Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 4, 2014

Original Article

SIMULTANEOUS HPLC DETERMINATION AND VALIDATION OF AMPHETAMINE, METHAMPHETAMINE, CAFFEINE, PARACETAMOL AND THEOPHYLLINE IN ILLICIT SEIZED TABLETS

DR. MOHAMED SULTAN*

***National Organization For Drug Control and Research, 51 St. wezarat elzerah, Dokki, Giza, Egypt, *Poison Control and Medical forensic Chemistry center, Riyadh, KSA**

Email: mmsultan_2000@yahoo.com

Received: 17 Feb 2014 Revised and Accepted: 3 Mar 2014

ABSTRACT

Objective: The main objective of current study develop and validate HPLC method, rapid, precise, accurate and specific for the separation and determination of paracetamol (PAR), theophylline (THE), amphetamine (AM), methamphetamine (MAM) and caffeine (CAF) in pure form and in illegal formulations.

Method: This method is based on HPLC separation of the five drugs on ZORBAX ODS column (250×4.6mm, 5µ). The mobile phase was prepared by mixing buffer (containing 1% of orthophosphoric acid 85% and 1% diethylamine 99%, pH 2.6), acetonitrile and methanol in the ratio 85:10:5 v/v/v. The flow rate is 1.5 ml/min, with isocratic elution and UV detection at 210nm. The retention times of paracetamol, theophylline, amphetamine, methamphetamine and caffeine were found to be at 3.67, 4.27, 5.44 6.72 and 8.40 min, respectively.

Results: The linear regression analysis data for calibration plots showed a good linear relationship over a concentration range of 0.39-100 µg/ml for paracetamol, 0.78-100 µg/ml for theophylline, 1.5-100 µg/ml for amphetamine, 1.25-100 µg/ml for methamphetamine and 0.39-100 µg/ml caffeine respectively. The mean values of the correlation coefficient, slope and intercept were 9996, 36.540 and -10.164 for paracetamol, 9999, 37.324 and -19.787for theophylline, 9998, 14.688 and -12.797 for amphetamine, 9995, 13.355 and 14.013 for methamphetamine and 1, 35.229 and 0.048 for caffeine. The method was validated as per the ICH guidelines. The limit of detection (LOD) and limit of quantification (LOQ) was 0.18 and 0.53 µg/ml for paracetamol, 0.21 and 0.63 µg/ml for theophylline, 0.788 and 2.365 µg/ml for amphetamine, 0.717 and 2.15 µg/ml for methamphetamine and 0.22 and 0.66 µg/ml for caffeine.

Conclusion: The developed and validated HPLC method and the statistical analysis showed that the method is repeatable and selective for estimation of the five studied drugs.

Keywords: Paracetamol, Theophylline, Amphetamine, Methamphetamine, Caffeine, Chromatography

INTRODUCTION

Paracetamol (PAR) (acetaminophen), N-(4-hydroxyphenyl)-acetamide (Fig. 1) is one of the most popular and widely used drugs for the treatment of pain and fever. It occupies a unique position among analgesic drugs. According to a recent update of the American College of Rheumatology (ACR) guidelines for osteoarthritis, PAR remains a first-line therapy because of its cost, efficacy and safety profiles [1]. Unlike non-steroidal anti-inflammatory drugs, it is considered to have an anti-inflammatory activity and does not produce gastrointestinal damage [1]. Unlike opiates it is almost ineffective in intense pain and has depressant effect on respiration [2]. PAR determined in combination with other drugs by HPLC methods [3,4,5,6,7,8].

Theophylline (THE) has maintained an important role as a potent and useful bronchodilator and used to treat asthma [9]. Chemically name as 1,3-dimethyl-2,6-dione as shown in (Fig. 2). THE determined alone or in combination with other drugs by HPLC [10,11,12,13]. Amphetamine (AM) α-Methylbenzeneethanamine (Fig. 3) is a potent central nervous system (CNS) stimulant of the phenethylamine class) and is synthetic stimulant. The original drug is called amphetamine but the group includes dextroamphetamine (dexies), methamphetamine (, crystal, meth), and smokeable methamphetamine (ice). These drugs, all of which have similar effects, are available as tablets and capsules that can be taken orally[14].

