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

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

ABSTRACT: *New, rapid, simple, and precise reversed-phase high-performance liquid chromatographic method was validated and developed for the simultaneous determination of caffeine, Paracetamol and Orphenadrine citrate. Good resolution and quantization were attained on reversed-phase. The mixture containing KOH buffer: Acetonitrile = (60: 40 v/v) was chosen as Mobile Phase. The flow rate balanced was 1 mL/ min, UV quantization was set at 220 nm. Satisfactory validation results were ascertained in terms of, percentage recovery (99.1) for Paracetamol, (98.2) for Caffeine and (98.9) for Orphenadrine Citrate precision and Specificity. This method was proved to be specific and accurate for the determination of mentioned drugs in pharmaceutical formulations along with their degradation products*.

Key words: Orphenadrine citrate; Paracetamol; caffeine; Reversed-phase HPLC; Acetonitrile; Potassium Hydroxide

INTRODUCTION

Caffeine is a naturally occurring substance belongs to the methyl xanthine family and commonly found in seeds, leaves, and fruits worldwide. The systematic name of Caffeine is 1, 3, 5 Trimethylxanthine and the chemical formula is $C_8H_{10}N_4O_2$ (Fig: 1) [1]. For cardiovascular, respiratory and the central nervous system caffeine is used as a drug. It is also used with aspirin, decrease the cerebral eye blood flow and also used for headaches treatment [2]. The density of caffeine is 1.2/cm, melting point is 237 °C and boiling point is 178 °C (sublimes) [3,4,5]. Caffeine enhances heartbeat, blood pressure and causes an irregular heart rhythm.

Paracetamol or 4-hydroxyacetanilide N-(4-hydroxyphenyl) acetamide is a para-aminophenol derivative (Fig: 2). It has antipyretic and analgesic properties and do not show any anti-inflammatory activities [6,7]. Paracetamol is a white crystalline powder with 151.16256 g/mole molecular weight and 169-171C^o melting point. Paracetamol reduces the temperature of patients suffering from fever and it is a mild painkiller. In different formulations many of these products are available for the relief of cold and influenza. Allergic skin reactions and gastrointestinal problems are often caused by paracetamol. Nephropathy can be caused by Paracetamol with drugs combinations containing phenacetin [8].

Orphenadrine citrate also known as dimethyl [2-(2-methylbenzhydrylox ethyl] amine dihydrogen citrate (Fig:3). Molecular formula of orphenadrine citrate is $C_{18}H_{23}NO.C_6H_6O$. Orphenadrine is an anti-muscarinic, anticholinergic, centrally acting skeletal muscle relaxant [9]. Orphenadrine is a white crystalline powder and its molecular weight is 461.5 gm [10,11,12]. It is use to relieve pain due to spasm of voluntary muscle [13]. Orphenadrine citrate is used as an alternative to quinine nocturnal leg cramps [14].

MATERIALS AND METHODS

Materials

The Standards of Paracetamol, Caffeine and Orphenadrine Citrate were used of Analytical grade and obtained from Medipak Company Lahore. All reagents and chemicals such as Acetonitrile, methanol, HCl and KOH of HPLC grade were got from Merck, Karachi Pakistan. The tablets named Medigesic were obtained from SP, Pakistan. The tablets were commercially available and labeled to contain 450 mg Paracetamol, 30 mg Caffeine and 50 mg Orphenadrine Citrate.

Method Development

A RP-HPLC method was improved for the simultaneous determination of Caffeine, Paracetamol and Orphenadrine citrate. Equipments used for the development of method were HPLC system LC 100 UV visible spectrophotometer and Column for strongly acidic molecules Purespher RP-18 endcapped, 5.0 µ, 100 A$^\circ$ 4.6 x 250 mm. The development of Analytical method has been done step by step. Various types of solvents were used for checking solubility of Caffeine, Paracetamol and Orphenadrine citrate. From different compositions the Mobile phase selected was Potassium hydroxide buffer: Acetonitrile = (60: 40). The mobile phase was filtered with the 0.45 µm Nylon membrane after sonication of 10 mins. The UV detection was checked at 220 nm. The flow rate was 1min/ml at ambient temperature. Injection Volume was 20 µL.

Preparation of 0.1 M Buffer

In 1000 ml volumetric flask 4.0 g Potassium Hydroxide was added. The distilled water was used to dissolve it and volume was up to the mark with distilled water. pH was maintained to 3.8+ 0.1 with Hydrochloric Acid.

Preparation of Mobile Phase

In 1000 ml volumetric flask 600 ml of Potassium Hydroxide buffer was transferred and then 400 ml Acetonitrile to prepare a mobile phase.

Standard Solution of Caffeine

In 100 ml measuring flask 30 mg of caffeine was transferred, added 80 ml mobile phase, sonicated to dissolve and again mobile phase was added to made volume up to the mark . 10 ml of this standard solution was taken into another 100 ml measuring flask and mobile phase was used to make up the volume. It contains 30 µg/ml.

