Research Article **CODEN: AJPAD7** **ISSN: 2321 - 0923**

Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry

Journal home page: www.ajpamc.com

METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF IBUPROFENAND CODEINE PHOSPHATE IN TABLET DOSAGE FORMS BY RP-HPLC

P. Rajavel*

[1*]Department of Pharmaceutical Analysis, A.M. Reddy Memorial College of Pharmacy, Narasaraopet, Guntur, Andhra Pradesh, India.

ABSTRACT

A validated reverse phase HPLC method has been developed for the simultaneous estimation of ibuprofen and codeine phosphate in Pharmaceutical dosage forms. The chromatographic separation was carried out on Kromasil 100, $C_{18,}$ 5 μ (250 x 4.6 mm) column at ambient temperature (25^{o}C) and Buffer: Acetonitrile (gradient) was used as mobile phase at the flow rate of 1 ml/min with PDA detection at 220 nm. The retention time of ibuprofen and codeine phosphate were found to be 18.003min and 9.676 min respectively. Linearity of both drugs were found to be in the concentration range of 360-480 µg/ml for ibuprofen and 20.48-30.72 µg/ml for codeine phosphate. The developed HPLC method was validated by determining its sensitivity, selectivity, linearity, accuracy and precision. The accuracy of the method was assessed by percentage recovery studies at three different levels at 80%, 100% and 120% of its working concentration. The percentage recovery of both drugs in the developed method was found to be in the ranges of from 98.5-101% that indicates the good accuracy of the method. This developed method can be used for the routine analysis for the estimation of ibuprofen and codeine phosphate in tablet dosage form.

KEYWORDS

Ibuprofen, Codeine Phosphate and RP-HPLC.

Author for Correspondence:

Rajavel P,
Department of Pharmaceutical Analysis,
A.M. Reddy Memorial College of Pharmacy,
Narasaraopet, Guntur, Andhra Pradesh, India.

Email: velraj512@gmail.com

INTRODUCTION[1-2]

Ibuprofen is chemically 2- (4 isobutyl phenyl) propionic acid. It is a non-steroidal anti-inflammatory drug and it is used as an analgesic, antipyretic and anti-inflammatory in the treatment of pain. It is official in IP, BP, and USP. Codeine Phosphate is chemically known as (5R, 6S) - 4,5 epoxy- 3 methoxy - N -methyl morphin-7 en -6-ol and it is used as Narcotic Analgesic, Anti-

diarroheal, Cough Suppressant. The structure of ibuprofen and codeine phosphate is shown in Figure No.1 and 2. Literature review revealed that several methods have been reported for the quantification of Ibuprofen and Codeine Phosphateindividually[3-5]. However there is no HPLC method have been reported for the simultaneous estimation of Ibuprofen and Codeine Phosphate in Pharmaceutical formulations. The present work describes a new, simple, rapid, accurate and precise RP-HPLC method developed and validated for the estimation of Ibuprofen and Codeine Phosphate simultaneously.

MATERIAL AND METHOD

Chromatographic separation was carried out on waters 2695 separations module, 2998 PDA detector and auto injector. All chemicals used were analytical grade and the solvents which are used in the mobile phase were HPLC grade.

Preparation of Mobile Phase

Preparation of buffer (Pump A)

3.12g sodium dihydrogen ortho phosphate was dissolved in 1000 ml distilled water and p^H was adjusted to 3.2 with orthophosphoric Acid.

Pump B: Acetonitrile (Table No.1).

Diluent

Distilled water and methanol in the ratio of 50:50.

Standard solution of ibuprofen

100mg of ibuprofen standard was accurately weighed in 100ml standard flask and 10ml of methanol was added to it. Then it was sonicated to dissolve and the volume was made up with diluent 10ml was taken in a 25ml standard flask and the volume was adjusted to 25ml with diluent to get a concentration of 400 µg/ml of ibuprofen. 10 µl of the solution was injected and the chromatogram was recorded.