Methamphetamine (MAM))-*N*,α-Dimethylbenzeneethanamine (Fig. 4) is a synthetic stimulant drug used for both medicinal and illicit purposes. Like most stimulants, MAM may induce strong feelings of euphoria and can be addictive. MAM is a popular recreational drug. It increases alertness and energy, and in high doses, can induce mental/emotional, enhance self-esteem, and increase sexual pleasure[15].

AM and MAM were determined together or with another drugs by Chromatographic methods, GC-MS [16,17,18], LC-MS [19,20,21,22,23] and HPLC [24].

Caffeine (CAF) 1,3,7-trimetyl-1H-purine-2,6(3H,7H)-dione (Fig. 5) is a central nervous system stimulant which is used both recreationally and medically to restore mental alertness when unusual weakness or drowsiness accurs. Dose of 200mg/day result in increased focus and better general body coordination [25]. CAF has been determined using, LC-spectrophotometer [26], spctrophotometric [27,28] and HPLC [29,30,31,32,33].

According to our detalied literature survey as on date, there are no HPLC methods avaliable for simaltenous determination of amphetamine, methamphetamine, caffeine, paracetamol and theophylline combination togther in any matirx either in formulation dosage forms or in biological fluids. Thus, it is essential to develop a suitable HPLC method, rapid, precise, acuurate, linear, a convenient HPLC method and validiated according to ICH guidelines [34].

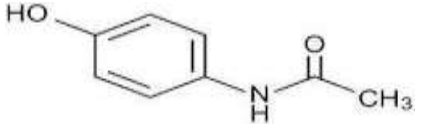

Fig. 1: Chemical Structure of Paracetamol (PAR)

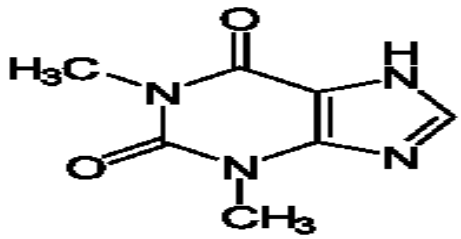

Fig. 2: Chemical Structure of Theophylline (THE)

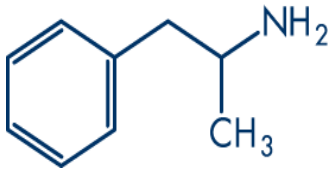

Fig. 3: Chemical Structure of Amphetamine (AM)

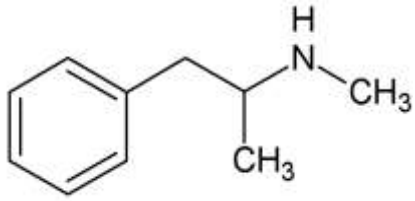

Fig.4: Chemical Structure of Methamphetamine (MAM)

Fig. 5: Chemical Structure of Caffeine (CAF)

MATERIALS AND MTHODS

Reagents and Chemicals

All solvents were of HPLC grade and Chromatographic-grade water was produced by a Milli-Q system (Millipore, Billerica, MA). The reference standards of PAR, THE were obtained as gift samples from Saudi Food & Drug Authority and AM, MAM and CAF were obtained from Lipomed AG, CH-4144 ARLESHEM, SWITZERLAND. Illicit seized tablets obtained from Poison Control and Medical forensic Chemistry center, Riyadh, KSA.

Preparation of buffer

The buffer solution consist of water containing 1% of orthophosphoric acid 85%, 1% diethylamine 99% and adjust pH to 2.6.

Preparation of mobile phase

The mobile phase was prepared as mixture of buffer: acetonitrile: methanol in the ratio 85: 10: 5 v/v/v. The mobile phase was filtered on 0.45µm nylon filter.

Preparation of standard stock solutions

The standard stock solution of PAR (100 µg/ml), THE (100 µg/ml), AM (100 µg/ml), MAM (100 µg/ml), and CAF (100 µg/ml). were prepared by transferring 10 mg of PAR, 10 mg of THE, 10 mg of AM, 10mg of MAM or 10 mg of CAF into 100 ml volumetric flask containing 40 ml of methanol, sonicated for 5 min; and cooled to room temperature. The volume was made up to the mark by methanol to give 100 µg/ml to each standard as individual. The stock solutions were stored at 2-8°C.

Preparation of sample solution

Weight of ten tablet seized as group, were accurately weighed, transferred to a clean and dry mortar and ground to fine powder. A weight of the powder equivalent to one tablet content was accurately weighed, then transferred to a clean 100 ml volumetric flask, 40 ml of methanol was added, the mixture was then sonicated for 10 min; and diluted to the volume with methanol. This solution was filtered through 0.45µm pore size nylon filter membrane.

