INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF RP HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF THIAMINE AND CAFFEINE IN TABLET DOSAGE FORM

Chanduluru Hemanth kumar, K. Kanaka Parvathi,

Department of Pharmaceutical Analysis, Aditya Pharmacy College, Aditya Nagar, ADB Road, Surampalem-533437, East Godavari Dist., Andhra Pradesh, India.

ARTICLE INFO

Article history
Received 31/05/2017
Available online
14/06/2017

Keywords
Thiamine,
HPLC,
Caffeine,
Validation.

ABSTRACT

The main objective of the study is to develop a simple, accurate and explicit RP-HPLC method for the simultaneous determination of Thiamine and Caffeine in tablet dosage form. An Agilent C18, 25 cm x 4.6mm X 5 µm column is used for the estrangement of drugs by a mobile phase consisting of water : methanol : ACN mixture in the ratio of 68:29:03 v/v. The flow rate maintained was 1.0 mL/min and the wavelength used for detection was 275 nm. The linearity was observed in the range of 10-200µg/ml for Thiamine (TH) and Caffeine (CF) with a correlation coefficient of 0.9995 and 0.9992 respectively. The mean percentage recoveries for 80%, 100% and 120% accuracy were found to be 101.7% ± 2.09, 100% ± 2.49 and 101.5% ± 1.61 respectively for TH and CF. Linearity, accuracy, precision and robustness parameters were estimated for validation. The developed method was simple, precise, less time consuming with accurate results can be utilized in the routine analysis and quantification of TH and CF tablets.

Corresponding author
Chanduluru Hemanth Kumar
Department of Pharmaceutical Analysis,
Aditya Pharmacy College, Aditya Nagar, ADB Road,
Surampalem-533437, East Godavari Dist.,
Andhra Pradesh, India.
hemanth.kumar361@gmail.com
+919494767997

*Please cite this article in press as **Chanduluru Hemanth Kumar** et al. Analytical Method Development and Validation of RP HPLC Method for the Simultaneous Estimation of Thiamine and Caffeine in Tablet Dosage Form. Indo American Journal of Pharmaceutical Research.2017:7(05).*

INTRODUCTION

Chemically caffeine designated as 1, 3, 7-Trimethyl-1H-purine-2, 6(3H, 7H)-dione3, 7-Dihydro-1, 3, 7-trimethyl-1H-purine-2, 6-dione and it is the most widely consumed central nervous system stimulant. The structural formula of caffeine is shown in Figure 1 (a) and soluble in water and organic solvents. Thiamine chemically called as 2-[3-[(4-Amino-2-methyl-pyrimidin-5-yl) methyl]-4-methyl-thiazol-5-yl] ethanol, a water soluble vitamin, combines with ATP to form thiamine pyrophosphate, an essential coenzyme in carbohydrate metabolism.

Literature survey states very minor analytical methods like UV spectrophotometry [1-2], RP-HPLC [3-7] and HPTLC [8-9] for the assessment of caffeine and Thiamine individually or combo with other drugs. No simple RP-HPLC method has disclosed for the estimation of caffeine and Thiamine in tablet dosage form. The aim of the suggested method is to develop a simple and accurate method for the simultaneous determination of CF and TH using RP-HPLC technique in tablets.

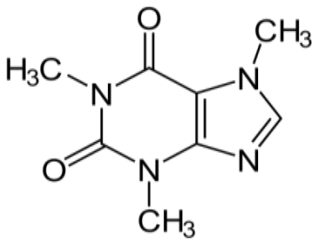


Figure 1(a)

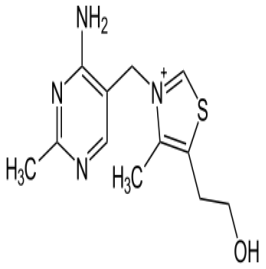


Figure 1 (b)

