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UV-SPECTROPHOTOMETRIC AND RP-HPLC METHODS FOR THE SIMULTANEOUS ESTIMATION OF ACETAMINOPHEN AND CAFFEINE: VALIDATION, COMPARISON AND APPLICATION FOR MARKETED TABLET ANALYSIS

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ABSTRACT

In the present study UV-spectrophotometric and RP-HPLC methods were validated for the simultaneous analysis of acetaminophen and caffeine in marketed tablets. The methods were validated in terms of linearity, accuracy (% Recovery), precision (inter day, intra day and reproducibility) and robustness. Both the methods were linear (R^2 = 0.998-0.999) and accurate (% recovery was 99.29% - 100.19% for UV method and 99.14% - 100.25% for HPLC method). The method was also found precise (% RSD< 2%) and robust. Potency of five marketed brands was determined by both the methods and no statistically significant difference was noticed between the potency obtained from UV-spectrophotometric and RP-HPLC methods by paired *t* test at 5% significance level. Drug release from the marketed products complied compendia specification that indicates that test products are equivalent. Any one of the validated methods can be used for the analysis of acetaminophen and caffeine tablets.

Keywords: Acetaminophen, Caffeine, UV, RP-HPLC and Method validation

INTRODUCTION

Multi-component formulations have gained a lot of importance now days due to greater patient acceptability, increased potency, multiple activity, fewer side effects and quick relief. Pharma Market is flooded with combination of drugs with various dosage form used for various diseases. The use of the mixture of acetaminophen and caffeine as an analgesic and antipyretic is well established in pharmaceutical formulation. Acetaminophen is one of the most popular over-the-counter drugs. It has analgesic and antipyretic properties with weak anti-inflammatory activity and it is used in the symptomatic management of moderate pain and fever. When taken at recommended doses it has an excellent safety profile. It is available in different dosage forms: tablet, capsules, drops, elixirs, suspensions and suppositories[1]. The drug is official in different pharmacopeia[2-3]. Caffeine is a drug that is naturally produced in the leaves and seeds of many plants. It is also produced artificially and added to certain foods. It stimulates the central nervous system causing increased alertness. It gives most people a temporary energy boost and elevates mood. The efficacy of any combination tablet formulation depends on the release of all the active pharmaceutical components. So drug release of both the component should be studied. In this study an initiative was taken to study the dissolution study of acetaminophen and caffeine tablet. Many reports about the dissolution of multi-component formulation revealed that not all the products release the drugs uniformly. Inter batch and intra batch variation were

observed. So the most widely used combination product is selected to study for their quality in terms of potency, DT and dissolution.

Selective and sensitive analytical method for quantitative determination of drugs and their metabolites are essential for successful evaluation of clinical pharmacology, pharmacokinetics (PK), bioavailability (BA) and bioequivalence (BE) studies. Acetaminophen and caffeine tablet is official in USP. Analysis methods are also described in the pharmacopeia. Several methods have been reported for the determination of caffeine in food or brverages[4-7]. Simultaneous determination of acetaminophen with other drugs has also been reported[8-9]. But only few methods have been reported for the simultaneous determination of acetaminophen and caffeine in tablet dosage form[10-11].

So development of analysis method for the simultaneous estimation of acetaminophen and caffeine is still required. In this study we have validated UV spectroscopic method by using dissolution media as solvent and we used simultaneous equation method for the calculation. We also validate RP-HPLC method by using simple solvent system and compare these two methods by paired *t* Test, so that one can test acetaminophen and caffeine tablet with their available facility. The proposed methods were validated for the parameters like linearity, accuracy, precision and robustness as per ICH guidelines [12].

MATERIALS AND METHODS

***Reagents and Chemicals*:** Methanol was of HPLC grade and was purchased from E. Merck, Darmstadt, Germany. Potassium di-hydrogen phosphate, sodium hydroxide and other reagents were of analytical-reagent grade and purchased from E. Merck, India. Water was deionised and double distilled. Working standard of acetaminophen and caffeine were collected from Eskayef Bangladesh Ltd as gift samples. Marketed tablets containing acetaminophen 500 mg and caffeine 65 mg were purchased from local drug store in Dhaka city after checking their manufacturing license number, batch number, production and expiry date.