Instrument and Chromatographic conditions

HPLC system consists Agilent HP 1100 system equipped with an auto sampler (Waldbronn Germany), quaternary pump, auto injector, column compartment and photodiode array(PDA) detector was used. The analytical column was ZORBAX ODS (250mmx4.6mm-4µm), the mobile phase was (Buffer: Acetonitrile: Methanol in ratio 85: 10: 5 v/v/v). The mobile phase was filtered through 0.45µm pore size nylon filter membrane with the flow rate was 1.5 ml/min, isocratic elution and injection volume 10µl. The column temperature was at room temperature, the UV- detection was at 210 nm wavelength and the run time was 10 min.

Optimization of HPLC method

All drugs were subjected to chromatographic analysis using mobile phases of different pH, flow rate and different stationery phases. The changes in the retention time, sensitivity and selectivity of all drugs were noted as a function of changing mobile phase, pH and stationary phase.

Initially methanol : water in different ratios were tried but incomplete separation of peaks were observed, then acetonitrile : water in different ratio was tried but splitting of CAF peak and low sensitivity of AM were observed. Later acetonitrile and methanol with buffer as (1%phosphoric acid 85% + diethylamine 99%) in different pH were tried, the best results found at pH 2.6 then the different ratios of buffer: Acetonitrile: Methanol were tried until get the best separation, resolution, peak symmetry, selectivity and sensitivity were in the ratio 85:10:5 v/v/v of mobile phase with flow rate 1.5 ml/min on ZORBAX ODS column (250×4.6mm - 5µm) as shown in (Fig. 6).

RESULTS AND DISCUSSIONS

Validation of the method

Validation of the optimized HPLC method was carried out with respect to the following parameters.

Linearity and range

Linearity of the method was studied by injecting six concentrations of drug prepared in the mobile phase in concentration range from 0.39 –100 µg/ml for PAR, THE and CAF and from 1.5- 100 µg/ml for AM and from 1.25- 100 µg/ml for MAM in triplicate into the HPLC system keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration curves and the linearity data as recorded in (Table 3).

Precision

The precision of the method was verified by repeatability and intermediate precision studies. Repeatability was performed by analysis of three different concentrations six times on the same day. The intermediate precision of the method was checked by studying two different analyst on three different days [34] results were recorded in (Table 1).

Table 1: Results of precision for standard drugs

Drugs	Concentration (µg/ml)	Repeatability (n = 6) R.S.D%	Intermediate Precision (n = 6) R.S.D%
PAR	10	0.119	1.696
	20	0.179	1.249
	60	0.411	1.025
THE	10	0.226	1.790
	25	0.219	1.156
	100	0.107	1.011
AM	25	0.975	1.465
	50	0.415	1.520
	100	0.379	1.285
MAM	25	0.983	1.375
	50	0.401	1.520
	100	0.222	1.169
CAF	15	0.296	1.660
	30	0.196	1.045
	75	0.204	0.769

Accuracy

Accuracy of the method was verified by studying recovery at three different concentration for PAR, THE, AM, MAM and CAF, by replicate analysis (n = 3). Samples of known concentration (reference standard solutions) were analyzed and the measured values, from the respective area counts and calculate the measured concentration of drugs and compared with the true values. The results obtained from the determination of accuracy, expressed as percentage recovery, were recorded in (Table 2).

Table 2: Results of accuracy for standard drugs

Drugs	Amount added (µg/ml)	Amount found (µg/ml)	Recovery %
PAR	25	24.60	98.40
	50	50.50	101.00
	100	103.00	103.00
THE	10	9.90	99.00
	25	25.15	100.60
	100	101.00	101.00
AM	10	10.20	102.00
	20	20.20	101.00
	60	58.80	98.00
MAM	15	15.08	100.50
	30	30.06	100.20
	75	75.75	101.00
CAF	25	25.5	102.00
	50	49.00	98.00
	100	97.00	97.00

Limit of detection(LOD) and limit of quantification(LOQ): The LOD and LOQ were separately determined on the basis of standard calibration curve. The residual standard deviation of the regression line or the standard deviation of y-intercepts of regression lines was used to calculate LOD and LOQ.

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD = 3.3 SD/S and LOQ = 10 S.D/S, where S.D is the residual standard deviation of regression line and S is the slope of the line. The results of LOD and LOQ obtained for studied drugs were recorded in (Table 3).

