International Journal of Pharmacy and Industrial Research (IJPIR)

ISSN:2231-6567

IJPIR |Volume 13 | Issue 3 | July - Sept - 2023
Available online at: www.ijpir.com

Research article ***Pharmaceutical Analysis***

A new analytical method development and validation for the estimation of aspirin and caffeine in active pharmaceutical ingredient and tablet dosage form by RP-HPLC

Inumarthy Meenakshi*[1], Dr. Ch. Prasad [1], A. Venkateswara Rao[1]

[1]*Department of Pharmaceutical Quality Assurance, Pydah College of Pharmacy Patavala, Andhra University, Kakinada, Andhra Pradesh, India.*

Corresponding Author: Inumarthy Meenakshi
Published on: September 11, 2023

ABSTRACT

A new, simple, rapid, accurate and precise Reverse Phase High Performance Liquid Chromatographic method has been developed for the validated of Aspirin and Caffeine, in Active pharmaceutical Ingredient form as well as in combined tablet dosage form. Chromatography was carried out on Symmetry ODS C18 (4.6mm × 250mm, 5µm) column using a mixture of Methanol: Acetonitrile (35:65v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 273 nm. The retention time of the Aspirin and Caffeine, was 2.085, 5.262 ± 0.02min respectively. The method produce linear responses in the concentration range of 30-70mg/ml of Aspirin and 6-14mg/ml of Caffeine,. The mean % assay of marketed formulation was found to be 100.04%, and % recovery was observed in the range of 98-102%. Relative standard deviation for the precision study was found <2%.The developed method is simple, precise and rapid, making it suitable for estimation of Aspirin and Caffeine in API and combined tablet dosage form. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Aspirin and Caffeine, RP-HPLC, Validation, ICH Guidelines.

INTRODUCTION

Analytical methods development and validation play important roles in the discovery, development, and manufacture of pharmaceuticals. The current good manufacturing practice (CGMP) and food drug administration (FDA) guidelines insist for adoption of sound methods of analysis with greater sensitivity and reproducibility. Development of a method of analysis is usually based on prior art (or) existing literature, using the same (or) quite similar instrumentation .It is rare today that an HPLC-based method is developed that does not in same way relate (or) compare to existing, literature based approaches. Today HPLC (high performance liquid chromatography) is the method of choice used by the pharmaceutical industry to assay the intact drug and degradation products. The appropriate selection and chromatographic conditions ensure that the HPLC method will have the desired specificity. UV spectroscopy is also a simple analytical tool widely used for routine assay of drugs. Hence for the assay of the selected drugs HPLC and UV spectroscopy has been chosen for these proposed methods.

The developed chromatographic methods further validated as per ICH or USFDA guidelines for all the critical parameters. To access the precision and to evaluate the results of analysis the analyst must use statistical methods. These methods include confidence limit, regression analysis to establish calibration curves. In each analysis the critical response parameters must be optimized and recognized if possible.

Pharmaceutical analysis plays a major role today, and it can be considered as an interdisciplinary subject. Pharmaceutical analysis derives its principles from various branches like chemistry, physics and microbiology etc. Pharmaceutical analytical techniques are applied mainly in two areas, quantitative analysis and qualitative analysis, although there are several other applications.

Drugs and pharmaceuticals are chemicals or like substances, which or of organic inorganic or other origin. Whatever may be the origin, we some property of the medicinal agent to measure them quantitatively or qualitatively.

In recent years, several analytical techniques have been evolved that combine two or more methods into one called "hyphenated" technique e.g. GC/MS, LC/MS etc. The complete analysis of a substance consists of four main steps.

The concept of analytical chemistry lies in the simple, precise and accurate measurements. These determinations require highly sophisticated instruments and methods like mass spectroscopy, gas chromatography, high performance thin layer chromatography, high performance liquid chromatography etc. The HPLC method is sensitive, accurate, precise and desirable for routine estimation of drugs in formulations.

Thereby it is advantageous than volumetric methods. Many HPLC methods has been developed and validated for the quantitative determination of various marketed drugs.

Analytical method development and validation places an important role in drug discovery and manufacture of pharmaceuticals. These methods are used to ensure the identity, purity, potency and performance of drug products majority of analytical development effort goes into validating a stability indicating method. So it is a quantitative analytical method based on the structure and chemical properties of each active ingredient of the drug formulation.

