DEVELOPMENT & VALIDATION OF ANALYTICAL METHOD USED FOR SIMULTANEOUS DETERMINATION OF PARACETAMOL, CAFFEINE AND CODEINE PHOSPHATE BY HPLC, IN PHARMACEUTICAL FORMULATION.

Ammara Nazir[1], Yasra Naseer[2], Roohi Shahid[3], Sahar Raza[4]
Department of Chemistry, Minhaj University Lahore, Lahore, Pakistan
Correspondence Author: ammara_nazir9@yahoo.com

ABSTRACT: *A Reverse Phase high performance liquid chromatographic method was developed and validated for simultaneously determination of active ingredients like Caffeine, Paracetamol and Codeine Phosphate in the pharmaceutical formulation. A mobile Phase of Water: Acetonitrile: Methanol = (60: 15: 25 v/v/v) was run on a C18 column, at the flow rate of 1min/ml. UV detection was performed at 240 nm. The retention times were 9.13, 7.78 and 6.42 for Caffeine, Paracetamol and Codeine Phosphate, respectively. The R.S.D qualities are less than 2 %, which showed that developed method was accurate and suitable for expected utilization. The method was validated with respect to the precision, accuracy and specificity of the Caffeine, Paracetamol and Codeine Phosphate in the pharmaceutical formulation.*

Key words: *Codeine Phosphate; Caffeine; Paracetamol; Reverse-Phase HPLC*

INTRODUCTION

The systematic name of caffeine is 1, 3, 5-trimethylxanthine (Fig: 1). $C_8H_{10}N_4O_2$ is the chemical formula of caffeine [1]. Caffeine is a white, odorless, fleecy masses, glistening needles like powder in pure form. Caffeine has physiological and pharmacological properties because it is a coffee compound. Caffeine may stimulate the central nervous system. Caffeine does not store into the body for a long time and is excrete out after taking it [2]. Caffeine imparts diuretic effect on kidney that disturbs the balance of fluid in the body. Caffeine also expands blood vessels, increase heart beat and raise the intensity of glucose and free fatty acids in plasma [3].

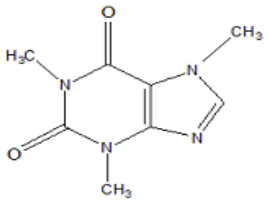


Fig: 1. Caffeine

Paracetamol is also called Acetaminophen. Paracetamol chemically known as 4- hydroxyacetanalide (Fig: 2) [4,5]. The anti-inflammatory effect of Paracetamol is weak but it is an effective and safe antipyretic and analgesic agent [6]. Paracetamol is a white crystalline solid in its pure form [7]. The Paracetamol has molecular formula of $C_8H_9NO_2$ and a molecular mass of 151.17 g/mol [8]. Paracetamol is used to prevent the fever, headaches, pain of arthritis, aches, colds, flu and period pain [9].

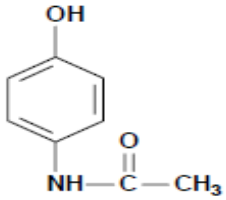


Fig: 2. Paracetamol

Codeine is an alkaloid [10]. In chemical terms the codeine is 7,8-didehydro-4,5alpha-epoxy-3-methoxy-17-methylmorphinan-6-alpha-olphosphate (1:1) (salt) hemihydrates (Fig: 3) [11]. The molecular formula of codeine is $C_{18}H_{21}NO_3$. The molecular weight of codeine is 406.4. Codeine is Colorless or white crystals or powder. [12]. Codeine is odor less and has bitter taste [13]. Codeine is used as a central analgesic, hypnotic, antinonciceptive, anti-peristaltic and sedative. Due to incessant coughing the codeine is recommended in insomnia and tuberculosis [106].

