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Development of Validated Spectrophotometric Method for Simultaneous Estimation of Acetylsalicylic Acid and Caffeine in Pure and Tablet Dosage Form

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ABSTRACT

A sensitive, rapid, and specific spectrophotometric assay method has been developed for the simultaneous determination of acetylsalicylic acid and caffeine in commercial tablets. Acetyl salicylic acid and caffeine showed absorbance maxima at 297 nm and 272 nm respectively in 0.1N NaOH solution. These drugs were estimated in formulations by simultaneous equation method (method A) and absorbance ratio method (method B). In method A, acetylsalicylic acid and caffeine at their respective λ_{max} of 297.0 nm and 272.0 nm showed linearity in the concentration range of 0-40 µg/mL and 0-25 µg/mL. Method B involved measurement of absorbance at isoabsorptive point of these two drugs i.e. at 289 nm with linearity in the concentration range of 0-40 µg/mL and 0-25 µg/mL for acetylsalicylic acid and caffeine respectively. Validation study revealed that the methods are specific, accurate, precise, and reproducible. The developed methods are simple, rapid, accurate, precise, reproducible, and economic and can be used for routine quantitative analysis of acetyl salicylic acid and caffeine in pure and tablet dosage form.

Keywords: Simultaneous estimation method, Absorbance ratio method, Acetyl salicylic acid, Caffeine, Validation

INTRODUCTION

Migraine and tension type headache, as defined by the International Headache Society[1], are very common diseases all over the world. The one-year prevalence of migraine in adults is 6% among men and 15–18% among women[2-3]. In surveys of the general population in North America and Western Europe, the one-year prevalence of episodic tension-type headache ranged from about 30% to about 80%[4]. The overwhelming majority of these patients used medication for their headache (e.g. 95% of men and 97% of women with migraine), but about two-thirds of migraineurs[5] and more than 80% of tension-type headache patients[6] never consulted a physician for their headaches and used over-the-counter (OTC) drugs for headache treatment[7-9].

Caffeine (CAF) in combination with acetylsalicylic acid (ASA) is used as an analgesic adjunct to enhance pain relief, although it has no analgesic activity of its own. Acute consumption of caffeine in combination with over-the counter (OTC) analgesics such as ASA or acetaminophen increases their activity by as much as 40%

depending on the specific type of pain involved. It is apparently due to the ability of caffeine to cause constriction of the cerebral blood vessels and possibly to facilitate the absorption of other drugs. The observed synergism of ASA and caffeine on the inhibition of PGE_2 synthesis in microglial cells[10], a common model for the COX-2 inhibiting activity of non-steroidal anti-inflammatory drugs, may partly explain these effects. Caffeine alone might have analgesic properties for specific types of pain in humans[11-13] and in human experimental pain models[14], but the overall evidence from clinical studies is weak.

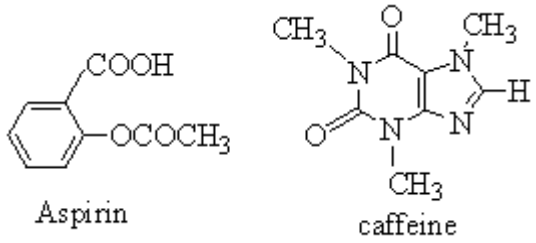

Figure 1: Chemical structure of acetylsalicylic acid and caffeine

The extensive use of these compounds in combined form and the need for clinical and pharmacological study require fast and sensitive analytical techniques for determination of their presence in pharmaceutical formulations[15]. For the simultaneous determination of ASA and caffeine (chemical structure shown in figure 1) in the mixture, different methods have been reported in the literature, including sequential injection chromatography[16], reversed-phase capillary electro chromatography[17], capillary zone electrophoresis based on the drug interactions with β-cyclodextrin[18], UV spectrophotometry with multivariate calibration[19-20], flow-through sensing method with UV detection[21], HPLC[22]and PLS-UV spectrophotometric method[23].

The review of the literature revealed that no UV-visible spectrophotometric method for determination of ASA and caffeine in pharmaceutical formulation is yet been reported. Herein, using UV-visible spectrophotometric method, ASA and caffeine were determined simultaneously in 0.1N NaOH. The method was successfully applied to the simultaneous determination of caffeine and ASA in pharmaceutical formulation.

MATERIALS AND METHODS

Material

Jasco V-530 UV-Visible spectrophotometer was used. A Denver electronic analytical balance (TB-214) was used for weighing the sample. Pure drug sample of ASA and caffeine were procured from S.D. Fine Chemicals Ltd., Mumbai. Tablet formulation (Micropyrin tablets) containing ASA (350 mg) and caffeine (20 mg) was procured from local medical store. 0.1N NaOH was used as a solvent.