Table 3: System Suitability Parameters

Parameters	PAR	THE	AM	MAM	CAF
Linearity	0.39-100 µg/ml	0.78-100 µg/ml	1.5-100 µg/ml	1.25-100 µg/ml	0.39-100 µg/ml
Regression equation	y = 36.54x- 10.16	y = 37.33x-19.79	y = 14.69-12.79	y = 13.36 + 14.013	Y = 35.23 + 0.05
Correlation coefficient	R^2 = 0.9996	R^2 = 0.9999	R^2 = 0.9998	R^2 = 0.9995	R^2 = 1
Retention time	3.698	4.292	5.444	6.724	8.406
Resolution		**2.1**	**3.46**	**2.84**	**3.26**
Theoretical plates*	**12814.54**	**13439.80**	**13597.85**	**9959.53**	**18583.16**
Peak width	0.1309	0.1451	0.1866	0.2694	0.2459
Symmetry	0.791	0.734	0.784	0.627	0.852
LOD	0.18	0.21	0.79	0.98	0.20
LOQ	0.53	0.63	2.37	2.95	0.60

*Tangent Line Method

Robustness of the method

The robustness of the proposed HPLC method was assessed by the ability to remain unaffected by small changes in experimental conditions. Change in flow rate by ± 0.2% and small changes in mobile phase organic strength and in pH by ±2% has no significant effect on chromatographic resolution.

Specificity

The specificity of the method was accessed from the chromatogram where complete separation of PAR, THE, AM, MAM and CAF was achieved. The peaks obtained were sharp, well separated at the baseline as shown in (Fig. 6) and the resolution for all peaks more than 1.5 as recorded in (Table 3).

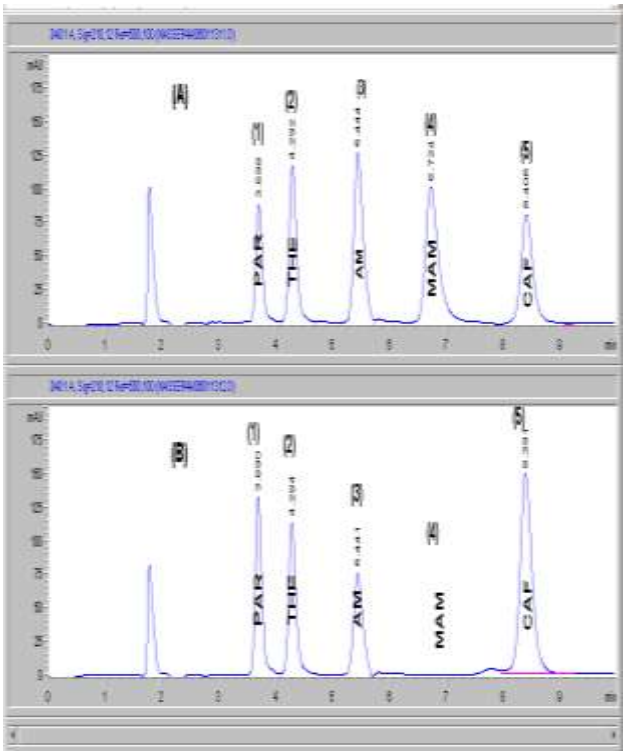

Fig. 6: (A) HPLC Chromatogram for Standard solutions of, (1)PAR (25µg/ml), (2)THE (25µg/ml), (3) AM (100µg/ml), (4)MAM (100µg/ml), and (5)CAF (40µg/ml); (B) HPLC Chromatogram of Illicit Seized Tablet Containing, (1)PAR, (2)THE, (3)AM, and (5)CAF.

Applications

Analysis of illicit seized tablets to determine the content of illicit tablets, by grind the tablet after weighing it and transfer definite weight and dissolve in methanol. A 10µl volume of sample solution was injected into HPLC, three times, under the conditions described above and calculate the concentration of drugs in tablet by using calibration curve for each Drug and the results were recorded in (Table 4)**.**

Table 4: Results for application of method on Illicit seized tablets

Drugs	Group I(content mg/tablet)	Group II (content mg/tablet)	Group III (content mg/tablet)
PAR	50		
THE	25	25	
AM	20	25	25
MAM			
CAF	55	50	60

CONCLUSION

RP-HPLC method of 10 min runtime was successfully developed for the simultaneous determination of five drugs with good resolution between all drugs more than 2 and shoes heigh degree of accuracy and precision with less than 2% RSD. The method is simple, accurate, rapid, precise and can easily use for routine analysis of the five drugs in any formulated forms(tablets, capsules and powder) and matrix.