Most of the drugs can be analyzed by HPLC method because of several advantages like rapidity, specificity, accuracy, precision, reproducibility, ease of automation and eliminates tedious extraction and isolation procedures.

On the literature survey, it was found that most of the analytical method available for the above mentioned drug is applicable for quantification in plasma samples, the most widely used method being liquid chromatography-mass chromatography. So it is felt that there is a need to develop accurate, precise analytical methods for the estimation of the drug in solid dosage formulation.

Newer analytical methods are developed for these drugs or drug combinations of the below reasons

- There may not be suitable method for a particular analyte in the specific matrix.
- Existing method may be too error prone or unreliable (have poor accuracy and precision).
- Existing method may be expensive, time consuming, energy intensive and may not be provide sensitive or analyte selectivity, and not easy for automation.
- Newer instrumentation and techniques may have evolved that provide opportunities for improved methods.
- There may be need for an alternate method to confirm, for legal and scientific reasons.

The newly developed analytical methods having their importance in different fields that include, research and development centre (R&D), quality control department (QC), approved testing laboratories, chemical analysis laboratories etc. For analysis of these drugs different analytical methods are routinely being used.

The analytical methods are classified as classical and instrumental. These methods signal measured in those methods was mentioned in following table.[16]

Table 1: Classification of analytical methods

Measurement signal	Analytical method
Chromatographic techniques	
Electrical	Gas chromatography (Thermal conductivity detector)
Increase in electrical current	Gas chromatography (Flame ionization detector)
Decrease in electrical current	Gas chromatography (Flame capture detector)
Electromagnetic radiation absorbed	Liquid chromatography (Ultraviolet light detector, diode array detector)
Electrical	Ion chromatography
Spectrophotometric method	
Emission radiation	Emission spectroscopy (X-ray, UV, Visible), Fluorescence and phosphorescence (X-ray, UV, Visible), radiochemistry.
Absorption of radiation	Spectrophotometry (X-ray, UV, Visible, IR) NMR and electron spin resonance spectroscopy.
Scattering of radiation	Turbidimetry, nephelometry, raman spectroscopy
Refraction of radiation	Refractometry, interferometry
Diffraction of light	X-ray and electron diffraction
Rotation of radiation	Polarimetry, optical rotatory dispersion
Mass to charge ratio	Mass spectroscopy
Electro chemical techniques	
Electrical potential	Potentiometry
Electrical current	Polarography, amperometry
Electrical resistance	Conductometry
Miscellaneous techniques	
Rate of reaction	Kinetic method
Thermal properties	DTA, DSC
Classical methods	
Mass	Gravimetric analysis
Volume	Volumetric analysis

MATERIALS AND METHODS

Caffeine from Sura labs, Aspirin from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK). Acetonitrile for HPLC from Merck,

HPLC method development
Trails

Preparation of standard solution
Accurately weigh and transfer 10 mg of Caffeine and Aspirin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.
Further pipette 0.5ml of the above Aspirin and 0.1ml of Caffeine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure
Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:
Initially the mobile phase tried was Methanol: Water and Water: Acetonitrile and Methanol: TEA Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Methanol: Acetonitrile in proportion 35:65 v/v respectively.

Optimization of Column
The method was performed with various columns like C18 column, Symmetry and Zodiac column. Symmetry ODS C18 (4.6mm × 250mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions
Instrument used : Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature : Ambient
Column : Symmetry ODS C18 (4.6mm × 250mm, 5µm)
Mobile phase : Methanol: Acetonitrile (35:65v/v)
Flow rate : 1ml/min
Wavelength : 273 nm
Injection volume : 20 µl
Run time : 10 min

Validation
Preparation of mobile phase

Preparation of Mobile Phase
Accurately measured 350 ml (35%) of Methanol, 650 ml of Acetonitrile (65%) were mixed and degassed in digital ultra sonicater for 20 minutes and then filtered through 0.45 µm filter under vacuum filtration.