Validation of UV Method

Instrumentation: A double-beam UV-Visible spectrophotometer (Model UV-1700 PC, Shimadzu, Japan) equipped with wavelength accuracy of +0.5 nm (with automatic wavelength correction) was used. The drug analyses data were acquired and processed using UV Probe software (Version 2.0, Shimadzu, Japan) running under Windows XP on a Pentium PC.

Preparation of standard solution and derivation of simultaneous equation: Standard solution of acetaminophen and caffeine across the range of 2-20 µg/ml were prepared by diluting stock solution of acetaminophen (100µg/ml) and caffeine (100 µg/ml) prepared in water. Solution containing mixture of acetaminophen and caffeine (10, 12, 15, and 18 µg/ml acetaminophen along with 10 µg/ml caffeine and vise versa) were also prepared by diluting standard solutions. Standard solutions of acetaminophen and caffeine were scanned separately in the range of 200-400 nm. Acetaminophen and caffeine showed absorbance maxima at 243.5 nm and 273 nm respectively. Absorptivity values for acetaminophen at 243.5 nm and 273 nm were 640 (ax_1) and 140 (ax_2) while respective values for caffeine were 130 (ay_1) and 510 (ay_2). Spectra for both the drugs are shown in Figure 1.

Now simultaneous equations were derived by replacing the absorptivity values of acetaminophen (ax_1= 640 and ax2= 140) and caffeine (ay_1= 130 and ay2= 510) in the following equations:

$C_x = A2\ ay1 - A1\ ay_2 / ax_2 ay_1 - ax_1\ ay_2$ ……(1)

$C_y = A1\ ax2 - A2\ ax_1 / ax_2\ ay_1 - ax_1\ ay_2$ ……..(2)

where, A1 and A2 are absorbance of sample solution at λmax of acetaminophen (243.5nm) and λmax of caffeine(273nm) respectively; ax_1 and ax_2 are the absorptivities of acetaminophen at 243.5nm and 273 nm respectively and ay_1 and ay_2 are the absorptivities of caffeine at the two wavelengths respectively.

Validation: The proposed method was validated for the parameters like linearity, accuracy, precision and robustness as per ICH guidelines [12]. Linearity of the method was determined by constructing calibration curves from the absorbance of standard solutions of acetaminophen and caffeine of different concentrations level (4-20 µg/ml) at 243.5 nm and 273 nm. Accuracy was determined by means of recovery experiments. Absorbance of solution containing known concentration of acetaminophen and caffeine was measured at two selected wave length. Then potency was calculated and accuracy was assessed from the test results as recovered by the assay.

The precision of the method was investigated with respect to repeatability (inter assay precision), intermediate precision (inter day precision) and reproducibility. Repeatability was determined by

performing three repeated analysis of the three standard solutions (4, 6, 8 µg/ml acetaminophen along with 10 µg/ml caffeine and vise versa) of standard mixture solution on the same day, under the same experimental conditions. Intermediate precision of the method was assessed by carrying out the analysis of standard solutions on three different days (inter-day) in the same laboratory. For reproducibility analysis was carried out in another lab. To determine the robustness different solvent was used. Percent recovery was calculated for both the drug. Analytical methods is generally known as robust if percent recovery is within 98-102%

Validation of HPLC Method

HPLC Instrumentation: A Shimadzu (Japan) HPLC system consisting of a CMB-20 Alite system controller, two LC-20AT pumps, SIL-20A auto-sampler and CTO-10ASVP column oven were used. Ultraviolet detection was achieved at 273 nm with a SPD-20A UV-VIS detector (Shimadzu, Japan). The drug analyses data were acquired and processed using LC solution (Version 1.3, Shimadzu, Japan) software running under Windows XP on a Pentium PC. The mobile phase, Phosphate buffer (pH 5.5): methanol (60:40 v/v) pumped at a flow rate of 1.0 ml/min through the column (C_{18}; 250 mm X 4.6 mm, 5µ shim-pack, Japan) at 30^0C. The mobile phase was filtered through a 0.2µ nylon membrane filter and degassed prior to use under vacuum.

Preparation Standard solution for HPLC analysis: Different concentrations (80%, 90%, 100%, 110% and 120% of target concentration) of acetaminophen and caffeine were prepared in mobile phase from stock solution of acetaminophen (100µg/ml) and caffeine (100µg/ml). Solution containing mixture of acetaminophen and caffeine of five different concentrations (80%, 90%, 100% 110% and 120% of target concentration) were also prepared by dilution as required.

