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Simultaneous Determination and Quantification of Paracetamol, Caffeine and Orphenadrine Citrate using Stability Indicating HPLC Method in a Fixed Dose Combination Tablet Dosage Form

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Abstract

The paper describes the development of novel and robust HPLC method for the determination and quantification of Paracetamol, Caffeine, and Orphenadrine Citrate in a solid dosage form. The chromatographic separation was achieved with RP C_{18} column Merck (5 µm, 4.6 mm × 250 mm) using buffer and methanol (680:320 v/v) as a mobile phase. The flow rate was set at 1.0 ml/min with column oven temperature maintained at 50°C. The method validation was performed as per ICH guidelines. The results of the validation showed satisfactory results and complied with the acceptance criteria. The Correlation coefficient (R_2) was greater than 0.999 for all the three components. The retention time was found to be 3 min ± 0.5 min (Paracetamol), 5.6 ± 0.02 min (Caffeine), 33 ± 1 min (Orphenadrine Citrate) respectively. The limit of detection for Paracetamol, Caffeine, and Orphenadrine Citrate was 2.8091, 2.837, and 1.3408 respectively. Stress degradation studies were performed by subjecting the analytes to various stress conditions, acidic and alkaline hydrolysis, photolytic degradation, Thermal degradation. The samples proved to be stable under all conditions except photolytic degradation. The method developed effectively separates the analytes from the degradation products if any without much variation in their retention time. Finally, the method developed proved to be suitable for the routine analysis of Paracetamol, Caffeine and Orphenadrine Citrate under isocratic conditions.

Keywords: Stability indicating HPLC; Paracetamol; Caffeine; Orphenadrine citrate; Solid dosage form

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Introduction

With recent advancements in dosage forms, combination of drugs is being used as therapeutic regimens to treat different diseases whereby more than one drug is prescribed to the patient either as a single tablet/capsule (fixed dosage form) or as a separate multiple pills [1]. One of the major benefits of the combination therapies is that they reduce drug resistance, improve drug response, and minimize adverse reactions and incidences [2,3]. In general, the concept of drug combination therapy is not new with significant increase in the clinical use of fixed dose combinations over the past 10 years for various ailments [4,5]. Numerous attempts have been made to develop a fixed dose combination to be used in migraine headache, muscular pain, toothaches and dysmenorrheal [6]. Fixed-dose combinations, compared to individual medications, Loose Pill Combinations (LPCs), offered four key advantages; simplified dosing/timing, greater efficacy with lower doses in combination, low risk of ADRs and pocket friendly [7].

Migraine, a highly prevalent disorder, manifested as highly disabling primary headache with socioeconomic burden and high prevalence, highest in United States, followed by Europe, Africa and Asia [8]. Combination of Orphenadrine citrate, Paracetamol and Caffeine has been utilized in the treatment of migraine [9-11]. Paracetamol is one of the famous over the counter drug. Paracetamol is a Para-aminophenol derivative (N-acetyl-para-aminophenol) and has analgesic and antipyretic properties. The Paracetamol has high targeted action in the brain, it blocks an enzyme involved in

the transmission of the pain [12,13]. Caffeine is 1,3,7-Trimethyl-3,7-dihydro-1H-purin-2,6-dion central nervous system stimulant. The caffeine has the tendency to increase the analgesic efficacy because of its stimulating effect on the CNS, relieving frequent pain-associated depression [14-17]. Caffeine in combination with the Paracetamol in a fixed combined dosage form has proved to be effective in curing numerous diseases like a migraine headache, dysmenorrhea, cancer pain, postpartum pain, sore throat, and dental post-surgery pain [18]. Orphenadrine Citrate is one of the anticholinergic drugs that is used to treat painful muscle spasms (spasm of skeletal muscles), other similar conditions. It belongs to the ethanolamine category and antihistamine (allergic relief) class [19,20].

Extensive literature searches showed that paracetamol, and caffeine is investigated either alone or in combination with other drugs in a pharmaceutical form [21-24].

The development of analytical method for the estimation of fixed-dose combinations is pivotal to ensure more effective and safe use of already prevalent and novel combinations. Many researchers have reported analytical methods base on the use of instrument techniques and high tech instruments on the estimation of Paracetamol, Caffeine and Orphenadrine Citrate either separately or in combination with other drug(s) in pharmaceutical dosage forms or biological fluids by spectrophotometry [24-32], liquid chromatography-mass spectrometry [33], High-Performance Liquid Chromatography (HPLC) [34-53], capillary electrophoresis [54], voltammetry [54,55], and Thin-Layer Chromatography (TLC) [56,57].

In the modern era and with the more stringent requirements from the regulatory bodies a stability indicating method has become essentially necessary for the simultaneous determination of the multiple drugs in one dosage form.