Diluent Preparation
The Mobile phase was used as the diluent

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)
Mobile phase : Methanol: Acetonitrile (35:65v/v)
Column : Symmetry ODS C18 (4.6mm × 250mm, 5µm)
Flow rate : 1 ml/min
Wavelength : 273 nm
Column temp : Ambient
Injection Volume : 20 µl
Run time : 10 minutes

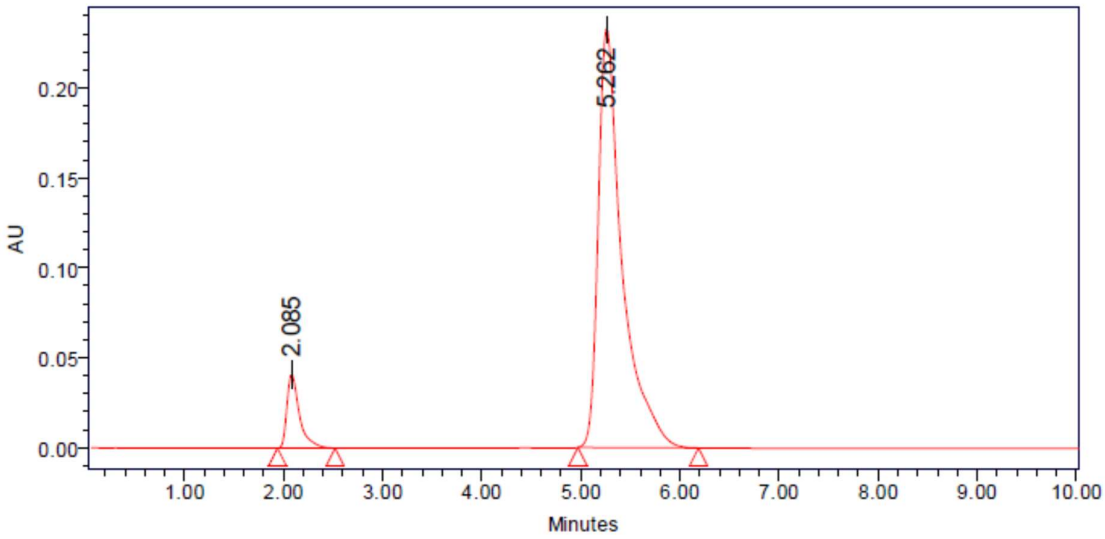

Fig 1: Optimized Chromatogram

Table 1: Peak Results for Optimized Chromatogram

S. No.	Peak Name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Aspirin	2.085	289658	3526		1.65	6745
2	Caffeine	5.262	4658749	28547	8.59	1.82	8638

From the above chromatogram it was observed that the Aspirin and Caffeine peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

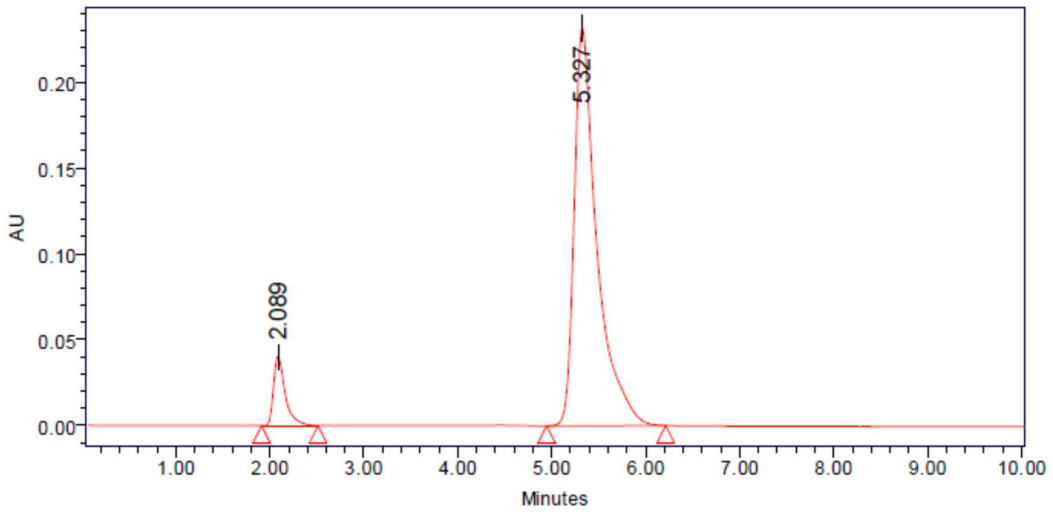

Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S. No.	Peak Name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Aspirin	2.089	298698	3658		1.68	6859
2	Caffeine	5.327	4758695	29586	8.64	1.85	8789

- *Resolution between two drugs must be not less than 2.*
- *Theoretical plates must be not less than 2000.*
- *Tailing factor must be not less than 0.9 and not more than 2.*
- *It was found from above data that all the system suitability parameters for developed method were within the limit.*

