	RT	Area	% Area
1	3.17	48989	93.50
2	3.87	3404	6.50

Fig.3: Linearity solution (25% level) of paracetamol and caffeine



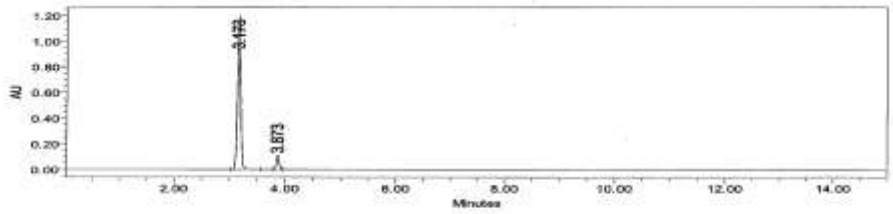
	RT	Area	% Area
1	3.16	2375171	92.93
2	3.86	180612	7.07

Fig.4: Linearity solution (50% level) of paracetamol and caffeine



	RT	Area	% Area
1	3.17	3521630	92.90
2	3.87	268991	7.10

Fig.5: Linearity solution (75% level) of paracetamol and caffeine



	RT	Area	% Area
1	3.17	4732205	92.89
2	3.87	362368	7.11

Fig.6: Linearity solution (100% level) of paracetamol and caffeine

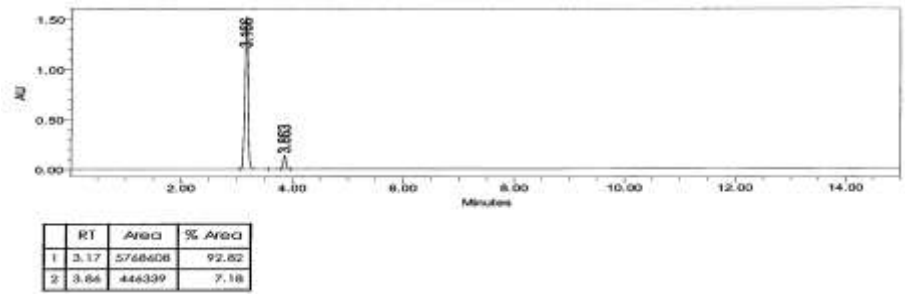


Fig.7: Linearity solution (120% level) of paracetamol and caffeine

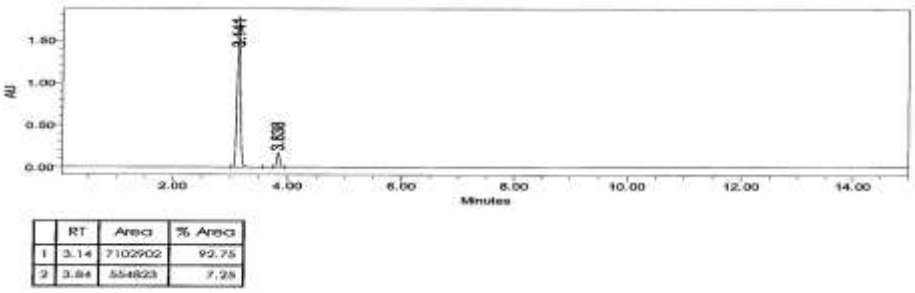


Fig 8: Linearity solution (150% level) of paracetamol and caffeine

3.3 Precision

The precision of the method expressed as the relative standard deviation not more than 1.0% of both compounds (Table-3)

Table.3: Precision test parameters for paracetamol and caffeine

Run No.	Paracetamol		Caffeine	
	Retention Time	Area	Retention Time	Area
1	3.14	4658530	3.84	357491
2	3.13	4658585	3.83	358676
3	3.15	4669660	3.85	358952
4	3.15	4672514	3.85	359059
5	3.14	4667571	3.84	358580
AVG	**3.14**	**4665372**	**3.84**	**358551**
SD	**0.007**	**5781**	**0.007**	**558**
RSD	**0.24**	**0.12**	**0.19**	**0.16**

3.4 Accuracy

Recovery of paracetamol and caffeine from placebo was determined at 100 % level concentrations. Average recovery was 99.0% for paracetamol and 98.9% for caffeine (Table- 4)

Table.4: Accuracy test parameters for paracetamol and caffeine

Run No	Paracetamol				Caffeine			
	Area of LOQ level	Area of 100 % level	Area of spiked level	Recovery (%)	Area of LOQ level	Area of 100 % level	Area of spiked level	Recovery (%)
1	49390	4658530	4707148	99.0	3805	357491	363000	98.9
2	48906	4658585	4703408		3711	358676	362811	
3	48537	4669660	4628371		3870	358952	357105	
Average	**48944**	**4662258**	**4679642**		**3795**	**358373**	**360972**	

3.5 Limit of Detection

The limit of detection was evaluated based on signal to noise ratios. The LOQ for paracetamol and caffeine were found to be 1.5 and 0.075 µg/ml respectively. The LOD solution prepared from LOQ solution diluted one third diluted (Fig .9).

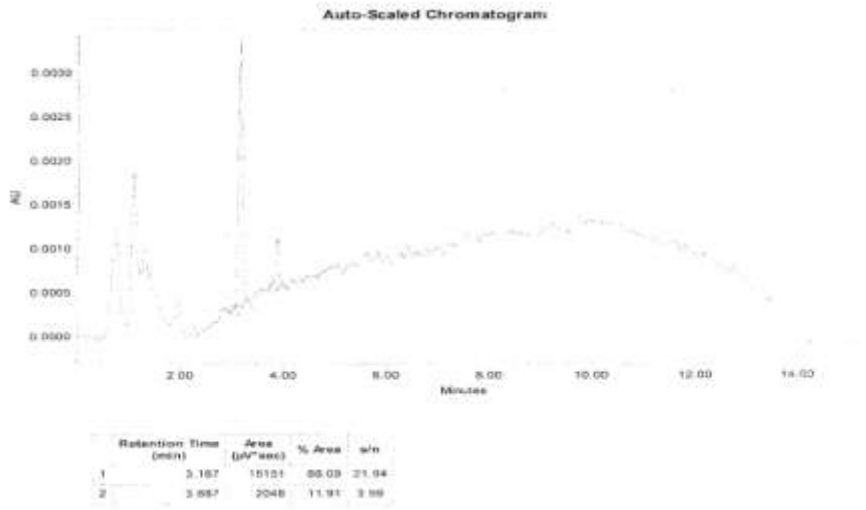


Fig 9: Chromatogram of LOD level solution of paracetamol and caffeine

IV. CONCLUSION

The validated reverse phase liquid chromatographic method developed in this study proved to be simple, accurate, precise and sensitive. The developed method is suitable for the identification and quantification of the combination of paracetamol and caffeine. A high percentage of recovery shows that the method can be successfully used on a routine basis.

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