Methods

Preparation of sample solution

According to the solubility characteristics of drug, 0.1N NaOH was selected as solvent for analysis. Standard stock solutions having 100 µg/ml of ASA and caffeine were prepared by dissolving separately 10 mg of each drug in 100 ml 0.1N NaOH. The stock solutions were individually diluted to get final concentration of 25 µg/ml each and the diluted solutions were scanned in 200-400 nm range to find out the maximum absorbance (λ_{max}).

Preparation of calibration curve

The overlay of spectra was used to find out isoabsorptive point of both the drugs which was 289 nm. For calibration curve, different aliquots were taken from stock solutions and diluted to prepare series of

concentrations *viz*. 0-40 and 0-25 µg/mL for ASA and caffeine. Calibration data for ASA and caffeine is given in table1.

Analysis of ASA and caffeine by UV-visible spectrophotometer

Simultaneous equation method: Method A

The absorptivity values of the drugs were determined at λ_{max} of ASA and CAF. The absorptivity value of the drug is the ratio of absorbance at selected wavelengths with the concentration of drug (µg/mL). The concentration of ASA and CAF was determined by equation 1 and 2 respectively.

$$c_x = \frac{A_2 a_{y1} - A_2 a_{y2}}{a_{x2} a_{y1} - a_{x1} a_{y2}} \quad \text{----------} \quad (1)$$

$$c_y = \frac{A_1 a_{x2} - A_2 a_{x1}}{a_{x2} a_{y1} - a_{x1} a_{y2}} \quad \text{----------} \quad (2)$$

Where, The absorptivities of X at λ_1 and λ_2, a_{x1} and a_{x2} respectively
The absorptivities of Y at λ_1 and λ_2, a_{y1} and a_{y2} respectively
The absorbances of the diluted sample at λ_1 and λ_2, A_1 and A_2 respectively
C_x and C_y –concentration of X and Y in the diluted sample

Assay of tablet formulation by simultaneous equation method was performed as follows, 20 tablets were weighed and crushed to fine powder. Powdered tablet equivalent to 50 mg of ASA and 2.85 mg of CAF was accurately weighed and transferred to 50 ml volumetric flask. The contents were sonicated for 15 minutes in 30 ml 0.1N NaOH to dissolve the drugs. The volume was made up to the mark to 50 mL using the same solvent. Resulting solution was filtered through whatman filter paper and from the filtrate; 1 ml was diluted to 10 mL to get final concentration of 100 µg/mL and 5.7 µg/mL of ASA and CAF respectively.

Absorbance of sample solution was recorded at 272 nm and 297 nm and the concentration of two drugs in the sample was determined using equation 1 and 2. Criteria for obtaining maximum precision based upon absorbance ratios were also calculated using equations 3 and 4. The results of estimation of both drugs by simultaneous equation method are shown in Table 2.

$$\frac{A_2/A_1}{a_{x2}/a_{x1}} \quad \text{----------} \quad (3)$$

$$\frac{a_{y2}/a_{y1}}{A_2/A_1} \quad \text{----------} \quad (4)$$

Absorbance ratio method (Q method): Method B

ASA and CAF exhibited λ_{max} at 297 nm and at 272 nm respectively at concentration of 25 µg/mL. Both the drugs showed iso-absorptive point at 289 nm. In the quantitative assay of two components in admixture by the absorbance ratio method, absorbances are measured at two wavelengths, one being the λ_{max} of one of the component and other being the wavelength of equal absorptivity of the two components i.e. an isoabsorptive point. The concentration of ASA and CAF was determined by equation 5 and 6 respectively.

$$c_x = \frac{Q_M - Q_Y}{Q_X - Q_Y} X \frac{A1}{a_{x1}} \quad \text{----------} \quad (5)$$

$$c_y = \frac{Q_M - Q_X}{Q_Y - Q_X} X \frac{A1}{a_{y1}} \quad \text{----------} \quad (6)$$

Where, $Q_x = a_{x2}/a_{x1}$ $Q_y = a_{y2}/a_{y1}$ $Q_M = A_2/A_1$

Assay procedure for simultaneous estimation of ASA and CAF in tablet dosage forms was same as mentioned in method A. Absorbance of sample solution was recorded at 297 nm and 289 nm and the concentration of two drugs in the sample was determined by using equation 5 and 6. The results of estimation of both drugs by absorbance ratio method are shown in Table 2.

Method validation

Accuracy

Accuracy was confirmed by recovery study as per ICH guidelines at three different concentration levels 80%, 100%, 120% by replicate analysis (n = 3). To a preanalyzed sample solution, standard drug solutions were added and then percentage of drug content was calculated. The results of accuracy study were reported in table 3.