MATERIALS

CF and TH were earned from Ideal analytical and research institution puducherry, India. All chemicals worn were analytical standard. The pharmaceutical tablet dosage form used in this research was with a label claim of CF 80mg and TH 300mg were purchased from local pharmacy

INSTRUMENTATION AND APPARATUS:

The HPLC system used for advancement design and corroboration was Agilent 1260 & 1290 series equipped with auto Sampler and variable wavelength detector (VWD). Agilent C18, 25 cm x 4.6mm X 5 µm, Manual injections (20 µL) were applied column kept under ambient temperature wave length was set at 275nm for determination of CF and TH. With isocratic mode of mobile phase as water: methanol: ACN (65:30:05) at a flow rate of 1.0 mL/ min has given a satisfactory result of resolution for CF and TH with a total run time of 10 min. Ezchrome software in a computer system is used for the collection and analysis of the data

Preparation of Mobile Phase:

Prepared a mixture of 69 volume of Water, 28 volume of Methanol and 3 volume of Acetic acid, mixed well and filtered the solution through 0.45 µm Nylon filter and sonicated for 10 minutes.

Preparation of Diluent:

Prepared a mixture of 60 volume of Water and 40 volume of Methanol, mixed well and sonicated for 10 minutes

Procedure for preparation of analytical solutions:

Standard solution (0.100 mg/mL of Thiamine Hydrochloride and 0.027 mg/mL Caffeine):

Weighed accurately about 100 mg of Thiamine Hydrochloride WS and 27 mg of Caffeine WS, and transferred into a 100 mL VF. Added about 50 mL of diluent and sonicated for 5 minutes to dissolve. Cooled and diluted up to the volume with diluent.

Transferred 5 mL of the above solution through pipette into a 50 mL VF and diluted up to the volume with diluent. Filtered the solution through 0.45 µm Nylon filter and collect the solution in an HPLC vial after discarding about first 2 mL of filtrate

Sample solution :(0.100 mg/mL of Thiamine Hydrochloride and 0.027 mg/mL Caffeine):

Selected randomly 20 tablets and weighed, grinded into fine powder. Weighed accurately 100 mg equivalent of Thiamine Hydrochloride and 27 mg of Caffeine from fine powder, and transferred into a 100 mL VF, added 50 mL of diluent and sonicated for 10 minutes to dissolve and diluted up to the volume with diluent. Transferred 5 mL of the above solution through a pipette into 50 mL volumetric flask and diluted up to the volume with diluent. Filtered the solution through 0.45 µm Nylon filter and collected the solution in an HPLC vial after discarding about first 2 mL of filtrate.

Placebo solution:

Weighed accurately about 98 mg of Tablet Placebo (Thiamine Hydrochloride and caffeine) and transferred into a 100 mL volumetric flask, added 50 mL of diluent and sonicated for 10 minutes to dissolve and diluted up to the volume with diluent. Transferred 5 mL of the above solution through pipette into a 50 mL volumetric flask and diluted up to the volume with diluent. Filtered the solution through 0.45 µm Nylon filter and collected the solution in an HPLC vial after discarding about first 2 mL of filtrate.

Method development

System Suitability:

This study was performed by injecting six times of the standard solution into HPLC System. The system suitability parameters, Percentage relative standard deviation (RSD), Number of theoretical plates (N) and tailing factor (T) were assessed for peak area for 6 replicate injections was found to be within limits

System precision:

This study was performed by injecting 6 times of standard solution. The prescribed method is suitable to perform the estimation of Thiamine Hydrochloride and Caffeine from Thiamine and Caffeine Tablet Further there was no deviation had seen in the given method.

Linearity

The linearity response of Thiamine and Caffeine Tablets was determined across the range of 10-200µg/ml. The linearity was found within the acceptance criteria. Hence it is concluded that the range of concentrations, 10 % to 200% with respect to assay concentration for assay method is linear for Thiamine and Caffeine Tablets.