There are number of methods available for the determination of Paracetamol, Caffeine Orphenadrine Citrate either in double active combined dosage form i.e. Paracetamol and Caffeine or with two different active combinations i.e. [5,58,59]. Analytical methods for single dosage form and HPLC method with three different drug combinations, Paracetamol, Caffeine and Dipyrone, in solid dosage form have been reported previously [51,52]. To the best of our knowledge, no literature evidences exist on RP-HPLC Stability indicating method for simultaneous determination of Paracetamol, Caffeine, and Orphenadrine Citrate in a combined tablet dosage form. Thus, we aimed to develop and validate RP-HLPC stability indicating method for three analytes, Paracetamol, Caffeine, and Orphenadrine Citrate, for highly reproducible, accurate, economical and reliable routine analysis of tablet dosage form. Moreover, another aim was to elute three analytes in a single injection to make the preparation and its execution simple. The average weight of the in-house designed combined solid dosage form was 920 mg with label claim of Paracetamol 450 mg, Caffeine 30 mg and Orphenadrine Citrate 50 mg respectively per tablet (Figure 1).

Experimental

Chemical reagents and materials: All the standards for Paracetamol, caffeine and Orphenadrine citrate were purchased from Starna scientific. Analytical grade reagents like Tetrabutylammonium hydrogen sulphate were purchased from Sigma Aldrich. HPLC grade Methanol was procured from Merck (Germany). Membrane filter paper 0.45 µm was bought from Millipore Corporation. Filter paper Sartorius (393 & 389) and Whatman no. 42 were procured from Sigma

Figure 1: Chemical structure of a) Caffeine b) Paracetamol and c) Orphenadrine Citrate.

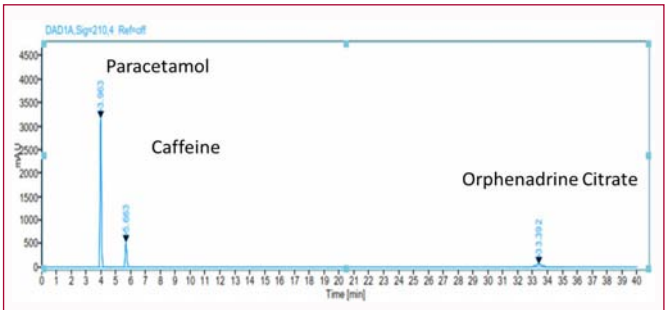

Figure 2: Overlay of the Chromatogram of Paracetamol, Caffeine and Orphenadrine Citrate.

Aldrich. Distilled water was produced locally. The Water filtered through 0.45 µm was used in complete study. Memmert Water Bath was used in the experiment.

Instruments: The analysis was carried out using HPLC system; 1260 Infinity II series, Agilent technologies USA, having Quaternary Pump (G7111B), Auto VialSampler (G7129A), Column Chamber (G7116A), Diode Array Detector DAD WR (G7115A). Other Instrument included Ultrasonic bath (Elma Germany), centrifuge (local) and LUX study chamber built in house to fulfill our requirements.

Methodology

The Mobile phase employed for the separation of three analytes (Paracetamol, Caffeine, and Orphenadrine Citrate) consisted of mobile phase A: Buffer (Tetrabutylammonium hydrogen sulphate 0.01 mol/L) and mobile phase B: Methanol in ratio (680:320 v/v). The Chromatographic separation was achieved using Merck 4.6 mm × 250 mm with particle size 5 µm at a flow rate of 1 ml/min and injection volume of 5 µl. The system applied was Isocratic and the column temperature was maintained at 50°C.

Preparation of standard calibration solution

Stock solutions were prepared by mixing adequate amount of the standards in their dissolving solvent (mobile phase). The individual stock solutions were used to prepare mix standard solution. Further the working mix stock solutions were prepared at different concentration levels. These working standard mixtures were obtained by diluting appropriate volume of stocks in mobile phase and attained the desired concentration levels.

Preparation of test stock and working test sample solutions

Took 20 tablet and crush & pulverized them in mortar and pestle. Took sample equivalent to average of tablet (920 mg) in a 250 mL volumetric flask. Add 100 mL of mobile phase, Shake and sonicate for 25 min to dissolve. Made the final volume with mobile phase and filtered the solution through a 0.45 micron membrane filter before

injecting.

Accurately weigh 50 mg of Orphenadrine Citrate, 30 mg of Caffeine & 450 mg of Paracetamol working standard in a 250 mL volumetric flask. Add 100 mL of mobile phase, shake and sonicate for 25 min to dissolve. Made the final volume with mobile phase and filtered the solution through a 0.45 micron membrane filter before injecting (Figure 2).

System suitability

The system suitability parameters such as symmetry, tailing factor and theoretical plates were automatically generated from the chromatogram using software open lab Agilent. These parameters ensured the accuracy of the system during the analysis.

Validation

Methods developed and used in the analytical chemistry laboratory must be evaluated and tested to ensure that they produce valid results appropriate for its intended use, i.e. they must be validated. The quantification or the analytical method validation is to check the characteristics of the method developed and to ensure that the developed method provides authentic results. The method validation was carried out by performing linearity, accuracy, precision, intermediate precision, LOD, LOQ, specificity, solution stability, and stress degradation studies as per ICH guidelines.

