Full Length Research Paper

Development of a UV-spectrophotometric method for the simultaneous determination of aspirin and paracetamol in tablets

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A specific, rapid and simple UV spectrophotometric method with good sensitivity was developed and validated for the simultaneous quantification of aspirin and paracetamol in standard solutions and tablets. The method employed solving of simultaneous equations based on the measurement of absorbance at two wavelengths, 265 and 257 nm, λ_{max} for aspirin and paracetamol, respectively. The calibration curve was linear for both drugs in a concentration range of 2 to 64 μg/ml. It can be concluded from the results that present method for the simultaneous determination of aspirin and paracetamol in tablets is specific, rapid and simple with good sensitivity. This analytical method is also applicable in ordinary laboratories also. It can also be adopted for quality control tests for these drugs in tablets.

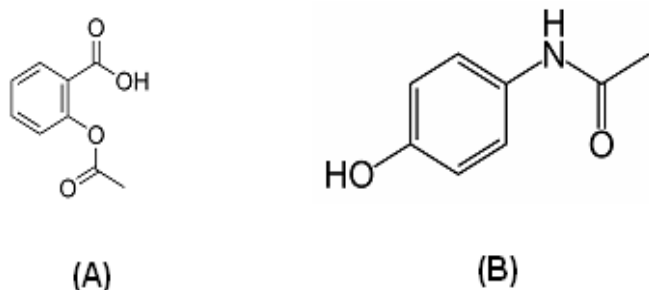**Key words:** UV spectrophotometric method, aspirin, paracetamol, simultaneous determination.

INTRODUCTION

Aspirin (Acetyl salicylate, Figure 1A) and paracetamol (N-acetyl-p-aminophen, Figure 1B) have been extensively used as antipyretic and analgesic drugs (Sweetman, 2002; Hardman and Limbird, 1996). They are frequently prescribed in admixture with each other or in combination with other drugs (Sweetman, 2002; Hardman and Limbird, 1996). Literature revealed that several methods have been reported for the quantification of aspirin and paracetamol individually but no analytical method using UV spectrophotometer for their simultaneous quanti-fication is reported. Ramos-Martos et al. (2001) applied liquid chromatography to the simultaneous determination of acetylsalicylic acid, caffeine, codeine, paracetamol, pyridoxine and thiamine in pharmaceutical preparations. Vidal et al. (2002) determined simultaneously acetaminophen, aspirin and caffeine using UV spectro-photometric flowthrough multiparameter sensor. Martos et al. (2001) conducted spectrofluorimetric determination of aspirin and codeine mixtures in pharmaceuticals. Szostak and Mazurek (2002) presented quantitative determination of aspirin and acetaminophen in tablets by FT-Raman spectroscopy. Criado et al. (2000) showed continuous flow spectrophotometric determination of paracetamol in pharmaceuticals following continuous microwave assisted alkaline hydrolysis. Pufal et al. (2000) determined paracetamol (acetaminophen) in different body fluids and organ samples after solid-phase extraction using HPLC and an immunological method. Vidal et al. (2003) studied simultaneous determination of paracetamol, caffeine and propyphenazone in pharmaceuticals by means of a single flow-through UV multiparameter sensor.

Khan et al. (2010) developed UV spectrophotometric method for the simultaneous estimation of Meloxicam and Paracetamol in tablet by simultaneous equation,

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Figure 1. Chemical structures of aspirin (1) and paracetamol (2).

absorbance ratio and absorbance correction method. Gondalia et al. (2010) developed and validated spectrophotometric methods for simultaneous estimation of ibuprofen and paracetamol in soft gelatin capsule by simultaneous equation method. Afkhami and Sarlak (2005) determined salicylamide and paracetamol simultaneously by spectrophotometric H-Point standard addition method and partial least squares regression. Akay et al. (2008) developed a rapid and simultaneous determination of aspirin, paracetamol, and their degradation and toxic impurity products by HPLC in pharmaceutical dosage forms. Abu-Qare and Abou-Donia (2001) produced a validated HPLC method for the determination of pyridostigmine bromide, acetaminophen, aspirin and caffeine in rat plasma and urine. Sinha et al. (2009) presented a validated stability indicating HPTLC method for determination of aspirin and clopidogrel bisulphate in combined dosage form.

Thus a successful attempt has been proposed in the article to quantify aspirin and paracetamol simultaneously by spectrophotometer. The UV spectrophotometric analyses are often preferred in quality control testing and ordinary laboratories due to its broad availability and suitability. The objective of this study was to develop and validate a simple and specific UV spectrophotometric method for the simultaneous determination of aspirin and paracetamol in tablets. This method exhibited precise, accurate and cost effective assay for these drugs in mixture.