PRECISION OF TEST METHOD

Repeatability of a method can be determined by multiple replicate preparations of the same sample. This is can be done either by multiple sample preparation in the same experiment or by preparing 3 replicates at 3 different concentrations. The precision of test method was evaluated by assaying six samples of a single batch of Thiamine Hydrochloride and Caffeine tablet formulations. All the performed samples showed results between 90.0 % and 110.0 %. The method precision parameter complies as per ICH.[10]

ACCURACY

The results obtained for accuracy are found within the acceptance criteria. Hence it is concluded that the assay method for Thiamine and Caffeine Tablets is proficient to recover the Thiamine Hydrochloride and caffeine drug material when the placebo is spiked in the range between 80 % and 120%.

Robustness

It should show the reliability of an analysis with respect to deliberate variations in method parameters. The condition may include of changing in Wave length, mobile phase flow rate and composition. The results obtained for all deliberately changed parameters are found within the acceptance criteria (90%-110%).

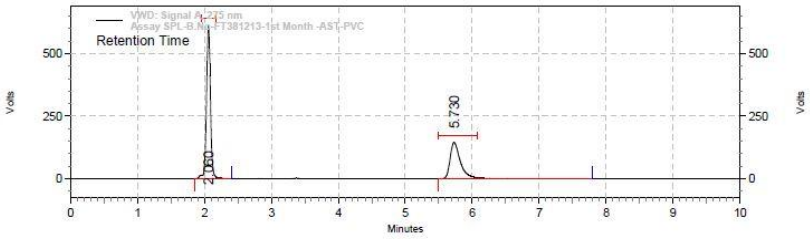
RESULTS AND DISCUSSIONS

Estimation of Thiamine and Caffeine in tablet dosage forms by RP-HPLC method was carried was validated as per ICH guidelines by using optimized chromatographic conditions. The standard and sample solutions were prepared. The chromatograms were recorded. The peak area ratios of standard and sample solutions were calculated. The results of analysis shows that the amount of drugs was in good agreement with the label claim of the formulation.

The quantitative estimation was carried out on tablet by taking the same concentration as for standard solution. The tablet shows percentage purity values ranging from 90-110% for Thiamine and Caffeine respectively.

Hear the separation was carried out by using a mobile phase consisting of water: Methanol: Acetic Acid in the ratio of (69:28:03). The column used was C_{18} 25cm × 4.6mm × 5µm with flow rate of 1.0mL/min using VWD detection at 275nm. The individual peaks of Thiamine and Caffeine was identified at retention time 2.060 and 5.730 minutes respectively.

The system suitability tests were carried out to evaluate the resolution and reproducibility of the system for the analysis. The results of the system suitability test were summarized in Table No.1.



VWD: Signal A, 275 nm Results

Peak Number	Name	Retention Time	Area	Area %	Theoretical plates (USP)	Asymmetry	Resolution (USP)
1	Thiamine Hydrochloride	2.060	42561133	61.51	6161	1.04873	0.0
2	Caffeine	5.730	26632996	38.49	7394	1.65460	19.8
Totals			69194129	100.00			

Fig 2: Chromatogram for Thiamine HCl and Caffeine.

TABLE 1: SYSTEM SUITABILITY PARAMETERS:

S. No	Parameters	Observation		Limit	Pass/fail
		Thiamine	Caffeine		
1	Retention time (min)	2.057	5.577	No limit	passed
2	No. of theoretical plates	6265	7638	NLT 2000	passed
3	Tailing factor	1.06	1.69	NMT 2	passed
4	Resolution factor	19.6	19.6	NLT 2	passed

Linearity was evaluated by visual inspection of the plot of peak area as a function of analyte concentration for Thiamine and Caffeine. The correlation co-efficient of Thiamine was found to be 0.9995 and the correlation co-efficient of Caffeine was found to be 0.9992, these are within limit The data regarding linearity for both the drugs were given in table 2 and corresponding calibration graphs were shown