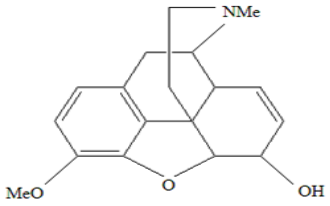


Fig: 3. Codeine

MATERIALS AND METHODS

Chemicals and reagents

The Analytical grade reference standards of caffeine, paracetamol were obtained from Medipak Pharmaceutical Company Lahore, Pakistan. The Powder form Codeine was obtained from the Forensic lab Lahore. Tablet named Femidol was commercially available that contains 30 mg Caffeine, 500 mg Paracetamol and 15 mg Codeine Phosphate with other excipients and placebos All the Chemicals used were of Analytical grade obtained from Merck Ltd. All the analytical grade chemicals and reagents were used in the experimental work without further purification.

Instrumentation

The development of method was carried out by the Reverse-Phase HPLC system that contains a column Purespher RP-18 endcapped, 5.0 µ, 100 A° 4.6 x 250 mm used as stationary phase and UV visible Spectrophotometer. The Mobile Phase consist of Water: Acetronitrile: Methanol (60: 15: 25 v/v/v) was used and pH was maintained at 2.6 with HCl. The Mobile Phase was filtered with 0.45 µm filter membrane after sonicated it for 10 mins. The flow rate was 1min/ml and the UV detection was performed at 240 nm. The isocratic elution was at 20 µL injection volume and the run time was 12 min.

Mobile Phase Preparation

In 1000 ml volumetric flask 600 ml of distilled water was transferred. Then 150 acetonitrile and 250 methanol were added to it. pH was adjusted to 2.6 with hydrochloric Acid.

Then it was syndicated and filtered with 0.45 µm nylon filter membrane. The ratio of mobile phase was (60: 15: 25 v/v/v).

Standard Solution of Caffeine

In 100 ml measuring flask 30 mg of caffeine was transferred and added 70 ml mobile phase then sonicated to dissolve and again added mobile phase to make volume up to the mark. 5 ml of this solution was taken into another 100 ml measuring flask and make volume upto the mark with mobile phase. It contains 15 µg/ml.

Standard Solution of Paracetamol

In 100 ml measuring flask 500 mg of Paracetamol was transferred, added 70 ml mobile phase then sonicated to dissolve and again added mobile phase to make volume up to the mark. 5 ml of this solution was taken, into another 100 ml measuring flask make volume up to the mark with mobile phase. It contains 250 µg/ml.

Standard Solution of Codeine Phosphate

In 100 ml measuring flask 15 mg of Codeine Phosphate was transferred, added 70 ml mobile phase then sonicated to dissolve and and again added mobile phase to make volume up to the mark. 5 ml of this solution was taken, into another 100 ml measuring flask make volume up to the mark with mobile phase. It contains 7.5 µg/ml.

Sample Preparation

Twenty tablets were weighed and ground in a fine powdered form then an accurate amount of this powder was taken equivalent to the weight of one tablet. This amount contains the 500 mg of Paracetamol, 15 mg of Codeine Phosphate and 30 mg of Caffeine. It was taken in 100 ml volumetric flask and added 80 ml of mobile phase and sonicated to dissolve, then make volume up to the mark with the mobile phase.

From this solution 5 ml of sample was taken into another volumetric flask of 100 ml, up to mark with the mobile phase and mixed well. The solution concentration was 15 mg/ml for caffeine, 250 mg/ml for Paracetamol and 7.5 mg/ml for Codeine Phosphate.

Preparation of Mixture of Standards

To made the solution that contains 15 mg/ml of Caffeine, 250mg/ml of Paracetamol and 7.5mg/ml of Codeine Phosphate. 30mg of Caffeine, 500mg of Paracetamol and 15mg of was taken in 100ml volumetric flask and added 80ml of mobile phase, sonicated to dissolve and then make volume up to the mark with mobile phase. From this solution 5ml of sample was transferred into another 100ml volumetric flask and up to mark with mobile phase and mixed well.