Assay (Standard)

Table 3: Results of system suitability for Aspirin

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Aspirin	2.090	289854	3526	8659	1.82
2	Aspirin	2.090	285745	3541	8642	1.83
3	Aspirin	2.089	289587	3612	8674	1.82
4	Aspirin	2.089	285466	3584	8692	1.83
5	Aspirin	2.085	285987	3572	8654	1.82
Mean			**287327.8**			
Std. Dev			**2194.024**			
% RSD			**0.763596**			

- *%RSD of five different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Table 4: Results of system suitability for Caffeine

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Caffeine	5.289	4658745	28564	8659	1.82	
2	Caffeine	5.289	4652587	28457	8647	1.83	
3	Caffeine	5.338	4674833	28952	8632	1.82	
4	Caffeine	5.327	4685825	28754	8645	1.83	
5	Caffeine	5.262	4652145	28964	8694	1.82	
Mean			4664827				
Std. Dev			14905.35				
% RSD			0.319526				

- *%RSD for sample should be NMT 2.*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Assay (Sample)

Table 5: Peak Results for Assay sample

S. No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Aspirin	2.088	296852	3659		1.66	6859	1
2	Caffeine	5.276	4785658	29865	9.75	1.83	8754	1
3	Aspirin	2.087	298545	3698		1.67	6874	2
4	Caffeine	5.268	4788982	29863	9.82	1.82	8785	2
5	Aspirin	2.085	296854	3674		1.67	6857	3
6	Caffeine	5.262	4789856	29865	9.78	1.83	8795	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Aspirin and Caffeine in pharmaceutical dosage form was found to be100.04%.

Linearity

Chromatographic data for linearity study

Aspirin

Concentration µg/ml	Average Peak Area
30	185658
40	245475
50	309658
60	365847
70	428698

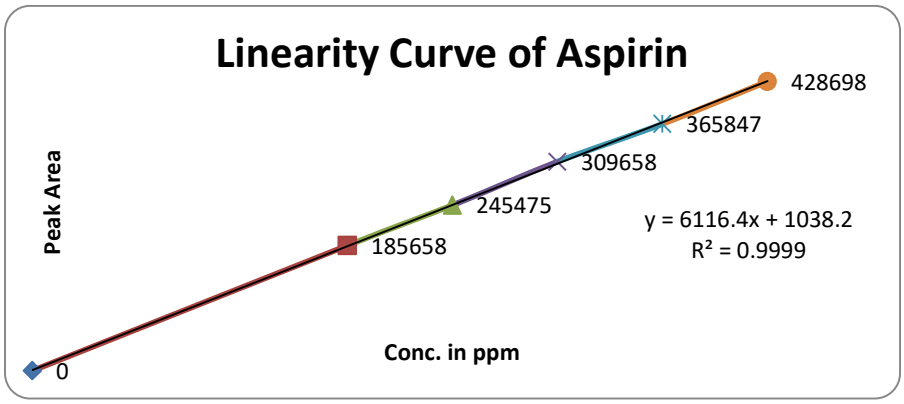

Fig 3: Linearity for Aspirin

Chromatographic Data for Linearity Study Caffeine

Concentration µg/ml	Average Peak Area
6	2658795
8	3556974
10	4458749
12	5265874
14	6169886