ACKNOWLEDGMENT

The author is thankful to Poison Control Center and Medical forensic Chemistry center, Riyadh, KSA, for providing the opportunity to carry out the research work and providing samples of amphetamine, methamphetamine and caffeine and illicit seized tablets.

REFERNCES

1. B. Hinz and K. Brune, Antipyretic Analgesic: NonsteroidalAntinflammatory Drugs, selective COX-2 inhibitiors, paracetamol and Pyrazolinones, Handbook of Experimental Pharmacology, 2006; 177: 65-93.
2. Tomas L. Lemke, David A.Williams, et al, FOY's Principle of Medicinal Chemistry, 2006; 6Edtion :963-964.
3. Carolin I n., Balan P., Chiranjeevi N and Vinnakota V. V. M. K., Development and validation of a reverse phase HPLC method of simultaneous estimation of Tolperisone hydrochloride and paracetamol in Dosage form, IJPPS, 2012; 4(5): 84-88.
4. Rajesh M. Kamble and Shrawan G. Singh, Stability –Indicating RP-HPLC Method for Analysis of Paracetamol and Tramadol in a Pharmaceutical Dosage Form E-Journal of Chemistry, 2012; 9(3): 1347-1356.
5. Boyka T., Ivanka P., Alexander Z., and Plamen P., Simulateneous high-performance liquid chromatography determination of Paracetamol and Ascorbic acid in tablet dosage forms, African Journal of Pharmacy and Pharmacology, 2012; 6(17): 1332-1336.
6. V. Maslarska and J. Tencheva, Simultaneous determination and validation of paracetamol and codeine phosphate in pharmaceutical preparation by RP-HPLC., IJPPS, 2013; 5(2): 417-419.
7. Abdulbari M. M and Ihasan M. SH, simultaneous determination and validation of chlorpheniramine maleate, paracetamol, phenylpropanolamine hydrochloride and caffeine in tablet dosage form by RP-HPLC., IJPPS, 2013; 5, Suppl 3: 668-670.
8. L. Suryan, V. K. Bhusari, K. S. Rasal, S.R. Dhaneshwar, Simultaneous quntitation and validation of paracetamol, phenylpropanolamine hydrochloride and cetirizine hydrochloride by RP-HPLC in bulk drug and formulation, IJPSDR, 2011; 3(4): 303-308.
9. Laurence L. Brunton, John S. Lazo and Keith L. Parker, The Pharmacological Basis of Therapeutics,2005; 11Edtion : 727-730.
10. Supriya Shidhaye, Sheetal Malke, Vilasrao Kadam; Validated stability indicating HPLC method for estimation of theophylline from a novel microsphere formulation, 2009; 3(1): 13-17.
11. Nirav P. M and Kaushal K. C; Method development, validation and stability study for simultaneous estimation of Etofylline and Theophylline by RP-HPLC in Markted formulation, J. Chem. Pharm. Res., 2011; 3(3): 597-609.
12. Siraj S., Buvaneswari, Selvadurai M., Mohamed S., Sd R., and Sundaram S., RP-HPLC method for a frequently prescribed bronchodilator-Theophylline, Asian J. Pharm Clin. Res., 2013; 6(2): 30-32.
13. Branishlava S., Vukosava D. M., Nervena G., Rade I., and Zika L., Simultaneous HPLC determination of Caffeine, Theobromine and Theophylline in Foods, Drinks and Herbal products, Journal of Chromatographic Science, 2008; 46: 144-149.
14. https://www. Drugs/Amphetamine and methamphetamine abuse.
15. Logan BK. Methamphetamine - Effects on human performance and behaviour. Forensic Sci. Rev., 2002; 14:133-51.
16. AdriennDobos, ElodHidvegi and Gabor Pal Somogy, comparision five reagent for the determination of amphetamine type Stimulants in urine by Extractive acylation and GC-MS, J. Anal. Toxicol, 2012; 36(5): 340-344.

