ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info/jasr

ISSN
0976-9595
Research Article

SPECTROPHOTOMETRIC AND CHROMATOGRAPHIC DETERMINATION OF ACETYLSALICYLIC ACID AND CAFFEINE IN PURE AND IN TABLET DOSAGE FORM

Sonali S. Bharate[1]*, Sandip B. Bharate[2]

[1]*Department of Pharmaceutics, P.E. Society's Modern College of Pharmacy (For Ladies), Dehu-Alandi Road, Moshi, Pune-412 105, Maharashtra, India*

[2]*Medicinal Chemistry Division, Indian Institute of Integrative Medicine (CSIR), Canal Road, Jammu Tawi – 180001, India*

**Corresponding author: sonalibharate@gmail.com*

ABSTRACT

In the present research paper, spectrophotometric and chromatographic assay method has been developed for the simultaneous estimation of acetylsalicylic acid and caffeine in bulk and commercial tablets. Acetylsalicylic acid and caffeine showed absorbance maxima at 228 and 275 nm in 0.1N HCl and at 225 and 272 nm in methanol, respectively. These drugs were estimated in formulations by simultaneous equation method (method A) and absorbance ratio method (method B). Acetylsalicylic acid and caffeine at their respective λ_{max} followed Beer-Lambert's law at concentration range of 0-28 and 0-1.6 µg/mL. Method B involved measurement of absorbance at isoabsoptive point of these two drugs i.e. at 244 nm in 0.1N HCl and at 240 nm in methanol respectively. HPLC method for simultaneous estimation of these two components employed a SMT-C18, OD-5 100/25 (250×4.6 mm) column with a mobile phase of methanol using a UV detector at the wavelength of 240 nm. The method was validated for linearity, accuracy, precision, repeatability and sensitivity. The method was linear for mixture of acetylsalicylic acid and caffeine over a concentration range 20-36 µg/mL (R^2 = 0.9973) and 1.14-2.05 µg/mL (R^2 = 0.9954), respectively. LOD and LOQ of acetylsalicylic acid were 1.70 µg/mL and 5.17 µg/mL and that of caffeine was 0.27 and 0.80 µg/mL, respectively. Both, the spectrophotometric and chromatographic methods were found to be linear, accurate, precise and reproducible with an added advantage of cost effectiveness which can be used for routine quantitative analysis of acetylsalicylic acid and caffeine in pure and tablet dosage form.

Keywords: LC method, Simultaneous estimation, Absorbance ratio method, Acetylsalicylic acid, Caffeine, Validation

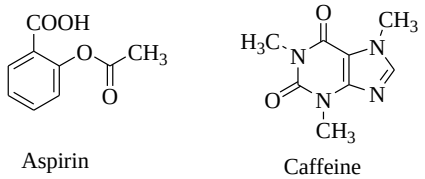
1. INTRODUCTION

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.

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 estimation of ASA (chemical structure shown in figure 1), 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, 23] and PLS-UV spectrophotometric method [24].



Aspirin Caffeine

Figure 1. Chemical structure of acetylsalicylic acid and caffeine

Review of the literature revealed that there is only one spectrophotometric method developed by us for simultaneous determination of ASA and CAF, which employed 0.1N NaOH [25]. Therefore, this study was designed in order to have comparative picture of developed and reported method for determination of ASA and caffeine in bulk and in pharmaceutical formulation. Herein, using UV-visible spectrophotometric method, ASA and caffeine were determined simultaneously in 0.1N HCl and methanol. Another objective of this research work was also to develop simple and economic LC method, which can be successfully applied in simultaneous determination of stated drugs in bulk and in pharmaceutical formulations.

2. MATERIAL AND METHODS

2.1. Material

Jasco V-530 UV-Visible spectrophotometer was used. A Shimadzu AUX220 analytical balance was used for weighing the samples. Pure drug sample of ASA and caffeine were procured from S.D. Fine Chemicals Ltd., Mumbai. Tablet formulation (Micropyrin tablets- Batch no. BD0168 by Piramal Healthcare Ltd.) containing ASA (350 mg) and caffeine (20 mg) was procured from local medical store.

HPLC apparatus

The HPLC analysis was carried out on SMT-C18, OD-5 100/25 (250×4.6 mm) connected to a HPLC (model CYBERLAB™) system consisting a LC-8A pump, UV detector and 20 µL injection loop.

2.2. Spectrophotometric analysis

2.2.1. Preparation of sample solution

To find out solubility profile of ASA and CAF, drugs were dissolved in water, 0.1N HCl and in methanol. It was observed that ASA and CAF exhibit absorbance maxima in 0.1N HCl and in methanol. Peak shape was nonsymetric when water was selected as solvent and therefore based on the solubility characteristics of the drugs, 0.1N HCl and methanol were selected as solvents for analysis. ASA and CAF are present in dosage form in the ratio of 17.5:1 and therefore both these drugs were mixed in geometric proportion and standard stock solution having 200 µg/mL of ASA and 11.428 µg/mL CAF were prepared by dissolving 5.3 mg of mixture in 25 mL of above mentioned solvents. Stock solutions were individually diluted to get final concentration of 20 µg/mL of ASA and 1.144 µg/mL of CAF and the diluted solutions were scanned in 200-400 nm range to find out the absorbance maxima (λ_{max}).