Linearity and range

Traditionally, methods are described as linear when there is a directly proportional relationship between the method response and concentration of the analyte in the matrix over the range of analyte concentrations of interest (working range). Linearity was performed by injecting mixture of standard solutions over five concentration ranges i.e. 60%, 80%, 100%, 120%, and 140%. Linearity was considered satisfactory when the coefficient of determination was R>0.999, based on their peak areas. Linearity was tabulated by visual inspection of concentration plot *vs.* peak area and confirmed from linear regression equation (Figure 3).

Sensitivity

The LOD is as the lowest concentration that can be distinguished from the background noise with a certain degree of confidence. It can be detected but not necessarily quantified under given conditions.

Similarly limit of quantification is the minimum concentration of the analyte that can be quantified with accuracy and precision under stated conditions. As per guidelines there are numerous methods to estimate LOD and LOQ i.e. visual evaluation, signal to noise ratio, based on standard deviation of the response and the slope, based on the standard deviation of the blank and from calibration curve. A minimum requirement for signal to noise of 3:1 is widely accepted for LOD and 10:1 for LOQ respectively. In this method LOD was calculated based on the Standard Deviation (SD) of the response and the slope of the calibration curve (S) of the analyte at levels approximating the LOD expressed by formula: DL=3.3 σ/S.

The quantification limit is expressed using the formula: QL=10 σ/S,

Accuracy

Accuracy was performed in terms of recovery. Accuracy was assessed using a minimum nine determinations over minimum concentration levels covering the specified range in triplicate. In recovery experiment added a known amount of standard to a sample

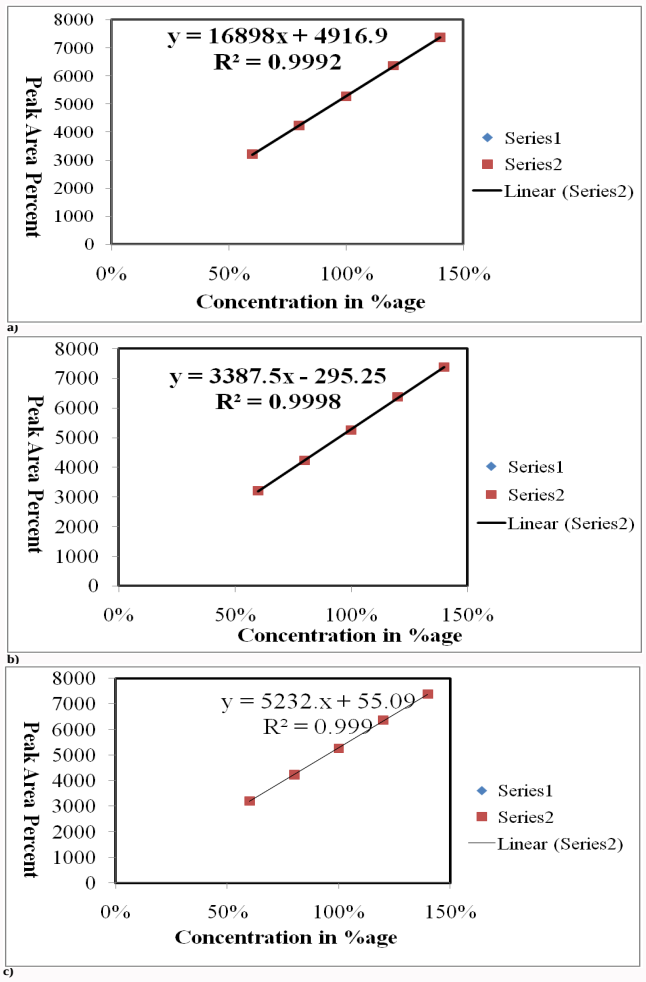

Figure 3: Linear Graph for a) Paracetamol b) Orphenadrine Citrate c) Caffeine.

(Placebo) and takes it through the entire method process to find out if you could recover whatever you added.

Precision and intermediate precision

Precision represents the random errors of a set of replicate measurements. Precision depends critically on the conditions! Repeatability and reproducibility conditions are a particular set of extreme conditions.

Repeatability is a set of conditions that include, the same measurement, procedure, operators, same measuring system, operating conditions, and location, and replicate measurements on the same or similar objects over a short period of time. Repeatability was evaluated over nine determinations covering the specified range at three concentrations with three replicates of each concentration.

Repeatability is a set of conditions that include the same experimental criteria but on consecutive days to evaluate the reproducible results on different days. Reproducibility was evaluated over three concentration ranges over three days period (n=2). Intermediate precision was evaluated by the same procedure as above but by different analyst on different days and using different equipment.

Specificity

Specificity was investigated to check the interference and the

Table 1: Shows the results of method validation.