Validation: The system suitability was assessed by six replicate analyses of standard solution at a 100% (50 µg/ml acetaminophen and 6.5 µg/ml caffeine) level to verify reproducibility of retention time, tailing factor and theoretical plates (Tangent) of the column. To determine the selectivity of the method standard samples and placebo formulation of acetaminophen and caffeine were injected one after another. Then the chromatograms were analyzed for retention time, peak area and peak shape to determine selectivity of the method. For linearity calibration graph was constructed from peak area obtained by injecting increasing amount of standard solutions (80%, 90%, 100%, 110% and 120% of target concentration). For accuracy determination standard solution containing acetaminophen and caffeine were injected and percent recoveries were calculated from peak area. The accuracy was calculated from the test results as the percentage of the drug recovered by the assay. The precision of the method was investigated by performing four repeated analysis of the three standard solutions (90%, 100% and 110% of target concentration) of standard solution on the same day (for repeatability) and different day for inter day precision. For reproducibility analysis was carried out in another lab. The relative standard deviation (% RSD) was determined in order to assess the precision of the method. The robustness of the method was assessed by altering the some experimental conditions such as by changing the flow rate from 0.9 to1.1 ml/min, amount of methanol (38% to 42%) and the temperature of the column (28 °C to 32 °C).

Comparison of analytical methods by potency determination: UV spectroscopic and RP-HPLC methods were compared by determining the potency of five different brands of acetaminophen and caffeine tablets. Average weight of acetaminophen and caffeine tablet was calculated. Then the tablets were grinded separately to fine powder with the help of mortar and pestle. Powder containing 50 mg acetaminophen and 6.5 mg caffeine was dissolved in water, shaken for about 10 minutes and filtered through filter paper. The filtered solution was further diluted with water and used for absorbance measurement in a double-beam Shimadzu (Kyoto, Japan) UV-Visible spectrophotometer (Model UV-1700 PC) to find out the potency. The samples were also prepared in mobile phase in the same way (50 µg/ml acetaminophen and 6.5 µg/ml caffeine) and injected in Shimadzu (Japan) HPLC system. Potency was calculated from peak area. Potency results from UV spectroscopic and RP-HPLC methods were compared by paired *t* Test.

Dissolution studies by the validated methods: The dissolution test was undertaken using tablet dissolution tester apparatus 2 (TDT-08L, Electrolab, India) in 5 replicates for each brand. Dissolution media was water. The medium was maintained at 37 ± 0.5°C. In all the experiments, 10 ml of dissolution sample was withdrawn at 0, 10, 30 and 60 min and replaced with equal volume to maintain sink condition. Samples were filtered and assayed by UV spectroscopic method. The concentration of each sample was determined from a calibration curve obtained from pure samples of caffeine. The samples were also analyzed by RP-HPLC to compare the analysis methods.

RESULTS AND DISCUSSION

Validation of UV method

The proposed method was linear with correlation coefficient 0.998-0.999 (Figures 2 and 3). Results of accuracy, precision and robustness are summarized in Table 1. From the validation results, the proposed UV spectroscopic method was found accurate, precise and robust.

Validation of HPLC Method

The proposed HPLC method was found selective, linear, accurate, precise and robust. Validation results are summarized in Table 2 and 3. The system was found suitable in respect of retention time (% RSD 0.022-0.028) mean theoretical plate count (more than 4500) and tailing factor (less than 1.5).

Correlation coefficient of calibration curve was 0.999 for both acetaminophen and caffeine. % recovery was found 99.14 % -100.25% and %RSD for precision study was less than 2%. All the results indicate that the method is linear, accurate and precise.

Peaks of acetaminophen and caffeine from standard placebo solution were on same time (Figure 4). On the other hand no additional peaks other than drugs were found within 7 min run time. Excipients did not change the retention time or interfere the analysis results. So the method is highly selective.

Robustne**ss** study was performed by making a slight variation in flow rate, amount of methanol and column temperature. No significant effect was observed in the recovery of drugs. % recovery was 98% to 102%. On the other hand changes in retention time, theoretical plate and resolution were also negligible. So we can say that the method is robust.

Potency of tablets: Potency determined by UV method and HPLC method was compared by paired *t* Test at 0.05 significance level (Table 4). The P-value was greater than the significance level, indicating that there was no statistically significant difference between the two methods.