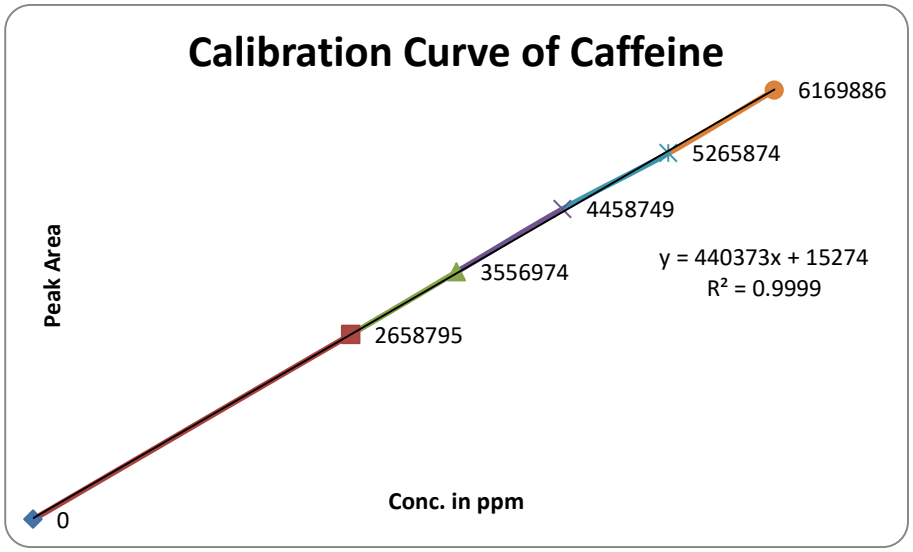

Fig 4: Calibration Curve for Caffeine

Repeatability

Table 6: Results of Repeatability for Aspirin

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Aspirin	2.086	289658	3569	6789	1.65
2	Aspirin	2.083	289547	3526	6758	1.66
3	Aspirin	2.083	285698	3598	6792	1.65
4	Aspirin	2.081	284579	3547	6749	1.66
5	Aspirin	2.081	285698	3598	6742	1.65
Mean			287036			
Std. Dev			2387.328			
% RSD			0.831717			

- *%RSD for sample should be NMT 2.*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Table 7: Results of method precession for Caffeine

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Caffeine	5.178	4685982	28569	8659	1.83	8.60
2	Caffeine	5.199	4698547	28574	8695	1.82	8.60
3	Caffeine	5.235	4658754	28598	8654	1.82	8.60
4	Caffeine	5.202	4635981	26985	8678	1.82	8.60
5	Caffeine	5.206	4658798	26857	8692	1.83	8.60
Mean			4667612				
Std. Dev			24754.3				
% RSD			0.530342				

- *%RSD for sample should be NMT 2.*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Intermediate precision

Table 8: Results of Intermediate precision Day1 for Aspirin

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Aspirin	2.083	298659	3659	6895	1.66
2	Aspirin	2.083	298574	3675	6847	1.67
3	Aspirin	2.089	296587	3698	6824	1.67
4	Aspirin	2.083	295684	3624	6856	1.66
5	Aspirin	2.082	296534	3698	6872	1.67
6	Aspirin	2.080	296528	3642	6895	1.66
Mean			297094.3			
Std. Dev			1226.273			
% RSD			0.412755			

- *%RSD of five different sample solutions should not more than 2*

Table 9: Results of Intermediate precision Day 1 for Caffeine

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Caffeine	5.229	4785698	298658	8798	1.83	
2	Caffeine	5.203	4785642	298624	8759	1.84	
3	Caffeine	5.133	4715266	293541	8762	1.83	8.65
4	Caffeine	5.229	4752143	298764	8754	1.84	
5	Caffeine	5.151	4715689	296534	8792	1.84	
6	Caffeine	5.112	4785982	295879	8764	1.83	
Mean			4756737				
Std. Dev			34512.01				
% RSD			0.72554				

- *%RSD of five different sample solutions should not more than 2*
  The %RSD obtained is within the limit, hence the method is rugged

Table 10: Results of Intermediate precision Day 2 for Aspirin

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Aspirin	2.078	278598	3785	6985	1.67
2	Aspirin	2.082	275985	3789	6925	1.68
3	Aspirin	2.080	274562	3795	6932	1.67
4	Aspirin	2.089	274154	3758	6954	1.68
5	Aspirin	2.083	274564	3746	6924	1.67
6	Aspirin	2.089	274584	3798	6984	1.68
Mean			275407.8			
Std. Dev			1684.552			
% RSD			0.611657			

- *%RSD of five different sample solutions should not more than 2*

Table 11: Results of Intermediate precision Day 2 for Caffeine

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Caffeine	5.077	4589852	27854	8547	1.81	
2	Caffeine	5.151	4526541	27463	8595	1.80	
3	Caffeine	5.112	4523654	27484	8523	1.81	8.62
4	Caffeine	5.133	4524571	27457	8574	1.80	
5	Caffeine	5.203	4526543	27658	8536	1.81	
6	Caffeine	5.133	4526587	27854	8542	1.80	
Mean			4536291				
Std. Dev			26268.18				
% RSD			0.579067				

- *%RSD of five different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is rugged.*

Accuracy

Table 12: The Accuracy Results for Aspirin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	153851	25	24.985	99.94%	100.00%
100%	306722.7	100	49.981	99.962%	
150%	460175.7	150	75.071	100.094%	

Table 13: The Accuracy Results for Caffeine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	233866.3	5	4.963	99.26%	99.94%
100%	455388.3	10	9.994	99.94%	
150%	680034	15	15.095	100.633%	