Validation Parameter			Paracetamol	Caffeine	Orphenadrine Citrate	Acceptance Criteria
Specificity			No peak	No peak	No peak	No peak should be present
Solution Stability	24 h 25°C	Absolute Difference	0.899%	0.138%	0.1748%	<2.0%
	48 h 25°C		-0.169%	-0.020%	-0.725%	<2.0%
	24 h 2°C-8°C	Absolute Difference	0.167	0.112	0.625	<2.0%
	48 h 2°C-8°C		0.289	0.425	0.499	<2.0%
Filter Compatibility		Filter 0.45µm	0	0	0	<2.0%
		Sartorius #393	-0.778%	0.555%	-0.894%	<2.0%
		Sartorius #389	9.320%	13.976%	21.27%	<2.0%
Filter Saturation		0.0 mL	0	0	0	<2.0%
		5.0 mL	-0.834%	-1.750%	-1.467%	<2.0%
		10.0 mL	-0.640%	-1.967%	1.574%	<2.0%
Linearity & Range		Correlation Coefficient	0.9992	0.9998	0.9998	R2 ≥ 0.999
Accuracy		Mean Recovery	98.13%	98.89%	101.60%	<2.0%
Robustness		Mobile Phase	%RSD<2.0%	%RSD<2.0%	% RSD < 2.0%	<2.0%
		Flow Rate	%RSD<2.0%	%RSD<2.0%	% RSD < 2.0%	<2.0%
		Wavelength	%RSD<2.0%	%RSD<2.0%	% RSD < 2.0%	<2.0%
		Column	%RSD<2.0%	%RSD<2.0%	%RSD<2.0%	<2.0%
		LOD	2.8091 ppm	2.8437 ppm	1.3408 ppm	-
		LOQ	8.5126 ppm	8.6173 ppm	4.0631 ppm	-
System Suitability		Symmetry	0.83	0.82	0.89	<1.5
		Plates	8657	12277	13702	≥ 2000
Precision		Repeatability	%RSD<2.0%	%RSD<2.0%	%RSD<2.0%	<2.0%
		Intermediate Precision	%RSD<2.0%	%RSD<2.0%	%RSD<2.0%	<2.0%

presence of other components that could interfere with the principle peak and cause dubious results.

Robustness

The robustness in this method was performed by making slight deliberate changes to the developed method. The changes were made to the flow rate, detection wavelength, column temperature, and mobile phase composition to monitor their impact on the percent recovery of the compound.

Solution stability

The test solution prepared of which one portion was protected and stored at room temperature 25°C and the other portion was stored in refrigerator at 2°C to 8°C for 24 h and 48 h and the recovery obtained was compared with the freshly prepared sample.

Stress degradation studies

In order to establish that the method developed is stability indicating stress degradation studies were conducted. The stress degradation studies were performed on the lines of ICH guidelines. The Working standard solution at a specified concentration was subjected to hydrolytic, oxidative, hydrolytic and thermal stress studies. Stress studies play a vital role in the determination of degradation pathways and to identify any impurities related to the drug substance/product, this in turns helps to identify possible degradation products and to make the formulation more stable. For this study, three samples and standards were prepared in triplicate for each degradation condition according to the developed analytical procedure. The evidence of the degradation was evaluated from the decreasing concentration trend.

Acidic and alkaline hydrolysis

From the individual standard stock solution of Paracetamol, Caffeine and Orphenadrine Citrate add 5 ml to the four 50 ml volumetric flask. Then 5 ml 2M HCL or 2M NaOH was added to three different volumetric flasks for acidic and alkali hydrolysis. Freshly prepared solution were neutralized with 2M HCL and 2M NaOH immediately and diluted to the mark with the diluting solvent. The solutions were filtered and injected into the HPC system. The previous three flasks with 0.1M HCL and NaOH along with standard stock solution were kept at the laboratory temperature for 24 h. The solutions were then treated in similar manner as freshly prepared sample i.e. 0 hr.

Oxidative degradation

Oxidative stress degradation studies were analyzed by treating the three analytes (Paracetamol, Caffeine, and Orphenadrine Citrate) in standard solution separately with 30% H_2O_2. The solutions in each flask were kept under laboratory temperature and humidity (25°C/51% RH) for 24 h. The 0 h sample was compared with the 24 h sample.

Photolytic stress

The photolytic degradation was carried out on the three analytes to determine the effect of light on each analyte (Paracetamol, Caffeine, and Orphenadrine Citrate). The standard solutions of individual molecule were kept in the photo stability chamber. The test sample was exposed to 1.2 million lux hour. The sample was kept for five days in the photo stability chamber as per the intensity of the chamber to

meet the requirements. The samples were than analyzed in similar fashion as 0 hour sample.

Thermal degradation

Thermal degradation is very important as the temperature has major effect on the stability of the drug substance/product. The standard or powdered sample solution was treated with temperature 70°C. The 0 hour sample was prepared and immediately analyzed on HPLC while the treated sample was heated at 70°C for 2 h and then analyzed.

A triplicate preparation of the composite sample or individual standard solution of Paracetamol, Caffeine and Orphenadrine Citrate was prepared from the stock solutions and treated with temperature and humidity 40°C and 60Rh-75RH. The samples were then analyzed according to the analytical procedure.