MATERIALS AND METHODS

Materials

Aspirin (purity 99.91%) and paracetamol (purity 99.99%) samples were provided by Atco Pharmaceuticals, Karachi and Efroze Pharmaceuticals, Karachi, Pakistan, respectively. Sodium hydroxide, monobasic potassium phosphate, acetonitrile, dichloromethane, hydrochloric acid (HCl, 37%) and methanol were procured from Merck, Germany. All chemicals were of analytical grade and were used without further purification. Tablets containing aspirin and paracetamol (100 tablets of each drug) were procured from local pharmacy (Servaid Pharmacy, Lahore, Pakistan). Double distilled water was used in the present study. UV-Vis spectrophotometer (1601, Shimadzu, Japan) with spectral bandwith

of 0.1 nm and wavelength accuracy of ± 0.5 nm with automatic wavelength correction and pH meter (Inolab, Germany) were used to determine the absorbance and pH of solutions, respectively.

Stock standard solution of aspirin

Stock standard solution of aspirin (100 µg/ml) was prepared by dissolving 100 mg aspirin in 1000 ml of 0.1 N HCl: methanol (1:1) in 1000 ml volumetric flask with vigorous shaking. This solution was further diluted to get various working solutions.

Stock standard solution of paracetamol

Stock standard solution of aspirin (100 µg/ml) was prepared by dissolving 100 mg aspirin in 1000 ml of 0.1 N HCl: methanol (1:1) in 1000 ml volumetric flask with vigorous shaking. This solution was further diluted to get various working solutions. The stock solutions were filtered through Whatman filter paper No. 41.

Working solutions of aspirin plus paracetamol

The calculated volumes of aspirin and paracetamol solutions were taken from stock standard solutions, mixed and prepared serial dilutions with 0.1 N HCl:methanol (1:1) containing 2, 4, 8, 16, 32 and 64 µg/ml of each, aspirin and paracetamol, alone and together.

Sample solutions

For the quantification of drugs from the commercial formulations, 20 tablets of Ascard 75 mg, Atco Pharmaceuticals, containing aspirin and 20 tablets of Panaram 500 mg, Efroze Pharmaceuticals, containing paracetamol were weighed to calculate mean tablet weight of each drug. There tablets were crushed into powder. A weighed quantity of tablet powder equivalent to 50 mg of aspirin and 50 mg of paracetamol were transferred into a 100 ml volumetric flask, diluted with 0.1 N HCl:methanol (1:1), sonicated for 20 min and made up to the volume with the same. Then the resulting solution was allowed to stand for 2 h, filtered through Whatman filter paper No. 41 and the filtrate was suitably diluted to produce the desired concentration (2, 4, 8, 16, 32 and 64 µg/ml) for both, aspirin and paracetamol, with 0.1 N HCl:methanol (1:1). The absorbance of these solutions was taken at appropriate wavelengths and the values were put in the respective formulas to determine concentrations.

Statistics

The comparison of various parameters was conduced to elaborate the significant difference using software, SPSS version 13.0. The level of significance was set at 0.05.

RESULTS

In recent years, the development of analytical methods for simultaneous determinations of drugs has gained considerable attention due to their importance in quality control testing of drugs and their products and in ordinary laboratories because of their wide availability and suitability. Since no method is reported in literature for the simultaneous quantification of aspirin and paracetamol in
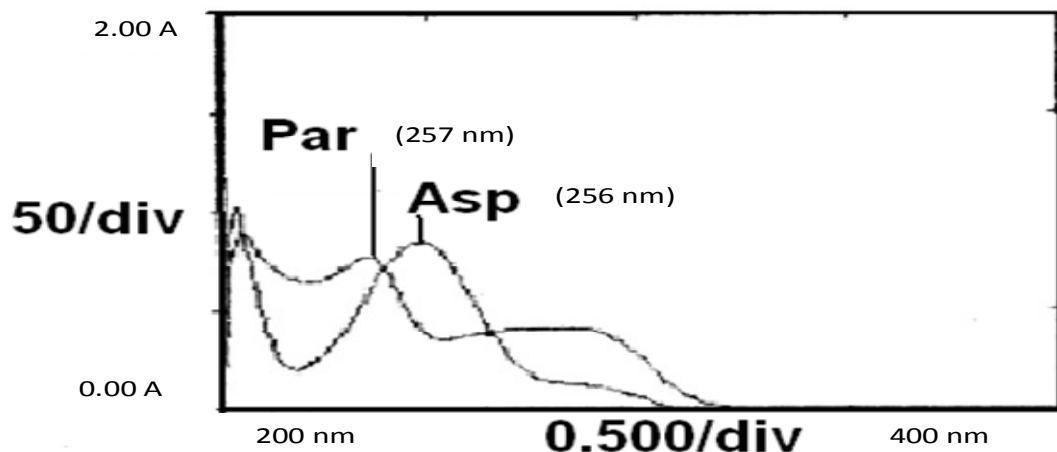
Figure 2. Overlain UV spectra of Paracetamol (Par) and Aspirin (Asp).

tablets for the routine quality control assay in ordinary laboratories, the development of such method could be appreciable.