Standard Solution of Paracetamol

In 100 ml measuring flask 450 mg of Paracetamol was transferred, added 80 ml mobile phase, sonicated to dissolve and again mobile phase was added to made volume up to the mark.10 ml of this standard solution was taken into another

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100 ml measuring flask the mobile phase was used to make up the volume. It contains 450 µg/ml.

Standard Solution of Orphenadrine Citrate.

In 100 ml measuring flask 50 mg of Orphenadrine citrate was transferred, added 80 ml mobile phase, sonicated to dissolve and again mobile phase was added to made volume up to the mark. 10 ml of this standard solution was taken into another 100 ml measuring flask the mobile phase was used to make up the volume. It contains 50 µg/ml.

Preparation of Sample

An accurately weigh amount of powdered drug equivalent to 450 mg of Paracetamol, 50 mg of Orphenadrine citrate and 30 mg of Caffeine was transferred in 100 ml volumetric flask and added 80 ml of mobile phase, sonicated to dissolve and again mobile phase was added to made volume up to the mark. From this 10 ml of sample solution was transferred into another 100 ml volumetric flask and up to mark with mobile phase and mixed well. The concentration of the above solution was Caffeine = 30 mg/ml, Paracetamol = 450 mg/ml and Orphenadrine citrate = 50 mg/ml

Preparation of Mixture of Standards

30 mg of Caffeine, 450 mg of Paracetamol and 50 mg of Orphenadrine citrate was added in 100 ml volumetric flask and 80 ml of mobile phase was added, sonicated to dissolve and volume was up to the mark with mobile phase. From this solution 10 ml of sample was transferred into another 100 ml volumetric flask and up to mark with mobile phase and mixed well. This solution contains 30 mg/ml of Caffeine, 450 mg/ml of Paracetamol and 50mg/ml of Orphenadrine citrate.

RESULTS AND DISCUSSION

A simple rapid and precise reverse phase HPLC method with UV detection was developed and validated for the simultaneous determination of Caffeine, Paracetamol and Codeine Phosphate in pharmaceutical formulations. Accuracy, precision and specificity were found from this validated method. Accuracy of the developed HPLC technique was assessed by ascertaining %age recovery and Relative standard deviation for distinctive dilution (80 %. 100 % and 120 %) of Paracetamol, Caffeine and Orphenadrine citrate. The accuracy of method was observed by standard addition method and the study was proceed in triplicate, at each level of concentration such as 80 %. 100 % and 120 %. The %age recovery and standard deviation of the %age recovery were calculated and results are mentioned in Table # 2.

To prove the sufficiency of the method, a laboratory mixture of Paracetamol, Caffeine & Orphenadrine citrate was prepared. This mixture was Prepared from the stock solutions that contains equal ratio of quantities in the dosage form. For quantitative determination of the mixture, a series of six replicas at 100 % concentration were prepared and twelve solutions for each concentration (twelve Replicas) were injected. There %age recovery, theoretical plates, tailing factor and resolution results are mentioned in shown # 1.

The specificity of the method, Paracetamol RS (single APIs 100 %), Caffeine RS (single APIs 100 %), and Orphenadrine citrate RS (single APIs 100 %) solutions were prepared. All of the APIs were mixed together. solutions of APIs, their mixture, placebo and sample solutions were run and results obtained. The chromatogram of mixture of standards was obtained which matched with others as shown in Fig 4.

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 Paracetamol, Caffeine and Orphenadrine citrate. The normal Recoveries for all dilutions were inside indicated limit.

The R.S.D qualities were under 2 %, which showed that developed method was accurate and suitable for expected utilization. No interference was found from placebo at the retention time of Paracetamol, Caffeine and Orphenadrine citrate.

CONCLUSION

A reverse phase high performance liquid charomatographic method was developed for simultaneously determined the Paracetamol, Caffeine and Orphenadrine citrate 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 simple method of standard and sample solutions preparation was many advantages of the developed RP-HPLC method. The method accurately determined the amounts of all APIs in the presence of impurities and excipients.