Results and Discussion

The novel method developed for Paracetamol, Caffeine and Orphenadrine citrate was initially optimized before finalizing the method. Preliminary trials were conducted using various mobile phases for better separation and resolution. The mobile phase initially investigated comprised of Acetonitrile: Water, Methanol with 0.1% formic acid: Water 0.1% formic acid, acetonitrile: Sodium dihydrogen phosphate (1:1) and methanol: Sodium dihydrogen phosphate (1:4). In most of the mobile phases the separation was poor and Orphenadrine citrate was not detected and the resolution between Paracetamol and Caffeine was very poor. The best resolution and separation was achieved using Methanol: Tibutylammonium hydrogen Sulphate under isocratic conditions. Two different C18 columns (Merck, 5 µm, 4.6 mm × 250 mm and Waters Symmetry®, 5 µm, 4.6 mm × 150 mm) were tested as stationary phases for better separation and determination of Paracetamol, Caffeine, and Orphenadrine Citrate peaks. The Merck, (5 µm, 4.6 mm × 250 mm) RP C18 exhibited better separation parameters. The mobile phase was used as a better diluting solvent in this study after optimization. The flow rate (1 ml/min to 1.2 ml/min), Wave length 210 nm to 260 nm), Injection Volume (5 µl, 10 µl and 20 µl) and, column temperature (30°C to 50°C) was studied and adjustments were made accordingly. The optimal flow rate, wavelength, Injection volume and column temperature for the method was 1 ml/min, 210 nm, 5 µl, and 50°C. The Optimal conditions were selected on the basis of cost effective preparation, ease of making mobile phase and god separation and chromatographic separation of the three analytes Paracetamol, Caffeine and Orphenadrine Citrate respectively with good system suitability values. Figure 2 depicts the HPLC chromatogram of standard and sample solution of Paracetamol, Caffeine, and Orphenadrine Citrate respectively.

The validation and the system suitability parameters were within the define acceptance criteria. The system suitability was conducting by analyzing the standard solution of paracetamol 0.2 mg/ml, Caffeine 0.12 mg/ml, and Orphenadrine Citrate 1.8 mg/ml in six replicate injections. Similarly equivalent dilutions were made for the samples and injected six replicas. The developed method proposed parameters for the three analytes were within acceptance limits and showed consistent results for both standard and samples. Excellent symmetry was achieved for Paracetamol (mean standard 0.83 and mean sample 0.82), Caffeine (mean standard 0.82 and means sample 0.83), Orphenadrine Citrate (mean standard 0.89, and mean sample 0.90). Mean theoretical plates for the standard of Paracetamol, Caffeine and Orphenadrine Citrate were 8657, 1227 and 13702 respectively. Similarly, Mean theoretical plates for the sample of Paracetamol, Caffeine and Orphenadrine Citrate were 8398, 12952 and 14614 respectively. The tailing factor of the three analytes were within acceptance criteria i.e. NMT 1.5 for both standard and sample.

The linearity curve drawn over the five concentration ranges. The linearity results were satisfactory and coefficient of determination for Paracetamol, Caffeine, and Orphenadrine Citrate were 0.9992, 0.9998 and 0.9998 respectively.

The accuracy was evaluated in terms of percent recovery by spiking the placebo with standard mixture of Paracetamol, Caffeine, and Orphenadrine Citrate over five concentration levels (60% to 140%). The minimum and maximum recovery obtained for Paracetamol was 97.40% and 100.21% respectively. The mean recovery for Paracetamol was 98.13%. Mean Recovery results for the Caffeine and Orphenadrine Citrate was 98.89% and 101.96% respectively.

The solution remained stable when kept at room temperature 25°C and refrigerator 2°C to 8°C for 24 h and 48 h. Few deliberate changes in the method's mobile phase composition, flow rate, Wave-length and column temperature, were made and there were no significant differences found in the peak area and the retention times of the analytes. The % RSD values were <2% for three analytes Paracetamol, Caffeine and Orphenadrine Citrate, which indicated adequate robustness of the method shown in the Table 1.

From the developed method it was evident that there was no interference from the tablet dosage form or any impurities in the drug retention time. In the stress degradation studies there was clear principal peaks from the degradation products. The developed method indicated a good specificity. The Limit of detection determined on the basis of the standard deviation of the response and the slope of the calibration curve from linearity test for this method was found to be 1.34 ppm Paracetamol, 2.81 ppm Caffeine and 2.84 ppm Orphenadrine Citrate. The Limit of Quantification was 4.063 ppm Paracetamol, 8.512 ppm Caffeine, and Orphenadrine Citrate 8.617 ppm respectively.

The precision in terms of repeatability and reproducibility and intermediate precision were also in compliance with the defined criteria. The % RSD of the Assay results of the six individual assays in the repeatability was not more than 2%. By changing the analyst and the system on different day also produced % RSD less than 2%. Hence, the proposed developed method is precise.

Stressed degraded samples were analyzed against freshly prepared samples placed for 0 h. The extent of degradation was evaluated in terms of percent recoveries. In case of acidic hydrolysis Paracetamol showed degradation up to 19% after exposure to 2M HCL for 24 h, whereas in alkali hydrolysis 2M NaOH both sample and standard of Paracetamol showed 22% degradation when exposed for 24 h. Caffeine was stable in acidic hydrolysis whereas in alkali hydrolysis peak did not appear as also indicated by Nafiu et al. [5]. Loss of peak may be attributed to the deprotonation started by NaOH because of which it becomes insoluble in mobile phase.