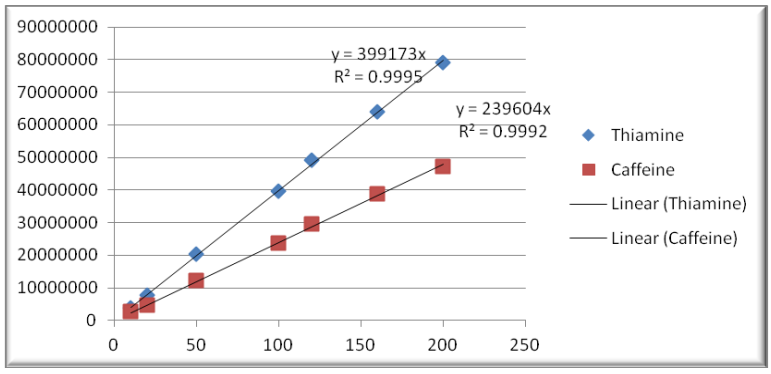


Fig 3: Linearity graph obtained.

Table 2: Linearity results.

S. No.	Parameter	Results obtained for Thiamine HCl	Results obtained for Caffeine
01	Correlation coefficient	0.9995	0.9992
02	Residual sum of squares	2479297252296.54	1240588797117.52
03	Intercept	246182.9554	228598.1
04	Slope of regression line	246182.9554	861046.6

ACCURACY:

The accuracy of the method was determined by recovery experiments. The recovery study was carried out and the percentage recovery range found to be within the limit, 90-110 percentages for Thiamine and Caffeine. The method shows precision of 0.10 for Thiamine and 0.19 for Caffeine.

Table No 3: Accuracy Results.

Accuracy Level in %	Thiamine Hydrochloride added in mg	Thiamine Hydrochloride recovered in mg	Caffeine added in mg	Caffeine recovered in mg	% Recovered Thiamine	% Recovered Caffeine	Mean of % Recovered Thiamine	Mean of % Recovered Caffeine
80	0.080450	0.080937	0.021240	0.021389	100.61	100.7	100.14	99.93
	0.081750	0.082377	0.021910	0.021897	100.77	99.94		
	0.079230	0.078477	0.020980	0.020804	99.05	99.16		
100	0.101350	0.102994	0.027800	0.027850	100.79	100.18	101.00	100.42
	0.101260	0.102430	0.027850	0.027884	101.05	100.12		
	0.101260	0.102430	0.027650	0.027913	101.16	100.95		
120	0.120680	0.121367	0.033100	0.033468	100.57	101.11	100.85	100.19
	0.121980	0.123214	0.032500	0.032509	101.01	100.03		
	0.120850	0.122023	0.032800	0.032612	100.97	99.43		

Robustness

The robustness of the method was performed by nanometer variation and change concentration of mobile phase, and the result was found to be within the limit. The method shows system suitability and precision within limits under given set of condition.

Table No 4: Robustness Results.

S. No.	Parameter Name	Results obtained: Thiamine Hydrochloride drug in mg	Thiamine Hydrochloride drug in %	Caffeine drug in mg	Caffeine drug in %
1		299.66	99.89	81.08	101.35
	Robust Wavelength 273 nm				
2		303.24	101.08	80.74	100.93
	Robust Wavelength 277 nm				
3	Robust flow rate 0.8mL	298.98	99.66	80.59	100.74
4	Robust flow rate 1.2mL	299.52	99.84	80.82	101.02
5	Robust mobile phase composition +5 %	302.95	100.98	80.30	101.62
	Robust mobile phase composition -5 %				
6		297.42000	99.14	79.79	99.74

CONCLUSION

The advanced chromatographic method for Thiamine and Caffeine is said to be rapid, simple, precise, accurate, and low cost solvents used so this method can be effectively applied for the routine analysis in research institution, QC department in industries, approved testing laboratories, biopharmaceutical studies, and in clinical pharmacokinetic studies.