- *The percentage recovery was found to be within the limit (98-102%).*

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Aspirin

Table 14: Results for Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	289658	2.090	6745	1.65
Less Flow rate of 0.9 mL/min	298659	2.736	6854	1.69
More Flow rate of 1.1 mL/min	275478	1.673	6685	1.62
Less organic phase	265397	2.736	6635	1.64
More organic phase	245876	1.673	6425	1.67

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Caffeine

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	4658749	5.289	8638	1.82
Less Flow rate of 0.9 mL/min	4875985	6.746	8759	1.81
More Flow rate of 1.1 mL/min	4525321	4.032	8452	1.80
Less organic phase	4425643	6.746	8695	1.83
More organic phase	4258675	4.032	8239	1.84

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the Quantitative estimation of Caffeine and Aspirin in bulk drug and pharmaceutical dosage forms.

This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.

Caffeine sodium is freely soluble in ethanol, methanol, and water and practically insoluble in Acetonitrile.

Aspirin is freely soluble in water, soluble in methanol, insoluble in acetone.

Methanol: Acetonitrile (35:65v/v) was chosen as the mobile phase. The solvent system used in this method was Economical.

The %RSD values were within 2 and the method was found to be precise.

The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectro photometric methods.

This method can be used for the routine determination of Caffeine and Aspirin in bulk drug and in Pharmaceutical dosage forms

ACKNOWLEDGEMENT

The Authors are thankful to the Management and Principal, Department of Pharmacy, Pydah College of Pharmacy, Osmania University, Kakinada, Andhra Pradesh for extending support to carry out the research work. Finally, the authors express their gratitude to the Sura Labs, Dilsukhnagar, Hyderabad, for providing research equipment and facilities.

REFERENCES

1. Hobart HW, Merritt LL, John AD. Instrumental methods of analysis. 7th ed. New Delhi: CBS Publishers; 1988. p. 580-610.
2. Sharma BK. Instrumental method of chemical analysis. 20th ed. Meerut: Goel Publishing House; 2001. p. 54-83.
3. Ashutoshkar. Pharmaceutical drug analysis. 2nd ed New Delhi. New Age International Publisher; 2005. p. 455-66.
4. Ahuja S, Michael WD. Hand book of Pharmaceutical Analysis by HPLC. 1st ed. London: Elsevier Academic Press; 2005. p. 44-54.
5. Snyder LR, Kirkland JL, Glajch JL. Practical HPLC method development. 3rd ed. New York: Wiley; 1988. p. 227.
6. Skoog DA, West DM. Principles of instrumental analysis Saunders Golden Sunburst Series. 2nd ed. Philadelphia; 1980. p. 674-5, 690-6.
7. Dr. Kealey, Haines PJ. Analytical chemistry. 1st ed. Bios Publisher; 2002. P. 1-7.
8. BraithWait A, Smith FJ. Chromatographic methods. 5th ed. Kluwer Academic Publishers; 1996. P. 1-2.
9. Weston A, Phyllisr. Brown, HPLC principle and practice. 1st ed. Academic press; 1997. P. 24-37.
10. Shethi PD. HPLC- quantitative analysis of pharmaceutical formulations. 1st ed New Delhi: CBS Publishers & Distributors; 2001. p. 8-10, 101-3.
11. Kasture AV, Mahadik KR, Wadodkar SG, More HN. Pune: Nirali Prakashan. J Pharm Anal. 8th ed. 2002;II:48-57.
12. Prajapati GA. Method development and validation for simultaneous estimation of Hypertensive drugs by RP-HPLC [M.Pharm thesis]. Gujarat, India: Maliba Pharmacy College, Gujarat Technological University. p. 7-28; 2011.
13. Gabor S. HPLC in pharmaceutical Analysis. 1st ed. Vol. I. London: CRC Press; 1990. p. 101-73.
14. Jeffery GH, Bassett J. Vogel's textbook of Quantitative Chemical Analysis. 5th ed. New York: John Wiley & Sons, Inc; 1991. p. 217-35.
15. Kazakevich Y, Lobrutto R. HPLC for pharmaceutical scientists. 1st ed. Wiley Interscience A JohnWiley & Sons, Inc Publishing House; 2007. P. 15-23.
16. Chromatography [online]. Wikipedia. Available from: http://en.wikipedia.org/wiki/Chromatography.