Orphenadrine citrate showed 15% deviation in the acidic hydrolysis and is stable in alkali hydrolysis exposed for 24 h. There was no significant effect on the concentration of both sample and standard of the Paracetamol in photolytic degradation studies whereas other two analytes Caffeine and Orphenadrine citrate showed slight

Table 2: Degradation studies conducted and their percent decrease in concentration in terms of recoveries.

Stress Studies	Condition	Time	Paracetamol	Caffeine	Orphenadrine Citrate
			% Recovery	% Recovery	% recovery
Acidic Hydrolysis	2M HCL	24 hr	80.67%	99.85%	85.06%
Alkaline	2M NaOH	24 hr	77.62%	No peak	100.12%
Oxidative Stress	30% H_2O_2	24 hr	55.89%	72.36%	68.45%
Photolytic Stress	Chamber	5 days	98.58%	95.59%	96.13%
Thermal Degradation	70°C	2 hr	100.01%	100.58%	101.01%
Temp/Humidity	40°C/&75 RH	2 hr	99.26%	98.82%	99.14%

degradation when placed for 5 days in the photo stability chamber. Decrease in all the analyte concentration was observed at 0 h in hydrogen peroxide solution and opposite trend was seen in the samples/standard placed for 24 h. Thermal degradation studies at temperature 70°C and temperature-humidity induced at 40°C-RH 75 studies did not show any additional peak in the chromatogram and the three analytes remained stable. In all the stress studies, there was not much change in the retention time. The above developed method was quantified and degradation pathways were investigated and are evident that the method has the stability indicating power (Table 2).

Conclusion

The results of the above study has exhibited that the method developed for the simultaneous determination of Paracetamol, Caffeine and Orphenadrine citrate is simple, robust, authentic and reproducible. Previously only two analytes Paracetamol and caffeine were determined by the methodology developed by other scientists. The method developed simultaneously analyses the three analytes in one injection instead of applying two different methods for the three analytes namely Paracetamol, Caffeine and Orphenadrine citrate. Under forced degradation studies, Paracetamol showed slight degradation in recovery in acidic and alkali hydrolysis. Orphenadrine showed slight degradation in acidic hydrolysis. Caffeine showed no peak in alkali hydrolysis. Analytes were decomposed when exposed to oxidative stress studies at 0 h. Orphenadrine citrate showed degradation in recovery in photolytic degradation studies. There was no interference of degradation products in the determination of the main compound. Hereby, the method developed is linear, precise, accurate, specific, robust as well as stability indicating and therefore suitable for routine analysis of the assay of the product (Paracetamol, Caffeine, Orphenadrine citrate) in combine solid dosage form in Quality Control laboratory.

References

1. Combination drug therapy. 2017.
2. Wu M, Sirota M, Butte AJ, Chen B. Characteristics of drug combination therapy in oncology by analyzing clinical trial data on Clinical Trials.gov. Pac Symp Biocomput. 2015:68-79.
3. What is cmbination therapy. Oncosec. 2017.
4. Wu M, Sirota M, Butte AJ, Chen B. Characteristics of drug combination therapy in oncology by analyzing clinical trial data on Clinical Trials. gov. Pac Symp Biocomput. 2014.
5. Nafiu A, Siok-Yee C, Hayat KN, Bassam FA, Nura UM, Seok-Ming T. A simple stability-indicating HPLC method for simultaneous analysis of paracetamol and caffeine and its application to determinations in fixed-dose combination tablet dosage form. Acta Chromatographica. 2019;31(2):85-91.
6. Mannix LK, Martin VT, Cady RK, Diamond ML, Lener SE, White JD, et al. Combination treatment for menstrual migraine and dysmenorrhea using sumatriptan-naproxen: Two randomized controlled trials. Obstet Gynecol. 2009;114(1):106-113.
7. Bell DSH. Combine and conquer: Advantages and disadvantages of fixed-dose combination therapy. Obstet Gynecol Metabol. 2013;15(4):291-300.
8. Lipton RB, Stewart WF, Diamond S, Diamond ML, Reed M. Prevalence and burden of migraine in the United States: Data from the American Migraine Study II. Headache. 2001;41(7):646-57.
9. Benjamin G, Michael M. Treatment of acute migraine headache. Am Fam Physician. 2011;83(3):271-80.
10. European pharmacopoeia. 2005;2184-5.
11. Martindale: The complete drug reference. 35th ed. London: Pharmaceutical Press; 2007.
12. Chandrasekharan NV, Dai H, Roos KL, Evanson NK, Tomsik J, Elton TS, et al. COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: Cloning, structure, and expression. Proc Natl Acad Sci USA. 2002;99(21):13926-31.
13. Jóźwiak-Bebenista M, Nowak JZ. Paracetamol: Mechanism of action, applications and safety concern. Acta Pol Pharm. 2014;71(1):11-23.
14. Nehlig A, Daval JL, Debry G. Caffeine and the central nervous system: Mechanisms of action, biochemical, metabolic and psychostimulant effects. Brain Res Brain Res Rev. 1992;17(2):139-70.
15. Liguori A, Hughes JR, Grass JA. Absorption and subjective effects of caffeine from coffee, cola and capsules. Pharmacol Biochem Behav. 1997;58(3):721-6.
16. Straube A, Aicher B, Fiebich BL, Haag G. Combined analgesics in (headache) pain therapy: shotgun approach or precise multi-target therapeutics? BMC Neurol. 2011;11:43.
17. Fiebich BL, Candelario-Jalil E, Mantovani M, Heinzmann M, Akundi RS, Hüll M, et al. Modulation of catecholamine release from rat striatal slices by the fixed combination of aspirin, paracetamol and caffeine. Pharmacol Res. 2006;53(4):391-6.
18. Ahmad Bhawani S, Fong SS, Mohamad Ibrahim MN. Spectrophotometric analysis of caffeine. Int J Anal Chem. 2015;2015:170239.
19. Budavaried S, editor. The merck index: An encyclopedia of chemicals, drugs and biologicals. 14th ed. 2006.
20. Sweetman S, editor. Martindale: The complete drug reference. 35th ed. Pharmaceutical Press; 2006.
21. Arayne MS, Sultana N, Siddiqui FA. Simultaneous determination of Paracetamol and orphenadrine citrate in dosage formulations and in human serum by RP-HPLC. J Chin Chem Soc. 2009;56(1):169-74.
22. Haj-Ali DN, Hamdan II. Development of a capillary electrophoresis method for the determination of orphenadrine citrate in tablets in the presence of paracetamol. Saudi Pharma J. 2010;18(4):233-7.