17. HeiHwa Lee, Jong Feng Lee, Sin Yu Lin, Ping Ho Chen and BaiHium Chen, Simultaneous Determination of HFBA-Derivatized amphetamines and Ketamines in urine by GC-MS, J. Anal. Toxicol., 2011; 35(3): 162-169.
18. Mariana Dadalto Peres, Fabricio Souza Pelicao, Bruno Caleffi and RunoSpinosa Martinis, Simultaneous Quantification of Cocaine, Amphetamines, Opiates and Cannabis in Vitreous Humor, J. Anal. Toxicol.,2014; 38(1): 39-45.
19. Huei-Ru Lin, Ka-Lan Choi, Tzu-Chieh Lin, anren Hu, Simultaneous quantification of amphetamines, opiates, ketamines and relative metabolites in urine for confirmatory analysis by LC-MS, Journal of Chromatography B, 2013; 929: 133-141.
20. Aliru Olajide Mustapha, Usman L. Ajao, Amphetamine analysis in waste waters-Method Performance SPE-HPLC MS/MS, EXCELI Journal, 2011; 10: 140-148.
21. Maria del Mar Ramirez Fenadez and NeleSamyn, J. Anal. Toxicol., 2011 35 (8): 577-582.
22. Belal KISS, Daniela-Saveta POPA, Marious BOJITA and Felicia LOGHIN, Development and validation of a HPLC-DAD/FID Method for the determination of MDMA, MDA, Methamphetamine, Morphine, Morphine-Glucuonides and 6-Monoacetylmophine in Human Plasma, Revue Roumaine de Chime, 2009; 54 (10): 833-840.
23. Dean Fritch, Kristen Blum, Sheena Nonnemacher, Brenda J. Haggerty, Matthew P. Sulilivan and Edward J. Cone, Identification and quantitation of Amphetamines, Cocaine, Opiates, and Phencyclidine in Oral Fluid by LC-MS, J. Anal. Toxicol., 2009; 33(9): 569-577.
24. V. Pavlova and S. Petrovska-Jovanovic, Simultaneous Determination of Amphetamine, Methamphetamine and Caffeine in Seized Tablets by HPLC, ACTA CHROMATOGRAPHICA. No. 18. 2007: 157-167.
25. United States Pharmacopeia, 2012, USP35-NF 30, P 3072.
26. Sonali S. Bharate and Sandip B. Bharate, spectrophotometric and chromatographic determination of Aceylsalicylic acid and Caffeine in pure and in tablet dosage form, journal of advanced Scientific Research, 2012; 3(1): 73-81
27. Ayman M Mohsen, Hayam M.Lotfy, Amr M. Badawey, Hesham Salem, and Sonia Z. Elkhateeb, Applicationof Three novel spectrophotometric methods manipulating ratio spectra for resolving a pharmaceutical mixture of chloropheniramine hydrochloride and caffeine, IJPPS, 2013; 5: 478-487.
28. Kuldeep Delvadiya, Ritukimbahune, PrachiKabra, Sunil K. and Pratik Patel, spectrophotometric simultaneous analysis of paracetamol, propyphenazone and caffeine in tablet dosage forms IJPPS, 2011; 3: 170-174.
29. Pandurang N. Patil, Caffeine in various samples and their analysis with HPLC, Int. J. Pharm. Sci. Rev. Res., 16(2), 2012; no 18, 76-83.
30. Sharam S., Sharam M.C., Sharam A. D., High Performance Liquid Chromatographic assay method for determination of paracetamol and caffeine in tablet formulation –in vitro dissolution studies,Journal of Pharmacy Research, 2011; 4(5): 1559-1561.
31. Viswanath Reddy pyreddy, Useni Reddy Mallu, VaraprasadBobbarala and SomasekharPenumajji, Develop a RP- HPLC Method for the determination Caffeine, Pseudoephedrine hydrochloride and chlorpheniramine maleate in pharmaceutical products, Journal pharmacy research, 2011; 4(4): 1225-1227.
32. Boyka G. T., Bistra D. K., Dimitar R. R., Lily P. P., and Lvanka p. p., HPLC assay and stability studies of tablet containing paracetamol and caffeine Int. Pharm. Sci. Rev. Res., 2013: 18(1):138-142.
33. Rahim A. A., Nofrizal S. and Saad B., Rapid tea catecins and caffeine determination by HPLC using microwave-assisted extraction and silica monolithic column, Food Chem., 2014; 147: 262-268.
34. Validation of Analytical Procedure, Text and Methodolgy. International Conference onHarmonisation (ICH Q2 (R1). 2005