Standard solution of codeine phosphate

32mg of codeine phosphate standard was accurately weighed in 100ml standard flask and 70ml of diluent was added to it. Then it was sonicated to dissolve and volume was made up with diluent. 2ml of this solution was taken in a 25ml standard flask and the volume was adjusted to 25ml with diluent to get a concentration of 25.6 µ g/ml of codeine phosphate. 10 µl of the solution was injected and the chromatogram was recorded.

Preparation of mixed standard solution

About 400mg of ibuprofen and 25.6mg of codeine phosphate were taken and transferred it in a 200ml volumetric flask, 10ml of methanol and 100ml of diluent were added to it, then it was sonicated for 15mins. The volume was made up with diluent and the solution was centrifuged. 5ml was taken and diluted to 25ml with diluent to get a concentration of 400 µ g/ml of ibuprofen and 25.6 µ g/ml of codeine phosphate[3-4]. 10 µ l of the solution was injected and the chromatogram was recorded and it is shown in Figure No.3.

Preparation of Sample solution

About 560mg of the powdered tablets (eqv to 400mg of Ibuprofen and 25.6mg of codeine phosphate) was weighed and transferred it in to a 200ml volumetric flask, 10ml methanol and 100ml of diluent were added to it. Then it was sonicated for 15 minutes. The volume was made up with diluent and solution was centrifuged. 5ml was taken and diluted to 25ml with diluent, to get a concentration of 400µg/ml of Ibuprofen and 25.6 µ g/ml codeine phosphate. The solution was filtered through 0.45 µ membrane. The amount of Ibuprofen and codeine Phosphate present in the tablet formulation was calculated by comparing the peak area of the standard.

Method development and validation[5-10]

The RP HPLC procedure was optimized with a view to develop an effective method for the simultaneous estimation of Ibuprofen and Codeine Phosphate in tablet dosage forms. Preliminary tests were performed in order to select the adequate and optimum chromatographic condition. Kromasil 100, $C_{18,}$ 5 µ (250 x 4.6 mm)column was used as a stationary phase and the separation was achieved by using mobile phase consisting of Buffer: Acetonitrile in gradient mode. Chromatogram of standard solution containing Ibuprofen and Codeine Phosphate is shown in Figure No.3. The developed HPLC method for the simultaneous estimation

Ibuprofen and Codeine Phosphate was validated as per the ICH guideline in terms of specificity, linearity, accuracy, precision, ruggedness and robustness, limit of detection and limit of quantification[11].

Specificity

The specificity of the method was determined by spiking the solution of placebo with the working standard solution containing Ibuprofen and Codeine Phosphate and this solution was analyzed as per the method described. The recorded chromatogram was compared with chromatogram of standard solution containing Ibuprofen and Codeine Phosphate to check the interference of the placebo with the response produced by the Ibuprofen and Codeine Phosphate.

System suitability

The system suitability of the method was determined by five replicate analysis of the standard solution containing Ibuprofen and Codeine Phosphate to check the reproducibility of the chromatographic system. In this method the reproducibility of peak area, retention time, theoretical plate and tailing factor of the peaks of Ibuprofen and Codeine Phosphate were checked.

Linearity

The linearity of the method was assessed by analyzing the standard solution containing Ibuprofen and Codeine Phosphate at 5 different levels (Ibu: 320-480 µg/ml, codeine: 20.48-30.72 µg/ml). The calibration curve of peak area (vs) concentration was plotted and correlation coefficient and regression line equation for both drugs were determined. The results for the linearity study are given in Table No.4. The calibration curve of Ibuprofen and Codeine Phosphate is shown in Figure No.4 and 5 respectively.

Accuracy

Accuracy of the method was assessed by analyzing the solutions containing Ibuprofen and Codeine Phosphate at three different levels 80%, 100% and 120% of its working concentration. Standard solutions were spiked with placebo and the percentage recovery of the drugs from the placebo was calculated. The results for the recovery study are given in Table No.5 and 6.