23. Darwish K, Salama I, Mostafa S, El-Sadek M. Validated stability-indicating reversed-phase-HPLC method for simultaneous determination of orphenadrine citrate, caffeine and aspirin. Chem Pharm Bull (Tokyo). 2012;60(11):1426-36.

24. Sharaf El-Din MK, Abuirjeie MA, Abdel-Hay MH. Simultaneous determination of acetaminophen with orphenadrine citrate, ibuprofen or chlorzoxazone in combined dosage forms by zero-crossing derivative spectrophotometry. Anal Lett. 1991;24(12):2187-206.

25. Erk N, Onur F. Simultaneous determination of analgine and paracetamol in tablets by spectrophotometric methods. Anal Lett. 1997;30(6):1201-10.

26. Dogan HN. Pharmazie. 1996;51(10):773-4.

27. Alves, JC, Poppi RJ. Simultaneous determination of acetylsalicylic acid, paracetamol and caffeine using solid-phase molecular fluorescence and parallel factor analysis. Anal Chim Acta. 2009;642(1-2):212-6.

28. Dinç E, Baleanu D. Two new spectrophotometric approaches to the multicomponent analysis of the acetaminophen and caffeine in tablets by classical least-squares and principal component regression techniques. Farmaco. 2002;57(1):33-7.

29. Blanco M, Alcala M. Simultaneous quantitation of five active principles in a pharmaceutical preparation: Development and validation of a near infrared spectroscopic method. Eur J Pharm Sci. 2006;27(2-3):280-6.

30. Dinç E, Ozdemir A, Baleanu D. An application of derivative and continuous wavelet transforms to the overlapping ratio spectra for the quantitative multiresolution of a ternary mixture of paracetamol, acetylsalicylic acid and caffeine in tablets. Talanta. 2005;65(1):36-47.

31. Sena MM, Poppi RJ. N-way PLS applied to simultaneous spectrophotometric determination of acetylsalicylic acid, paracetamol and caffeine. J Pharm Biomed Anal. 2004;34(1):27-34.

32. Ito M, Suzuki T, Yada S, Nakagami H, Teramoto H, Yonemochi E, et al. Development of a method for nondestructive NIR transmittance spectroscopic analysis of acetaminophen and caffeine anhydrate in intact bilayer tablets. J Pharm Biomed Anal. 2010;53(3):396-402.

33. Anna W, Jin S, Haijun F, Shuo G, Zhonggui H. Simultaneous determination of paracetamol and caffeine in human plasma by LC-ESI-MS. Chromatographia. 2008;67(3-4):281-5.

34. Acheampong A, Gyasi WO, Darko G, Apau J, Addai-Arhin S. Validated RP-HPLC method for simultaneous determination and quantification of chlorpheniramine maleate, paracetamol and caffeine in tablet formulation. Springerplus. 2016;5:625.

35. Verlag DA, editor. USP 38- NF 33 the United States Pharmacopeia and National Formulary 2015. Deutscher Apotheker Verlag, 2014.

36. Stationery Office (Great Britain). British Pharmacopoeia 2014. Stationery Office, 2013.

37. Sullivan C, Joseph S. Development and validation of an HPTLC-densitometry method for assay of caffeine and acetaminophen in multicomponent extra strength analgesic tablets. J Liq Chromatogr R Technol. 2003;26(20):3453-62.

38. Suzen S, Akay CS, Tartılmi RS, Onal E, Cevheroglu SJ. Ankara Univ. 1998;27(2):93-100.

39. XuX, Stewart JT. HPLC methods for aspirin-caffeine-butalbital and acetaminophen-caffeine-butalbital mixtures in tablet dosage forms using non-porous octadecylsilane columns. J Liq Chromatogr R Technol. 2000;23(5):769-79.