Method development

Selection of an appropriate solvent system

Various solvent systems like distilled water, 0.1 N HCl, phosphate buffer pH 6.8 and pH 7.4, methanol, distilled water: methanol (1:1), 0.1 N HCl: methanol (1:1), phosphate buffer pH 6.8-methanol (1:1) and phosphate buffer pH 7.4-methanol (1:1) were tried to select an appropriate solvent with good suitability and stability. A solvent system, 0.1 N HCl: methanol (1:1) was selected for the determination of aspirin and paracetamol, since both drugs were soluble in it. In this solvent, minimal interference in the absorbance of both drugs was observed. The standards of aspirin and paracetamol were scanned in the range of 200 to 400 nm against water as blank for obtaining overlain spectra. The over-lain UV spectra are shown in Figure 2. The absorbance and absorptivities of serial standard solutions of aspirin and paracetamol were carried out at selected wave lengths λ_1 265 and λ_2 257 nm, respectively. The UV absorption of aspirin and paracetamol interfered with each other, therefore law of additivity was employed for the determination of both drugs in mixture. The method employed solving of simultaneous equations using Cramer's rule and matrices. The absorptivity (a) is extinction coefficient which was calculated using equation as:

$$a = A/C \tag{1}$$

where A and C are the absorbance and concentration (g/100 ml) respectively. Equation 1 was used to derive following two simultaneous equations as:

$$A_1 = a_{X1} C_X + a_{y1} C_y \tag{2}$$

$$A_2 = a_{X2} C_X + a_{y2} C_y \tag{3}$$

where, C_X = Concentration of aspirin; C_y = Concentration of paracetamol; A_1 = Absorbance of mixture at λ_1; A_2 = Absorbance of mixture at λ_2; A_{X1} = Absorptivity of aspirin at λ_1; A_{X2} = Absorptivity of aspirin at λ_2; a_{y1} = Absorptivity of paracetamol at λ_1; a_{y2} = Absorptivity of paracetamol at λ_2.

Subsequently, the mean absorptivity values as:

$$A_{X1} = 67.7 \pm 2.092$$

$$A_{X2} = 52.2 \pm 1.112$$

$$a_{y1} = 60.7 \pm 2.130$$

$$a_{y2} = 61.5 \pm 1.099$$

were substituted to Equations 2 and 3, a set of 2 simultaneous equations was framed as Equations 4 and 5:

$$A_1 = 67.7 C_X + 60.7 C_y \tag{4}$$

$$A_2 = 52.2 C_X + 61.5 C_y \tag{5}$$

The concentration of aspirin and paracetamol in mixture were determined by solving Equations 4 and 5.

Method validation

Subsequently, the method was validated as given below. Working solutions of both drugs in a concentration range of 2 to 64 μg/ml were prepared and analyzed spectrophotometerically to develop calibration curve

Table 1. Validation parameters for aspirin and paracetamol.

Number	Parameter	Aspirin	Paracetamol
1	Purity assay (%)	99.16	99.09
2	LOD (µg/ml)	0.730	0.591
3	LOQ (µg/ml)	2	2
4	Linearity range (µg/ml)	2 - 64	2 - 64
5	Regression coefficient (R^2) for calibration curve	0.9925	0.9905
6	Y-equation	$y = 0.042x - 0.004$	$y = 0.036x + 0.0679$
7	Precision (%)	0.92	1.87
8	Molar absorptivity (0.001) Absorbance unit / mole cm/dm^3)	5.92×10^4	0.031029
9	Sandell's sensitivity (mg/cm^2/0.001 absorbance unit)	7.36×10^3	0.029137

between concentration and absorbance. The values of regression coefficient (R^2) for aspirin and paracetamol were 0.9925 and 0.9905, respectively which indicate a good correlation between the concentration and absorbance within the concentration range tested. The Y-equation for aspirin and paracetamol were $y = 0.042x - 0.004$ and $y = 0.036x + 0.0679$, respectively (Table 1).

To check precision (percentage RSD) of method, six replicate samples of the same concentrations of aspirin and paracetamol were analyzed. The precision for aspirin and paracetamol were 0.92 and 1.87%, respectively (Table 1). The results suggest good precision of this simultaneous method. The intra- and inter-day precision was also less than 2%. The molar absorptivity (0.001 absorbance unit / mole cm/dm^3) and Sandell's sensitivity (mg/cm^2/0.001 absorbance unit) were 5.92×10^4 and 0.031029, respectively, while for paracetamol were 7.36×10^3 and 0.029137, respectively. To evaluate the specificity and selectivity of method, the λ_{max} of standard solutions of aspirin and paracetamol were compared to their marketed formulations. No interference was observed from the tablets excipients which indicate the specificity and selectivity of method.