Inter-day and intra-day precision

Precision of the method was tested five times by analyzing a standard solution of ASA and CAF of 25 µg/mL. R^2 values were calculated and compared. Precision was expressed as relative standard deviation (RSD) which was found to be < 2% (Table 1).

Repeatability

Repeatability was studied by repeating assay of tablet formulation six times. The standard deviation and RSD were calculated. The results of statistical evaluation are given in Table 1.

Linearity

For both drugs, appropriate dilutions of standard stock solutions were analysed as per the developed method. Calibration curve was plotted in the concentration range of 0-40 µg/mL for ASA and 0-25 µg/mL for CAF. The linearity data for both methods are presented in Table 1.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ of ASA and CAF by proposed methods were determined using calibration standards. LOD and LOQ were calculated as 3.3σ/S and 10 σ /S respectively, where S is the slope of the calibration curve and σ is the standard deviation of response. The results of the same are shown in Table 1.

RESULTS AND DISCUSSION

The solubility of ASA and CAF was studied and 0.1N NaOH was selected as a choice of solvent. ASA and CAF showed well defined λ_{max} at 297 nm and 272 nm respectively therefore these two wavelengths were considered for development of simultaneous equation method and 289 nm as isoabsorptive point

for absorbance ratio method (Figure 1a, 1b and 1c). Two drugs individually followed Beer-Lambert's law over the concentration range of 0-40 µg/ml and 0-25 µg/mL for ASA and CAF respectively (Figure 1d, 1e, 1f and 1g). Coefficient of correlation for ASA at 297 and 289 nm was found to be 0.9998 and 0.9995 respectively with RSD < 0.1. For caffeine, coefficient of correlation at 272 and 289 nm was found to be 0.9994 and 0.9998 respectively with RSD < 0.1. The values of correlation coefficient suggest the level of precision of the method. Table 1 gives calibration data for both the drugs.

Table 1: Calibration data of ASA and caffeine

Parameter	At 272 nm	At 297 nm	At 289 nm	
	CAF	ASA	ASA	CAF
Beer Lambert's law limits (µg/ml)*	0-25	0-40	0-40	0-25
Regression equation	y = 0.046 x	y = 0.0185 x	y = 0.0163 x	y = 0.0159 x
Slope (m)	0.046	0.0185	0.0163	0.0159
Correlation coefficient (R^2)	0.9994	0.9998	0.9995	0.9998
Interday precision (%RSD)	0.0147	0.0206	0.0181	0.0723
Intraday precision (%RSD)	0.0061	0.0088	0.0049	0.0168
Repeatability	99.50±0.2321	100.10±0.6578	99.90±0.3452	100.40±0.2341
LOD (µg/ml)	0.6360	1.0896	0.9956	3.4907
LOQ (µg/ml)	1.9272	3.3018	3.0170	10.5779

*= *Average of 3 determinations*

Drug content in tablet (amount present) was directly found from the above mentioned equations for both the methods. Standard deviations, RSD were calculated and are given in table 2. Percentage estimation in tablet dosage form was 99.6573 and 103.3977 (%RSD < 2) for ASA and caffeine respectively by method A. Percent drug content was 98.5405 and 99.9815 (%RSD < 2) for ASA and caffeine respectively by method B.

The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures. Linear regression equations (intercepts and slopes) for ASA and CAF were established. The high values of the correlation coefficients and the values of Y-intercepts close to zero indicate the good linearity of the calibrations. The values of slope, intercept and correlation coefficient values are given in Table 1. Limit of detection (LOD) and limit of quantitation (LOQ) were determined by using the formula and are mentioned in Table 1.

To study the validation parameters accuracy, reproducibility, reliability and interference, recovery experiment was carried out by standard addition. The recovery of added standard was calculated at different concentration levels. From the total amount of drug found, the percentage recovery was calculated which was between 93-107 %w/w (RSD < 2.0).