Precision

The system precision was evaluated by measuring 6 successive injections of 10 µl of standard solution. The method precision was determined by preparing the sample of single batch of Ibuprofen and codeine Phosphate from the tablet formulation for six times and six successive injection of 10 µl of working sample solution were injected and the chromatograms were recorded and The % RSD of the obtained results was calculated.

Ruggedness and robustness

The ruggedness of the method was ascertained by carrying out the assay of the sample on different instrument by different analyst. The chromatogram which is recorded for ruggedness studies is shown in Figure No.6. Robustness of the method was determined by analyzing the sample by deliberately changed chromatographic conditions such as change in column, flow rate (0.2ml/min) and detection wavelength (±2 nm).

LOD and LOQ

The limit of detection and limit of quantification of Ibuprofen and Codeine Phosphate were calculated by using standard deviation of the responses and the slope of the calibration curve of Ibuprofen and Codeine Phosphate. LOD and LOQ were estimated by using the following formula,

$LOD = (3.3 \times \sigma) / S$

$LOQ = (10 \times \sigma) / S$

Where, σ is the standard deviation of the response

S is the slope of the calibration curve.

Analysis of Ibuprofen and Codeine Phosphate in Tablet formulation

For the assay of Ibuprofen and Codeine Phosphate in tablet formulations, twenty tablets were weighed and the average weight of the tablets was 281.4mg. The weighed tablets were crushed in to fine powder about 560mg of the powdered tablets (equivalent to 400mg of Ibuprofen and 25.6mg of codeine phosphate) was weighed and transferred it in to a 200ml volumetric flask, 10ml methanol and 100ml of diluent were added to it. Then it was sonicated for 15 minutes. The volume was made up with

diluent and solution was centrifuged. 5ml was taken and diluted to 25ml with diluent, to get a concentration of 400 µg/ml of Ibuprofen and 25.6 µg/ml codeine phosphate. The solution was filtered through 0.45 µ membrane. The amount of Ibuprofen and codeine Phosphate present in the tablet formulation was calculated by comparing the peak area of the sample with that of standard and reports are given in Table No.2.

RESULT AND DISCUSSION

A simple, accurate and precise RP HPLC method was developed for the simultaneous estimation of Ibuprofen and Codeine phosphate in Pharmaceutical dosage forms. All the results were summarized in Table No.3. Specificity of the method was found out through non-interference of the placebo in identical conditions of assay. This confirms the specificity of the developed method. Linearity of the drug was obtained in the range of 320 - 480 µg/ml for Ibuprofen and 20.48 - 30.7 µg/ml for Codeine Phosphate. The linearity coefficient and percentage curve fitting slope was found to be 0.99978 and 99.97% for Ibuprofen 0.9997 and 99.97% for Codeine Phosphate. The limit of detection of Codeine Phosphate was found to be 0.256 µg/ml. The limit of quantification of Codeine Phosphate was found to be 1.024 µg/ml. Accuracy of the method was determined through recovery studies of the drug. Recovery of the drug is well within acceptance limits (98.5% to 101%). Precision of the method was determined by assay of drug formulations by replicate injection and precision of system was determined by using standard solution. % RSD of the assays is found to be within the limits of 2%. Thus the developed method is found to provide high degree of precision and reproducibility.

Ruggedness was determined by performing the same assay on different days, assay being carried out by different analyst using different instrument. The test results were within the limits 98.5 to 101%. The result is found to be reproducible. In spite of variation in conditions which could be normally expected from analyst to analyst. Robustness was determined by carrying out the assay by changing flow rate and wavelength. Percentage purity was found to be within limit 98.5 to 101%. The values of percentage purity obtained with the change in flow rate, wavelength makes it possible to carryout the method for Ibuprofen and codeine Phosphate with a small variation in flow rate and wavelength. This indicates the lack of influence on test results by operational and environmental variables for developed method. System suitability was determined by performing the assay with the same sample repeatedly. The number of theoretical plates was found to be 150556 for Ibuprofen and 142290 for Codeine Phosphate. The tailing factor was found to be 1.05 for Ibuprofen and 1.19 for Codeine Phosphate and it is indicating good and complete separation of the two components from each other with well-defined base line.