***In vitro* drug release study and comparison of dissolution data:** The release profiles of acetaminophen and caffeine tablets (A-E) are shown in Fig 5 and 6. All dissolution data are based on the actual drug content of the test tablets as calculated from the assay results.

USP specification for acetaminophen and caffeine tablet is that not less than 75% (Q) of the labeled amount of acetaminophen and caffeine should dissolve in 60 minutes. All the brands complied with the USP specification. Initial drug release was different but with increase of time drug release became almost similar. More than 80% drug was released within 30 min and around 100% drug was released within 60.

Similarity of acetaminophen and caffeine release can be proved by comparison of % Dissolution Efficiencies (Table 6). Dissolution Efficiency (% DE) is the area under the dissolution curve within a time range (t1 - t2). %DE was calculated by using the following equation:

$$DE = \frac{\int_{t_1}^{t_2} y \cdot dt}{y_{100} \times (t_2 - t_1)} \times 100$$

Where *y* is the percentage dissolved at time *t*

% Dissolution efficiencies (% DE) of acetaminophen and caffeine were compared by paired *t* Test. P value is greater than the defined significance level indication no statistically significant difference in drug release.

CONCLUSION

From this validation study we can conclude that the developed UV and RP-HPLC methods are accurate, rapid, precise, reproducible and inexpensive with acceptable correlation co-efficient, RSD (%) and standard deviation. Any one of the methods can be used for simultaneous determination of acetaminophen and caffeine in bulk or pharmaceutical dosage form (individual or combine). Simplicity of sample preparation and use of low cost reagents are the additional benefit of this method. So this method can be used in the quality control department for potency and dissolution study. On the other hand all the tested brands are found equivalent in respected of potency and drug release.

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Table 1: Accuracy, precision and robustness result of the UV method

Validation parameters		Acetaminophen	Caffeine
Accuracy	% Recovery ± SD	99.29% ± 0.31	100.19%± 0.41
Precision (%RSD)	Repeatability	0.52	0.73
	Ruggedness	0.71	0.78
	Reproducibility Lab-I	0.52	0.78
	Reproducibility Lab-II	1.27	1.64
Robustness % Recovery ± SD	Water	100.67 ± 0.30	99.22± 0.19
	Phosphate Buffer pH 6.8	100.17 ± 0.61	99.47± 0.18

Table 2: Results of system suitability study of HPLC method

Parameters	Acetaminophen (50 µg/ml)		Caffeine(6.5 µg/ml)	
	Average ±SD	%RSD	Average ±SD	%RSD
Retention time	3.576±0.001	0.028	4.588±0.001	0.022
Area	420530±2978	0.708	20956.33±263	1.255
Theoretical plates	4723.54±3.141	0.066	4822.333±3.951	0.082
Tailing factor	1.33±0.004	0.301	1.27±0.005	0.394

Table 3: Linearity, accuracy and precision results of HPLC method

Validation parameters		Acetaminophen	Caffeine
Linearity (regression coefficient-R^2) (*Y = mX+C)	R^2 (mean ±SD)	0.9995±0.0002	0.9994±0.0002
	%RSD **	0.021	0.021
	Slope (mean ±SD)	88390.36±41.31	3300.74 ±65.42
Accuracy	% Recovery	99.14 ± 0.73	100.25± 0.49
	%RSD	0.736	0.489
Precision (%RSD)	Repeatability	0.55	0.49
	Ruggedness	0.77	0.79
	Reproducibility	1.35	1.71

***** R^2 = regression coefficient

******Y = mX+C; where Y = peak area, m = slope, X = concentration (µg/ml) and C = intercept.

Table 4: Potency of the acetaminophen and caffeine tablets

Brand	Acetaminophen % ± SD (n = 5)				Caffeine % ± SD (n = 5)			
	UV method	HPLC method	t-value	Sig. (2-tailed)	UV method	HPLC method	t-value	Sig.(2-tailed)
A	99.00 ± 0.89	99.23 ± 0.52			100.41 ± 0.52	101.94 ± 0.87		
B	99.37 ± 0.19	99.57 ± 0.62			99.64 ± 0.38	99.35 ± 0.74		
C	98.72± 0.48	98.92 ± 0.28	0.384	0.72	99.50 ± 0.79	100.82 ± 0.36	1.06	0.347
D	100.82 ± 0.48	100.36 ± 0.29			99.93 ± 0.71	100.57 ± 0.37		
E	100.51 ± 0.86	100.59± 0.23			100.47 ± 0.82	99.67 ± 0.66		