RESULTS AND DISCUSSION

Results

A Reverse Phase high performance liquid chromatographic method was developed and validated for simultaneously determination of active ingredients like Caffeine, Paracetamol and Codeine Phosphate in the pharmaceutical formulation. Excellent and sharp peak with good separation were obtained in short period of time by using HPLC Column as shown in Fig: 4. In this HPLC system the results of Specificity, Accuracy and the Precision were calculated accurately by this Validated method.

Specificity

Solutions of the Caffeine RS (single APIs 100%), Paracetamol RS (single APIs 100%), and

Codeine Phosphate RS (single APIs 100%) were prepared and mixed well together, spiked with the solution of placebo and the sample solution to determine the specificity of the method. This solution was run through the column to obtained Chromatograms. The obtained chromatograms were matched with the chromatograms of the standard solutions of the Caffeine, Paracetamol and Codeine Phosphate. There was no interference observed between the peaks of drugs and the placebo. Broad peaks and good resolution was obtained as shown in Fig 4. So because of no interference of placebo throughout the run the method has a good specificity for the Pharmaceutical formulations.

Accuracy

To determine the Accuracy of method the standard solution of the Caffeine, Paracetamol and Codeine Phosphate was analyzed at three different levels of concentration 80%, 100% and 120%.

The triplicates of the each level of the standards, spiked with placebo were run to observe the % age recovery of the standards. The Percentage Recovery, Tailing Factor and the Relative Standard Addition were observed and mentioned in Table no 1.

Precision

The precision of the method was observed by injected the six replicas of the mixture of the standards of the Caffeine, Paracetamol and Codeine Phosphate. This mixture of the standards was formed from the stock solution of the standards of Caffeine, Paracetamol and Codeine Phosphate contained the same amounts as in the dosage form. Then the six Replicas of the sample solution were injected and the Chromatograms were obtained. The Chromatograms were compared and calculated the results from it that were mentioned in table no 2.

DISCUSSION

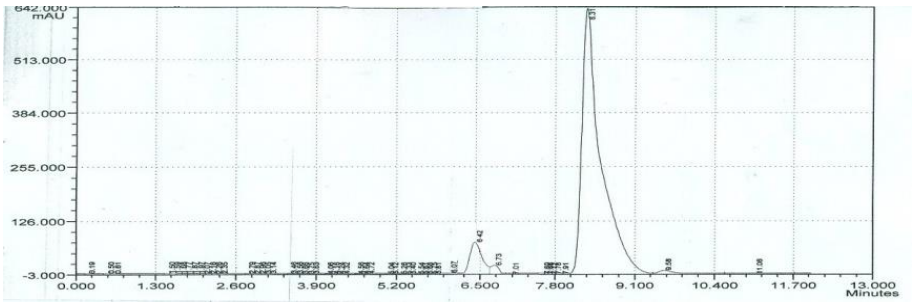
Accuracy of the developed HPLC technique was assessed by ascertaining %age recovery and Relative standard deviation for distinctive dilution (80 %. 100 % and 120 %) of Caffeine, Paracetamol and Codeine Phosphate. The normal Recoveries for all dilutions were inside indicated limit. The limit of recovery is ± 2 %.

The R.S.D qualities are less than 2%, which showed that developed method was accurate and suitable for expected utilization. No interference was found from placebo at the retention time of Caffeine, Paracetamol and Codeine Phosphate.

CONCLUSION

A reverse phase high performance liquid charomatographic method was developed for simultaneously determined the Paracetamol, Caffeine and codeine Phosphate in pharmaceutical formulations. The results proved that the developed method was simple, accurate and reproducible. The HPLC method was developed and validated by analytical parameters. The validation results shows good precision, accuracy, and specificity.

The simplicity of mobile phase, short run time, less expensive chemicals, isocratic mode of elution, good resolution and



.