Table No.1: Gradient information

S.No	Time	Flow	% A	% B
			Buffer	Acetonitrile
1	0.01	1.0	90	10
2	5	1.0	90	10
3	12	1.0	30	70
4	16	1.0	30	70
5	18	1.0	90	10
6	22	1.0	90	10

Table No.2: Quantitative estimation (Assay)

S.No	Content	Label claim (mg)	Peak area	Amount present (mg)	Percentage purity
1	Ibuprofen	200mg	9707657	199.79	99.90
2	Codeine Phosphate	20mg	714970	12.69	99.14

Acceptance criteria: 98.5 – 101% w/v.

Table No.3: Results of Validation of the developed HPLC Method

S.No	Parameters	Result for RP-HPC		Acceptance Criteria
		Ibuprofen	Codeine Phosphate	
1	Specificity	Complies	Complies	No interference of excipients
2	**Linearity / Range**			
	Correlation coefficient	0.99978	0.99997	0.997
	Percentage curve fitting	99.97%	99.99%	99.7%
3	LOD	-	0.256 ppm	-
4	LOQ	-	1.024 ppm	-
5	Accuracy	99.13-100.95%	98.64-99.60%	98.5 - 101%
6	**Precision**			
	System Precision	0.17	0.93	2% (RSD)
	Method Precision	0.27	0.18	2% (RSD)
7	**Ruggedness**			
	Instrument-1 Analyst-1	99.66	99.83	98.5-101%
		0.65	0.68	2% (RSD)
	Instrument-2 Analyst-2	99.33	100.55	98.5 - 101%
		0.23	0.16	2% (RSD)
8	Robustness Lower Parameter	100.11	99.83	-

	(flow rate 0.8 ml, wavelength 218 nm)	100.17	100.42	98.5 - 101%
	Higher Parameter (flow rate 1.2 ml, wavelength 222 nm)	99.97 99.66	100.38	-
	Column-1		100.75	-
	Column-2			
9	**System Suitability Parameters**			
	Theoretical Plates	150556	142290	-
	Taling factor	1.05	1.19	
	RSD%	0.19	0.15	
	Resolution	62		

Table No.4: Linearity data

S.No	Concentration (µg/ml)	Concentration (%)	Peak Area
Ibuprofen			
1	320.0	80	7600862
2	360.0	90	8519969
3	400.0	100	9443090
4	440.0	110	10443296
5	480.0	120	11679143
Codeine Phosphate			
1	20.48	80	557788
2	23.04	90	626719
3	25.60	100	695726
4	28.16	110	767363
5	30.72	120	838220

Table No.5: Recovery study of Ibuprofen

S.No	Sample - ID	Sample injected in (µg/ml)	Area obtained	Sample found in (µg/ml)	% Recovery
1	80%	323.90	7851256	326.53	100.81
		324.90	7886027	327.97	100.95
		324.30	7879221	327.69	101.05
2	100%	398.10	9495001	394.89	99.19
		398.00	9443783	392.76	98.68
		400.10	9536509	396.62	99.13
3	120%	481.50	11612257	482.95	100.30
		480.60	11523446	478.36	99.53
		480.20	11478979	477.40	99.42

Table No.6: Recovery study of Codeine Phosphate

S.No	Sample - ID	Sample injected in (µg/ml)	Area obtained	Sample found in (µg/ml)	% Recovery
1	80%	20.20	556018	20.07	99.34
		20.30	554830	20.02	98.64
		20.20	555695	20.05	99.28
2	100%	25.30	698163	25.20	99.59
		25.40	694969	25.08	98.74
		25.00	685326	24.73	98.93
3	120%	30.60	844339	30.47	99.58
		30.80	844770	30.49	98.98
		30.60	844530	30.48	99.60