In order to determine mean drug contents, aspirin 75 mg and paracetamol 500 mg tablets were tested. Their assay showed 99.16 and 99.09 % contents of aspirin and paracetamol, respectively, which were non-significantly (p>0.05) different from the stated purity for both drugs. This result shows good accuracy of method also. The limit of detection (LOD) and limit of quantification (LOQ) for aspirin and paracetamol were also evaluated from the slope (S) of their respective calibration curves and the standard deviation of blank (σ), using equation as:

$$LOD = 3.3\sigma/S \qquad (6)$$

$$LOQ = 10\sigma/S \qquad (7)$$

The LOD for aspirin and paracetamol were 0.730 and 0.591 µg/ml, respectively while the values of LOQ were 2 µg/ml for both drugs. The results of LOD and LOQ elaborate sufficient sensitivity of method.

DISCUSSION

Various researchers have studied the evaluation of aspirin and paracetamol separately or in combination with some other drug in various dosage forms and biofluids (Ramos-Martos et al., 2001; Vidal et al., 2002; Akay et al., 2008; Abu-Qare and Abou-Donia, 2001; Sinha et al., 2009). However, the literature survey has shown that no analytical study regarding simultaneous determination of aspirin and paracetamol has been conducted previously. This study is useful because these two drugs are commonly administered simultaneously (Szostak and Mazurek, 2002). This successful study has been conducted to determine aspirin and paracetamol simultaneously by spectrophotometer in tablets. The UV spectrophotometric analyses are often preferred in quality control testing and ordinary laboratories due to its broader availability, suitability and ease of use. The objective of this study was to develop and validate a simple and specific UV-spectrophotometric method for the simultaneous determination of aspirin and parace-tamol in tablets. This method exhibited precise, accurate and cost effective assay for these drugs in mixture. Based on previous studies, Ramos-Martos et al.(2001) have described a rapid reversed-phase liquid chromate-graphic method, with UV detection, for the simultaneous quantification of aspirin, caffeine, codeine, paracetamol,

pyridoxine, and thiamine in pharmaceutical dosage form. The calibration curves were linear for salicylic acid, caffeine, paracetamol, and pyridoxine in the range of 50 to 500 mg/L, and for codeine and thiamine in the range of 50 to 1000 mg/L. The method was applied to the analysis of 13 marketed pharmaceutical dosage forms. Recovery values ranged from 92.6 to 105.5% with relative standard deviations of 1.1 to 5.8%. Vidal et al. (2002) presented a single triparameter flow-through sensor with UV detection for the simultaneous determination of acetaminophen, aspirin and caffeine. The calibration curve ranged between 10 to 100, 40 to 500 and 4 to 50 µg/ml, for acetaminophen, aspirin and caffeine, respectively. The LOD values were from 0.3 to 0.8 µg/ml and the LOQ values were from 1.0 and 2.7 µg/ml with RSDs from 1.2 to 3.4, respectively. Cemal et al. (2008) developed a rapid and simultaneous determination method for parace-tamol and aspirin and their degradation and toxic impurity products by high performance liquid chromatography (HPLC) in pharmaceutical dosage forms. The developed method was linear in the ranges of 0.5 to 4.0 and 0.75 to 6.0 µg/ml for paracetamol and aspirin, respectively. Relative standard deviations for repeatability, repro-ducibility and recovery were below 2%. Abu-Qare and Abou-Donia (2001) developed a method for the separation and quantification of pyridostigmine bromide, acetaminophen, aspirin, and caffeine in rat plasma and urine. The LOD values were ranged between 100 and 200 ng/ml, while LOQ values were 150 to 200 ng/ml. Mean percentage recovery of five spiked plasma samples were 70.9±9.5, 73.7±9.8, 88.6±9.3, 83.9±7.8, and from urine 69.1±8.5, 74.5±8.7, 85.9±9.8, 83.2±9.3, for pyrido-stigmine bromide, acetaminophen, aspirin and caffeine, respectively. The relationship between peak areas and concentration was linear over range between 100 and 1000 ng/ml. Though above mentioned processes for the simultaneous analysis of aspirin and paracetamol are more sensitive than present method, however all the previous methodologies involved the use of highly sensitive apparatus like HPLC as compared to the UV-spectrophotometer as employed in this study.

Conclusion

It can be concluded from the results that this method for the simultaneous determination of aspirin and paraceta-mol in tablets is specific, rapid and simple with sufficient sensitivity. This analytical method is also applicable in ordinary laboratories also. It can also be adopted for quality control tests for these drugs in tablets.

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