Table 5: Comparison of % Dissolution efficiencies for acetaminophen and caffeine release

Brands	% DE		t-value	P Sig. (2-tailed)
	Acetaminophen	Caffeine		
A	89.61	82.77		
B	79.17	78.67		
C	85.01	90.40	0.147	0.889
D	89.68	94.75		
E	88.91	83.94		

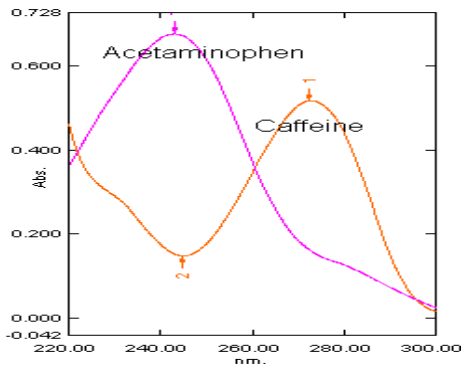

Figure 1: Spectrum of acetaminophen and Caffeine

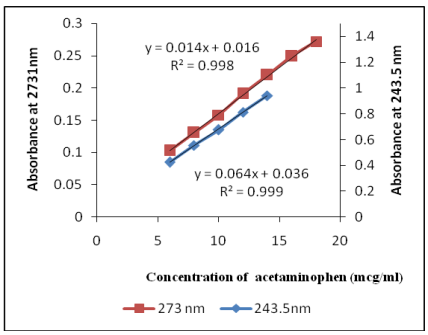

Figure 2: Calibration curve of acetaminophen at 243.5 nm and 273 nm

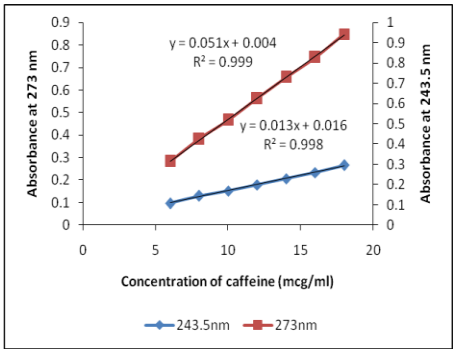

Figure 3: Calibration curve of caffeine at 243.5 nm and 273 nm

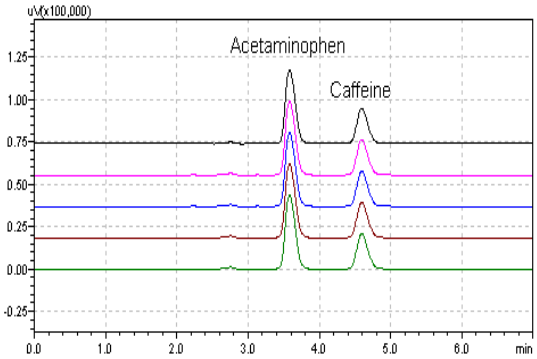

Figure 4: Chromatogram of acetaminophen and caffeine from standard and placebo formulation

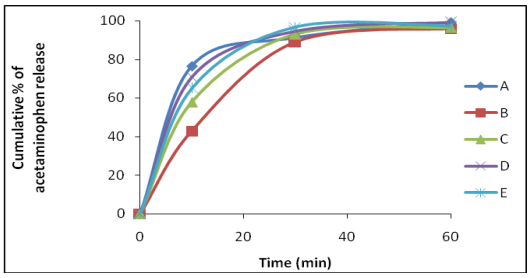

Figure 5: Drug release from acetaminophen and caffeine tablets (A-E)

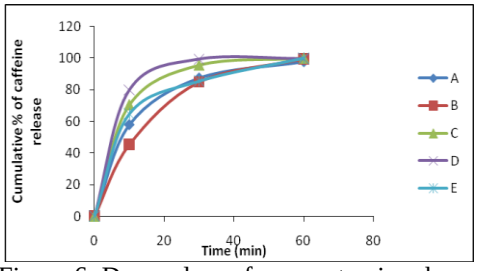

Figure 6: Drug release from acetaminophen and caffeine tablets (A-E)

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