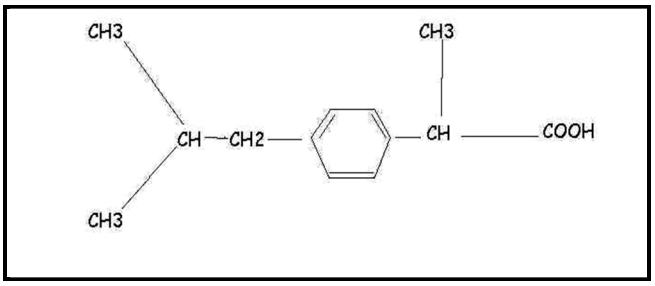

Figure No.1: Structure of Ibuprofen

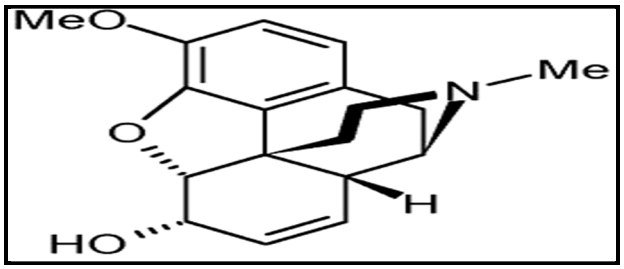

Figure No.2: Structure of Codeine phosphate

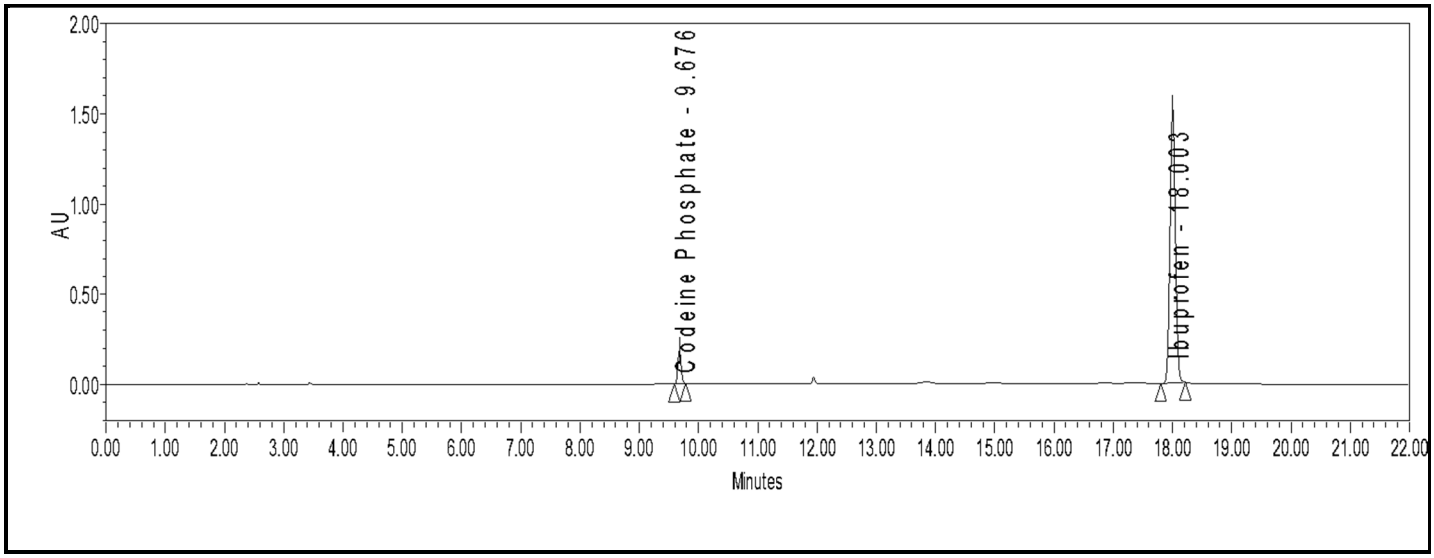