40. Arayne MS, Najma S, Farhan AS. Simultaneous determination of paracetamol and orphenadrine citrate in dosage formulations and in human serum by RP-HPLC. J Chin Chem Soc. 2009;56(1):169-74.

41. Vignaduzzo SE, Teodoro SK. Development and validation of a HPLC method for the simultaneous determination of bromhexine, chlorpheniramine, paracetamol, and pseudoephedrine in their combined cold medicine formulations. J Liq Chromatogr R Technol. 2013;36(20):2829-43.

42. Rasged NS, Abdallah OM, Farag RS, Awad SS. Validated bivariate calibration spectrophotometric and high-performance liquid chromatography methods for simultaneous determination of dantrolene sodium and paracetamol in pharmaceutical dosage form. Adv Anal Chem. 2014;4(1):1-8.

43. Hadad GM, Emara S, Mahmoud WM. Development and validation of a stability-indicating RP-HPLC method for the determination of paracetamol with dantrolene or/and cetirizine and pseudoephedrine in two pharmaceutical dosage forms. Talanta. 2009;79(5):1360-7.

44. Al-kaysiHanan N, Mutaz ASS. High pressure liquid chromatographic analysis of orphenadrine citrate and acetaminophen in pharmaceutical dosage forms. Anal lett.1987;20(9):1451-66.

45. Sisco WR, Rittenhouse CT, Everhart LA, McLaughlin AM. Simultaneous high-performance liquid chromatographic stability-indicating analysis of acetaminophen, codeine phosphate, and sodium benzoate in elixirs. J Chromatogr. 1986;354:355-66.

46. Hossain M, James WA. Relative bioavailability of a novel sustained-release acetaminophen molded tablet. Int J Pharm.1996;133(1-2):223-35.

47. Hassan MA, Mamoun MAH, Julia F, Carol AT, Clive JCR, Adnan AB. Paracetamol bioavailability from an elixir a suspension and a new alcohol-free liquid dosage form in humans. Int J Pharm. 1988;42(1-3):155-9.

48. Yuen KH, Peh KK, Quah YL, Chan KL. A novel simultaneous HPLC assay for serum paracetamol and sulfapyridine as markers of gastric emptying and orocecal transit. Drug Dev Ind Pharm. 1997;23(2):225-8.

49. De Orsi D, Gagliardi L, Bolasco A, Tonelli D. Simultaneous determination of triprolidine, pseudoephedrine, paracetamol and dextromethorphan by HPLC. Chromatographia. 1996;43(9/10):496-500.

50. McGuinness BW. A Double-Blind comparison in general practice of a combination tablet containing orphenadrine citrate and paracetamol ('Norgesic') with paracetamol alone. J Int Med Res. 1983;11(1):42-5.

51. Srikantha D, Ramesh Raju RR. Estimation of the orphenadrine citrate in tablet dosage form by RP-HPLC method. Int J Pharm Biol Sci. 2014;4(1):30-7.

52. Altun ML. HPLC method for the analysis of paracetamol, caffeine and dipyrone. Turk J Chem. 2002;26(4):521-8.

53. Cunha RR, Chaves SC, Ribeiro MM, Torres LM, Muñoz RA, Dos Santos WT, et al. Simultaneous determination of caffeine, paracetamol, and ibuprofen in pharmaceutical formulations by high-performance liquid chromatography with UV detection and by capillary electrophoresis with conductivity detection. J Sep Sci. 2015;38(10):1657-62.

54. Tefera M, Geto A, Tessema M, Admassie S. Simultaneous determination of caffeine and paracetamol by square wave voltammetry at poly (4-amino-3-hydroxynaphthalene sulfonic acid)-modified glassy carbon electrode. Food Chem. 2016;210:156-62.

55. Oi-Wah L, Shiu-Fai L, Yiu-Ming C. Simultaneous determination of ascorbic acid, caffeine and paracetamol in drug formulations by differential-pulse voltammetry using a glassy carbon electrode. Analyst. 1989;114(9):1047-51.

56. Tavallali H, Zareiyan SF, Naghian M. An efficient and simultaneous analysis of caffeine and paracetamol in pharmaceutical formulations using TLC with a fluorescence plate reader. J AOAC Int. 2011;94(4):1094-9.

57. Sheikh Salem MA, Alkaysi HN, Gharaibeh AM. Quantitation of orphenaorine citrate and acetaminophen in tablet formulation using thin layer chromatography densitometry. Anal Lett. 1989;22(3):585-96.

58. Cuervo ES, Rivera CL, Peña PC. Optimization and validation of a simple and fast RP-HPLC method for simultaneous determination of acetaminophen and caffeine in tablets. Indian J Pharm Sci. 2017;79(5):731-9.

59. Mohammad AA, Bashir E, Youssef AA. Simultaneous determination of orphenadrine citrate and paracetamol in tablets by using RP-HPLC coupled with UV detection. 2017;9(6).