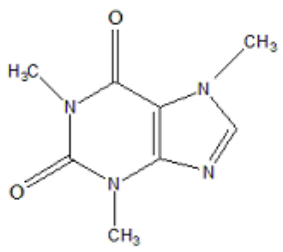

Fig: 1. Caffeien

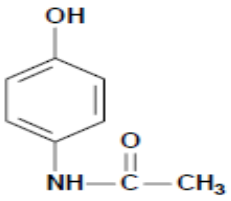

Fig: 2. Paracetamol

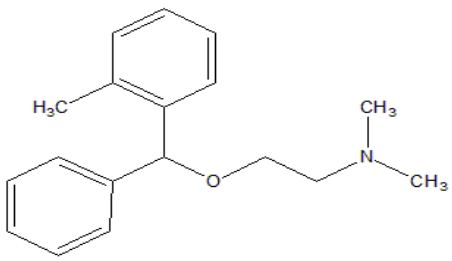

Fig: 3. Orphenadrine Citrate

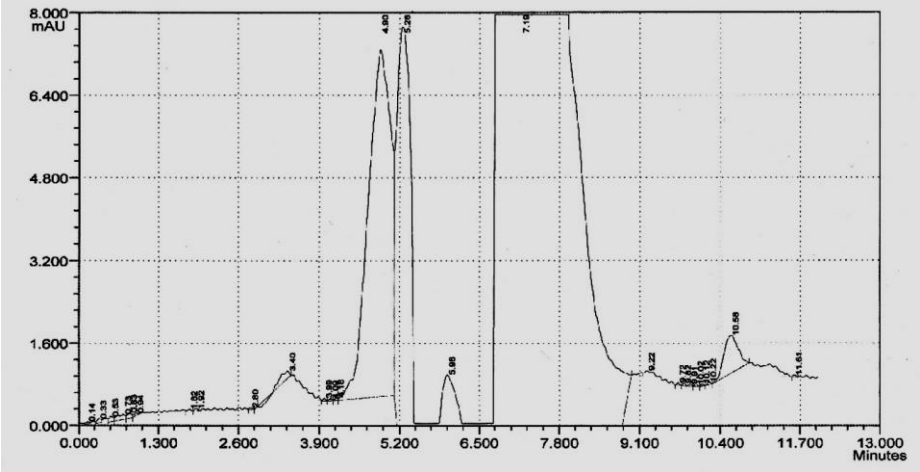

Fig: 4. Chromatogram of Mixture of standards

Table No. 1: Results of precision of Caffeine, Paracetamol and Orphenadrine citrate

Drugs	Peak Area	%age Recovery	% RSD	T.F.
Caffeine	662317	100.06	0.184	1.46
Paracetamol	120763232	100.12	0.286	1.63
Orphenadrine citrate	120588	100.14	1.86	3.79

T**able No. 2: Results of % age recovery of Caffeine, Paracetamol and Orphenadrine citrate**

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%	340 mg	24.04 mg	24 µg /mL	622177	80.63	0.66	2.44	22312
	100.2%	380 mg	30.06 mg	30 µg /mL	662105	100.38	0.61	3.64	23085
	120.2%	420 mg	36.06 mg	36 µg /mL	696775	120.07	0.68	4.47	23174
Paracetamol	80.2%	340 mg	360.9 mg	360 µg /mL	10566354.7	80.63	0.64	1.44	25281
	100.2%	380 mg	450.9 mg	450 µg /mL	12066315.5	100.38	0.51	1.78	51365
	120.2%	420 mg	540.9 mg	540 µg /mL	14066456.0	120.07	0.23	1.47	49843
Orphenadrine citrate	80.2%	340 mg	40.1 mg	40 µg /mL	100576	80.63	0.76	2.11	22321
	100.2%	380 mg	50.1 mg	50 µg / ml	120588	100.38	0.71	1.80	22578
	120.2%	420 mg	60.1 mg	60 µg / mL	140572	120.07	0.68	1.93	22432

REFERENCES

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

[2]. Waynoka, H. N., Gatebe, E. G., Gitu, L. M., Ngumba, E. K., Maritim, C. W. Determination of caffeine content of tea and instant coffee brands found in the Kenyan market. *Journal of Food Science,* **4(6)**: 353 – 358(2010)

[3]. Zubair, M. U. *Analytical Profiles of Drug Substances,* (-15thEd) pp.447, Academic Press, New York, London (1986)

[4]. Mills, T. *Instrumental Data for Drug Analysis,* (2nd Ed) pp.77-79, Elsevier Press, UK (1991)

[5]. Moffatt, A.C. *Clarke's Isolation and Identification of Drugs,* (2nd Ed) pp. 56, Pharmaceutical Press, London (1986)

[6]. Martindale. *The Extra Pharmacopoeia,* (31st Ed) pp.81-503, Royal Pharmaceutical Society, London, Britain (1996)

[7]. British Pharmacopoeia. The Stationary office under license from the controller of Her Majesty's stationary office for the department of health on behalf of the health Ministers, pp. 1385- 1417(2003)

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

[9]. Karim, A., Jumaa, F. A. A., Abdul, R., Hikmet, A. A. Formulation and Clinical Evaluation of Orphenadrine citrate as a Plain Tablet. *J.Pharm.Sci.*, **15**: 1-7(2006)

[10]. George, H., Cocolas. (1991). *Wilson and Gisvoldorganic medicinal and pharmaceutical chemistry,* (9th Ed) pp. 483, Lippincott Company, USA.

[11]. Lund. (1991). *The pharmaceutical codex, Principles and Practice of pharmaceutics,* (12th Ed) pp. 986 – 987, The pharmaceutical press, London.

[12]. Clarks. (1986). *Isolation and identification of drugs*, (2nd Ed) pp. 833, The pharmaceutical press, London.

[13]. Elenbass, J. K. (1980). Centrally acting oral skeletal muscle relaxants. *J. Hosp. pharm.*, **37**: 1313 – 1323.

[14]. Latta, D., Turner, E. (1989). An alternative to quinine nocturnal legs cramps. *Curr. Ther. Res.*, **45**: 833 – 837.