Figure No.3: HPLC Chromatogram of Ibuprofen and Codeine phosphate standard

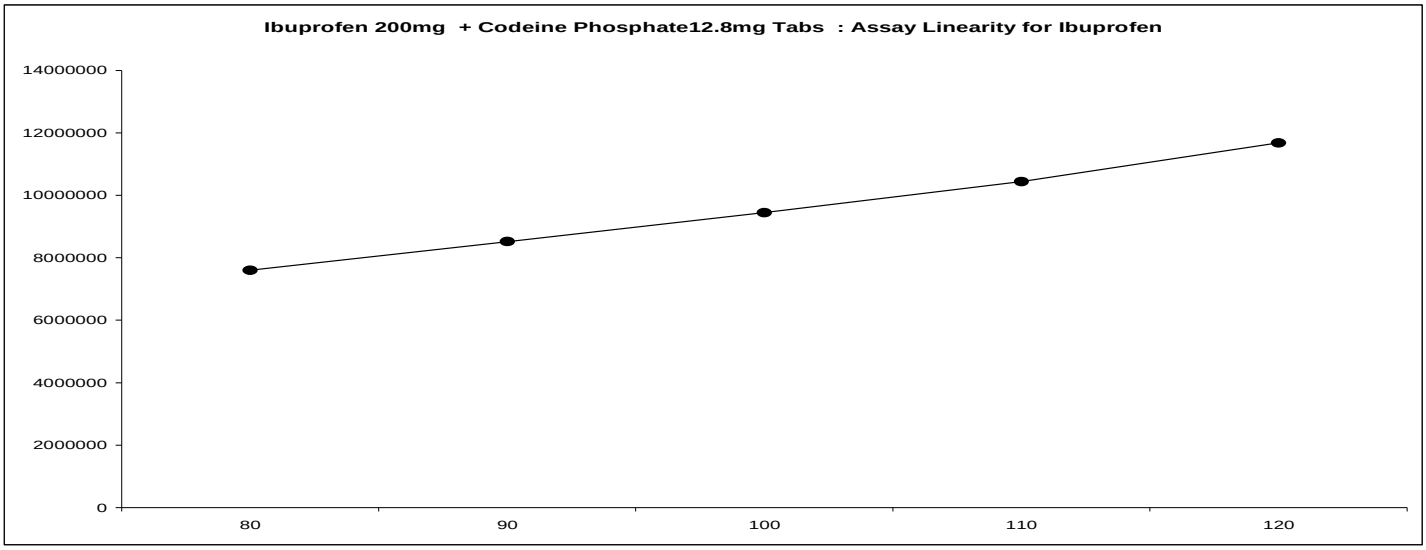

Figure No.4: Calibration curve of Ibuprofen

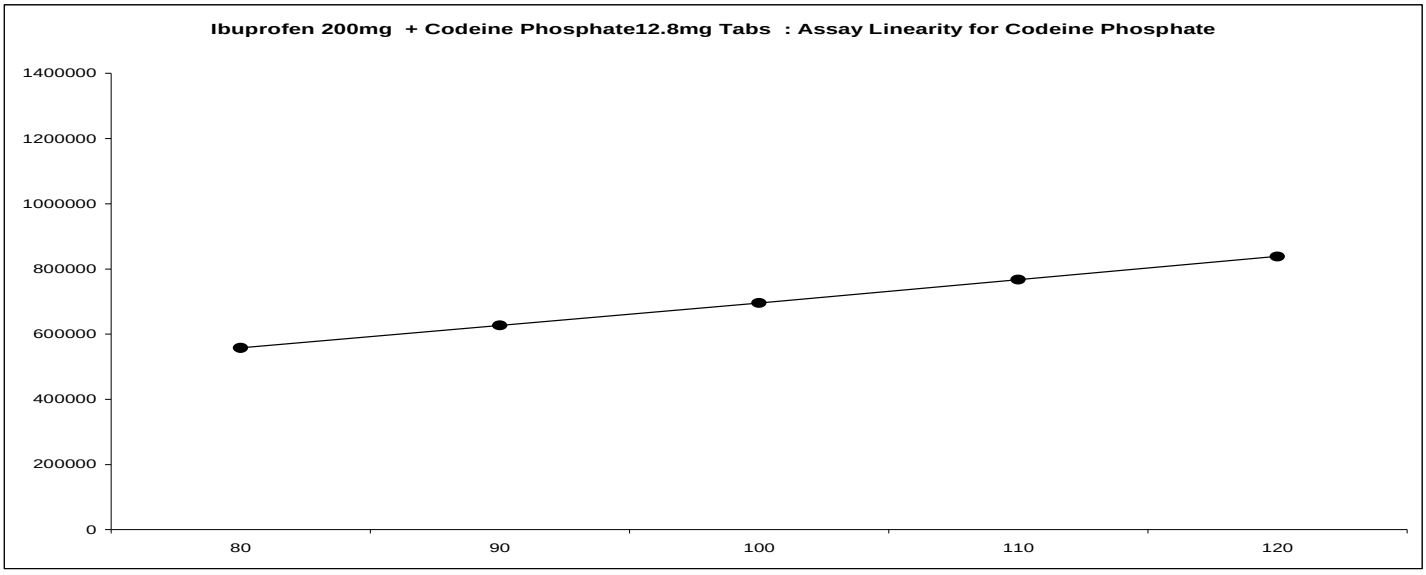