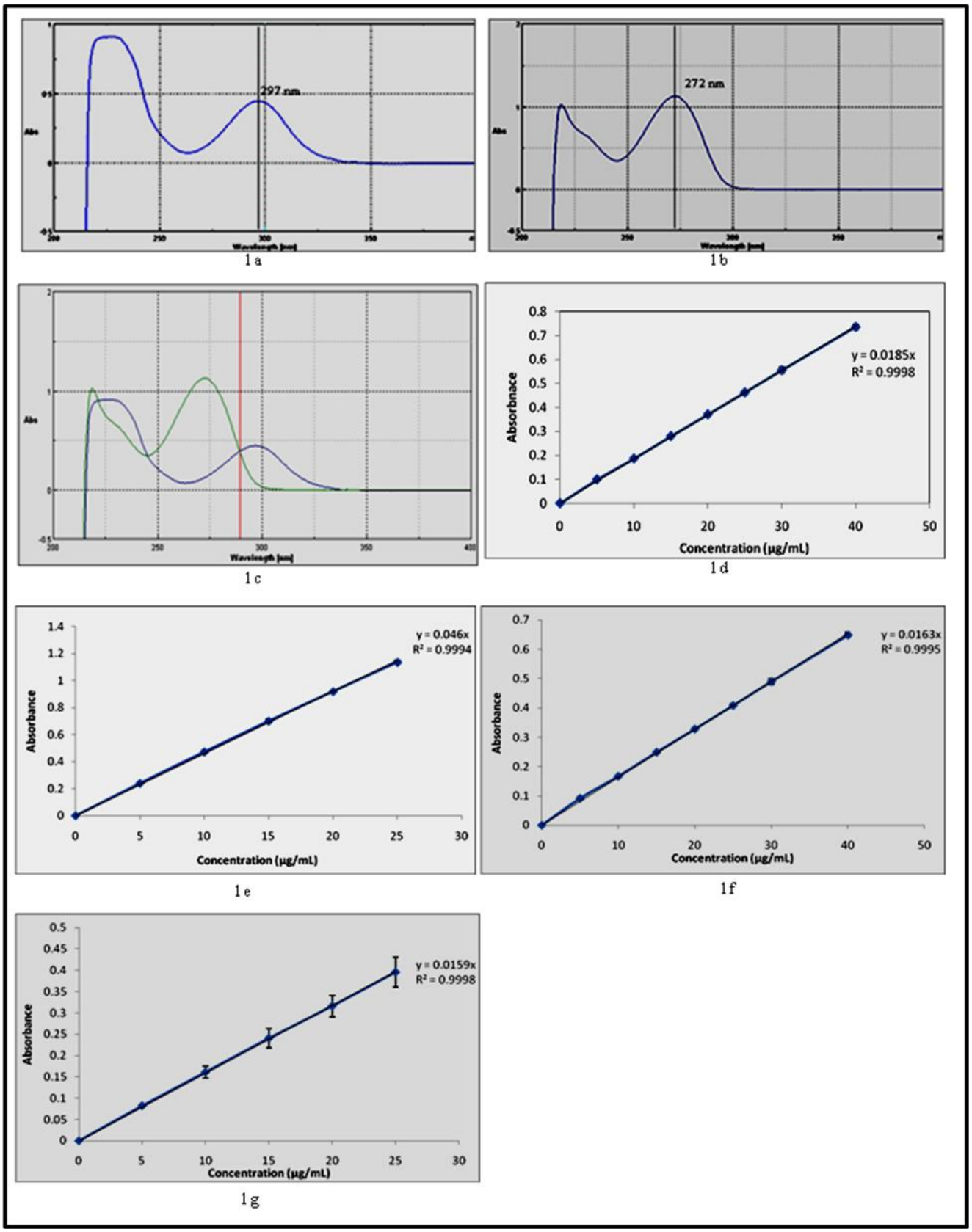

Figure 2: *Spectroscopic data of ASA and caffeine 1a. Acetyl salicylic acid spectrum; 1b. Caffeine spectrum; 1c. Overlay spectrum of ASA and CAF; 1d. Calibration curve of ASA in 0.1N NaOH at 297 nm; 1e. Calibration curve of CAF in 0.1N NaOH at 272 nm; 1f. Calibration curve of ASA in 0.1N NaOH at 289 nm and 1g. Calibration curve of CAF in 0.1N NaOH at 289 nm*

Table 2: Analysis of tablet formulation

Method	Tablet components	Labeled claim (mg/tab)	Amount found* (mg/tab)	% Purity	SD*	%RSD*
1. Simultaneous estimation method	ASA	350	348.8007	99.6573	0.7029	1.3483
	CAF	20	20.19745	103.3977	0.8147	0.1007
2. Absorbance ratio method	ASA	350	344.8918	98.5405	0.4781	0.7185
	CAF	20	19.9963	99.9815	0.5003	0.0949

*= *Average of 3 determinations*

Table 3: Results of recovery studies

Method	Recovery level (amount added) %	% recovery ± SD	
		ASA	Caffeine
A	80	100.7339 ± 0.1854	93.128 ± 0.0977
	100	106.3502 ± 0.1892	94.9698 ± 0.2789
	120	106.4086 ± 0.1007	93.9220 ± 0.2804
B	80	102.6770 ± 0.1995	103.6197 ± 0.5507
	100	107.1546 ± 0.2035	99.8780 ± 0.4871
	120	107.1699 ± 0.2031	104.7262 ± 0.4680

Thus, it can be concluded that the methods developed were simple, accurate, sensitive and precise. Hence, the above methods can be applied successfully in simultaneous estimation of acetyl salicylic acid and caffeine in marketed formulations.

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