Hence we believe that the future analytical methods will use this method for the determination of TH and CF in combined dosage form with no alterations.

RECOMMENDED FUTURE RESEARCH

Future research is endorse in any other pharmaceutical merged dosage form as this method is optimized to elute the analytes.

List of abbreviations

RP-HPLC - Reverse phase High Performance Liquid Chromatography
TH - Thiamine

CF	-	Caffeine
ACN	-	Acetonitrile
VWD	-	variable wavelength detector
RSD	-	Relative Standard Deviation
VF	-	Volumetric Flask

ACKNOWLEDGEMENT

Authors are thankful to Dr. Ravishankar, Dr. Diwakar, Dr. Sathis Kumar and the Management of Aditya pharmacy college, Surampalem for their backing and encouragement.

Conflict of interest:

All the financial need for the work has used by our own money

REFERENCE

1. Thamake SL, Jadhav SD, Pishawikar SA, Development and Validation of Method for Simultaneous Estimation of Caffeine and Ramipril from Capsule Dosage Form by First Order Derivative Spectroscopy, *Asian J. Research Chem.* 2(1): Jan-Mar. 2009 ISSN 0974-4169 Page no:52-53
2. Marcelo M. Senaa, Zahra F. Chaudhryb, Carol H. Collinsb, Ronei J. Poppia, Direct determination of diclofenac in pharmaceutical formulations containing B vitamins by using UV spectrophotometry and partial least squares regression *Indian journal of pharmaceutical sciences*, 2006 Vol. 68 (5) Page : 685-686
3. D Sathis Kumar, B.D.N Prasanthi, A Harini, P Anusha, method development and validation of valacyclovir hydrochloride and ritonavir in tablet dosage form using RP-HPLC *Jurnal Teknologi 76:1 (2015) 39–45*
4. Y.V. Sunil kumar, Usenni Reddy Mallu , I V Kasi Viswanath, RP-HPLC Method Development and Cleaning Method Validation for the Analysis of Triclabendazole in Veterinary Pharmaceutical Dosage Forms International Journal of ChemTech Research ISSN(Online):2455-9555 Vol.9, No.05 pp 507-520, 2016
5. Katikala. Prasanth N, Development and validation of a novel RP-HPLC method for simultaneous determination of paracetamol, phenylephrine hydrochloride, caffeine, cetirizine and nimesulide in tablet formulation, *Indian journal of pharmaceutical scinces*, 2008 Vol 70 (6) Page : 754-760
6. V.K. Redasani A.P. Gorle R.A. Badhan P.S. Jain S.J. Surana R.C. Patel, Simultaneous Determination Of Chlorpheniramine Maleate, Phenylephrine Hydrochloride, Paracetamol And Caffeine In Pharmaceutical Preparation By RP-HPLC, *Indian journal of pharmaceutical sciences* 2006 Vol. 68 (6) Page : 793-796
7. Mackenzie Ree and Erik Stoa Simultaneous determination of aspartame, benzoic acid, caffeine, and saccharin in sugar-free beverages using HPLC, *Indian journal of pharmaceutical scinces*, 2007 Vol. 69 (1) Page : 110-111
8. NJ Shah, BN Suhagia, RR Shah, PB Shah, Development and validation of a HPTLC method for the simultaneous estimation of Thiamine and hydrochlorothiazide in tablet dosage form, *Indian journal of pharmaceutical scinces*, 2007 Vol. 69 (2) Page : 202-205
9. P. B. Deshpande, G. Shridharan, Libi Anandi, D. Jadhav, M. C. Damle, S. V. Gandhi, Validated Method Development for Estimation of Caffeine and Fenofibrate in Fixed Dose Combination by HPTLC, *Kongposh publications THE PHARMA REVIEW* (MAY 2009)
10. ICH Topic Q2A, "Validation of Analytical Procedures": Methodology, 6th Nov 1996, www.ich.org.

5487847845**1170546**