Figure No.5: Calibration curve of Codeine Phosphate

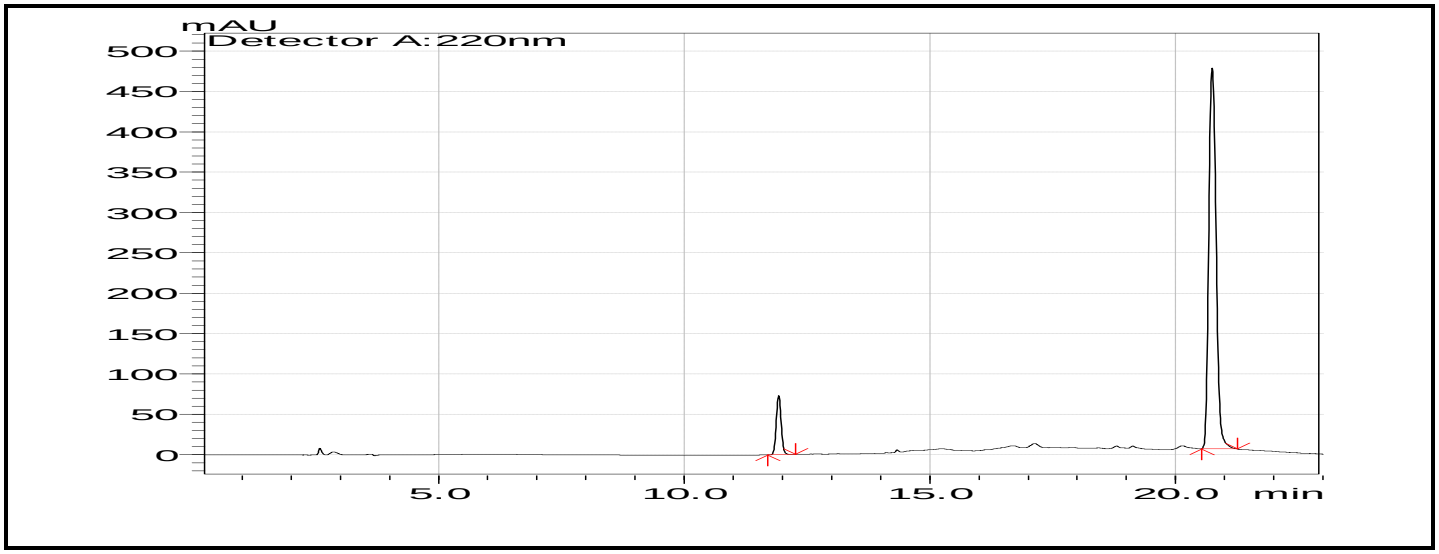

Figure No.6: Chromatogram of Ibuprofen and Codeine phosphate for Ruggedness test (Instrument -2, Analyst-2)