Fig: 4. Chromatogram of Mixture of standards

Table # 1: Results of % age recovery of Caffeine, Paracetamol and Codeine Phosphate

Drugs	Theoretical Contents (%	Weight of Placebo	Amount Added per 100 mL	Concentration injected into HPLC	Peak Area of Sample	%age Recovery	% RSD	T. F.	Theoretical Plates
Caffeine	80.2%	108mg	24.04 mg	12 µg /mL	29473	80.22	0.11	1.38	35305
	100.2%	135 mg	30.06 mg	15 µg /mL	36789	100.13	0.07	2.42	35326
	120.2%	162mg	36.06 mg	18 µg /mL	44087	119.98	0.13	1.44	35379
Paracetamol	80.2%	108mg	401 mg	200 µg/mL	1819634	80.63	0.33	2.14	29041
	100.2%	135 mg	501 mg	250 µg/mL	2274517	100.38	0.34	1.32	29066
	120.2%	162mg	601 mg	300 µg/mL	2729445	120.07	0.34	1.49	29128
Codeine Phosphate	80.2%	108mg	12.03 mg	6 µg /mL	75267	79.97	0.12	0.71	38351
	100.2%	135 mg	15.03 mg	7.5 µg /mL	94152	100.01	0.55	1.80	38461
	120.2%	162mg	18.03 mg	9 µg /mL	112987	120.05	0.10	1.93	38374

Table No. 2: Results of precision of Caffeine, Paracetamol and Codeine Phosphate

Drugs	Peak Area	%age Recovery	% RSD	T.F.
Caffeine	36771	100.07	0.20	1.53
Paracetamol	2274794	100.01	0.029	1.36
Codeine Phosphate	94317	100.16	0.135	0.79

Simple method of standard and sample solution preparation had many advantages of the developed RP-HPLC method. The method accurately determined the amounts of all APIs in the presence of impurities and excipients.

REFERENCES

[1]. Aurnaud, M. J. The pharmacology of caffeine. *Prog. Drug*, **31**: 273 − 313(1987)

[2]. Barone, J. J., Roberts, H. R. Caffeine consumption. *Food and chemical toxicology*, **34**: 119 – 129(1996)

[3]. Lawrence, E. L. *What you need to know about food and cooking for health*,(5th Ed) pp. 254 − 258. Viking company, New York, USA(1986)

[4]. Reynolds, J. E. F. *Martindale - The Extra Pharmacopoeia*, (34th Ed.) pp. 50 − 53, Pharmaceutical Press, London(2005)

[5]. Rockville, M. D. *The United States Pharmacopeia*, (24th Ed.) pp. 17 − 39, Pharmacopeial Convention, U.S(2000)

[6]. Rodenas, V., Garcia, M. S., Sanchez, P. C., Albero, M. Simultaneous determination of propacetamol and paracetamol by derivative spectrophotometry. *Talanta*, **52:** 517 − 523(2000)

)

[7]. Frank, E. *Paracetamol - a curriculum resource*, (1st Ed) pp. 1 − 24, Royal Society of Chemistry, Burlington house, Piccard illy, London(2002)

[8]. Allen, L. V. *Remington's Pharmaceutical Sciences*, **(**23rd Ed) pp.1109 − 1110, Mack Publishing Company, Easton, PA(1995)

[9]. McElhatton, P. R., Sullivan, F. M., Volans, G. N. Paracetamol overdose in pregnancy, analysis of the outcome of 300 cases referred to the Teratology Information Service. Reprod. *Toxicol.*, **11**: 85 – 94(1997)

[10]. Bhandari, M., Bhandari, A., Bhandari, A. Recent updates on codeine. *Pharm Methods*, **2(1)**: 3 – 8(2011)

[11]. Vree, T. B., Van, D. R. T., Koopman, K. P. M. Codeine analgesia is due to codeine-6-glucuronide, not morphine. *Int. J. Clin. Pract.*, **54(6)** :395 − 398(2000)

[12]. Lewis, R. J. *Hawley's Condensed Chemical Dictionary*, (12th Ed) pp. 298, Van Nostrand Rheinhold Co., New York(1993)

[13]. Casaret., Doull's. *Toxicology*, (2nd Ed) pp. 663, 678 − 691, Macmillan Publishing Co. Inc., New York(1980