CONCLUSION

As the literature survey reveals there is no method has been reported for the simultaneous determination of these two drugs, Ibuprofen and Codeine Phosphate in combination. The developed method is cheap, easy and it gives sharp peak with high resolution. The developed method is applied for the determination of Ibuprofen and Codeine Phosphate simultaneously. The assay results are with the label claim of the formulation. The developed method is validated as per ICH guidelines using parameters like Accuracy, Precision Linearity and Range, Specificity, Ruggedness, LOD, LOQ and Robustness. Hence the developed method is found to be satisfactory and it complies with all validation parameters. So

this developed method can be used for the routine analysis of Ibuprofen and Codeine Phosphate simultaneously in tablet dosage form.

ACKNOWLEDGEMENT

I'm very thankful to M/s Genovo development Services for providing the gift sample of pure drug of Ibuprofen, Codeine Phosphate and commercial tablet formulation. We would also like to thank the Manager, R and D department, Genovo development Services, Bangalore for providing the necessary facilities to carry out this work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBILIOGRAPHY

1. Beckett A H and Stenlake J B. "Practical Pharmaceutical chemistry", *CBS Publisher and distributor, New Delhi,* 4th edition, 1996, 85-157.
2. Sethi P D. "HPLC Quantitative Analysis of Pharmaceutical formulations", *CBS Publisher and distributor, New Delhi,* 1996.
3. Gasco-Lopez A I, Izquierdo-Hornillos R, Jimenez A. "LC method development for ibuprofen and validation in different pharmaceuticals", *Pharmaceutical Biomedical Analysis,* 21(1), 1999, 143-9.
4. Haikala V E, Heimonen I K, Vuorela H J. "Determination of ibuprofen in ointments by reverse-phase liquid chromatography", *Journal of pharmaceutical Sciences,* 80(5), 1991, 456-8.
5. Ko C Y, Marziani F C, Janicki C A. "High-performance liquid chromatographic assay of codeine with acetaminophen in tablet dosage forms", *Journal of Pharmaceutical Sciences*, 69(9), 1980, 1081-4.
6. Madelaine R Whelan, James L Ford, Mark W Powell. "Simultaneous determination of ibuprofen and hydroxypropylmethylcellulose (HPMC) using HPLC and evaporative light scattering detection", *Journal of Pharmaceutical Biomedical Analysis*, 30(4), 2002, 1355-9.
7. Murat Kartal. "LC method for the analysis of paracetamol, caffeine and codeine phosphate in pharmaceutical preparations", *Journal of Pharmaceutical and Biomedical Analysis*, 41(4), 2006, 1463-1467.
8. Nitsche V, MascherH. "Determination of codeine in human plasma by reverse-phase high-performance liquid chromatography", *Journal of Pharmaceutical Science*, 73(11), 1984, 1556-8.
9. Papadoyannis N Caddy. "Rapid analysis for Codeine by reversed-phase high-performance liquid chromatography", *Analytical Letters*, 19, (9 and 10), 1986, 1065 - 1081.
10. Rafifa Hamoudova and Marie Pospisilovaa. "Determination of ibuprofen and flurbiprofen in pharmaceuticals by capillary zone electrophoresis", *Journal of Chromatography*, 24(349), 1997, 112-118.
11. Whelan M R, Ford J L, Powell M W. "Simultaneous determination of ibuprofen and hydroxyl propylmethyl cellulose (HPMC) using HPLC and evaporative light scattering detection", *Journal of Pharmaceutical and Biomedical Analysis,* 7, 30(4), 2002, 1355-1359.

Please cite this article in press as: Rajavel P. Method development and validation for the simultaneous estimation of Ibuprofen and Codeine phosphate in Tablet dosage forms by RP-HPLC, *Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry,* 1(1), 2013, 8 - 17.