2.2.2. Preparation of calibration curve spectrophotometrically

The overlay of spectra was used to find out iso-absorptive point of both drugs which was 244 and 240 nm in 0.1N HCl and in methanol, respectively. The calibration curve for pre-mixed drugs, ASA: CAF in the ratio of 17.5:1 using spectroscopy was plotted. Different aliquots were taken from stock solutions and diluted to prepare series of concentrations *viz*. 12-28 µg/mL for ASA and 0.69-1.6 µg/mL for caffeine. Calibration data for ASA and caffeine in 0.1N HCL and in methanol is given in table 1 and 2. Using 0.1N HCl as a solvent, calibration curve of above dilutions was plotted at 228 (λ_{max} of ASA), 272 (λ_{max} of CAF) and at 244 nm (iso-absorptive point). In methanol, calibration curve in above stated combination was obtained at 225 (λ_{max} of ASA), 272 (λ_{max} of CAF) and at 240 nm (iso-absorptive point).

2.2.3. Spectrophotometric analysis of ASA and caffeine

A. Simultaneous equation method: Method A

The absorptivity values of 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.

$$c_x = \frac{A_2a_{y1} - A_2a_{y2}}{a_{x2}a_{y1} - a_{x1}a_{y2}} \quad \text{(1)}$$

$$c_y = \frac{A_1a_{x2} - A_2a_{x1}}{a_{x2}a_{y1} - a_{x1}a_{y2}} \quad \text{(2)}$$

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 HCl/ methanol to dissolve the drugs. The volume was made up to the mark to 50 mL using the same solvents. 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 λ_{max} of CAF and at λ_{max} of ASA 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 result is depicted in table 3.

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

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

B. Absorbance ratio method (Q method): Method B

In the quantitative measurement of two components 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 λ_{max} of CAF and at iso-absorptive point and the concentration of two drugs in the sample were determined using equations 5 and 6. The results are shown in table 3.

2.2.4. Method validation

A. Linearity

To find out the linearity, standard solutions of both drugs were prepared individually (4, 8, 12, 16 and 20 µg/mL), each in three replicates. Since both the drugs are available in combined dosage form in the ratio of 17.5:1 (ASA: Caffeine), calibration curve was plotted after mixing both of them in above mentioned proportion. For this, 20 and 350 mg of pre-sieved caffeine and ASA was weighed respectively and mixed in geometric proportion. Powder mixture equivalent to 5.3 mg (contained 5 mg of ASA and 0.29 mg of caffeine) was dissolved in either 0.1N HCl/ methanol in a 25 mL volumetric flask and volume was made with the respective solvent. From this stock solution, various concentrations were prepared for calibration curve; [(ASA; 12, 16, 20, 24 and 28 µg/mL) and (CAF; 0.69, 0.92, 1.14, 1.4 and 1.6 µg/mL)]. The linearity data for both methods are presented in table 1 and 2; correlation coefficient is expressed in figure 2 and 3.

B. Inter-day and intra-day precision

Precision of the method was tested five times by analyzing a standard solution of ASA and CAF of 20 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 and 2).

C. Repeatability

Repeatability was studied by performing assay of tablet formulation six times. The standard deviation and RSD were calculated and are depicted in table 1 and 2.

D. 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 $3.3\sigma/S$ and $10\sigma/S$ respectively, where S is slope of the calibration curve and σ is standard deviation of the response. Results of the same are shown in table 1 and 2.

2.3. Liquid chromatography (LC) method

2.3.1. HPLC conditions

The mobile phase consisted of methanol. The flow rate was 0.75 mL/min. The wavelength of detection was 240 nm (isoabsorptive point of ASA and CAF). The injection volume was 20 µL.

2.3.2. Preparation of sample solution

Mobile phase (methanol) was used as a solvent for preparation of both stock as well as standard solutions and prepared as described under the section 2.2.1.

2.3.3. Preparation of calibration curve

From the data obtained by spectrophotometric analysis, methanol was selected as the mobile phase for analyzing both the drugs simultaneously by LC system. Iso-absorptive point of both the drugs in methanol was 240 nm and therefore 240 nm was set as the detection wavelength in LC system. Standard calibration curve was plotted in a mixture form simultaneously by injecting different concentrations of ASA (20, 24, 28, 32 and 36 μg/mL) and CAF (1.14, 1.4, 1.6, 1.82 and 2.05 μg/mL). Calibration curves are shown in figure 4.

2.3.4. Method validation

A. Linearity

Standard solutions as mentioned in section 2.3.3, each in three replicates, were injected into the system. The method of linear regression was used for data evaluation. Area Under the Curve (AUC) was plotted against theoretical concentrations of standard (figure 4). Linearity was expressed as a correlation coefficient.

B. Precision

Precision of the method was tested by injecting a mixed standard solution consisting 20μg/mL and 1.14 μg/mL of ASA and CAF, five times respectively. Peak areas were determined and compared. Precision was expressed as percentage relative standard deviation (%RSD).

C. Repeatability

Repeatability was studied by performing assay of tablet formulation six times. The standard deviation and RSD were calculated and are depicted in table 5.

D. Determination of limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection (LOD) is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value. The limit of quantitation (LOQ) is the lowest amount of analyte, which can be quantitatively determined with suitable precision. LOD and LOQ of the developed method were determined by injecting progressively low concentration of the standard solution under the chromatographic conditions mentioned under the HPLC conditions section. The lowest concentrations assayed where the signal/noise ratio was at least 10:1, this concentration was regarded as LOQ. The LOD was defined as a signal/noise ratio of 3:1.

E. Assay of formulation

Marketed tablet formulation consisting both the drugs was evaluated for the drug content using developed HPLC method. 20 tablets were weighed and crushed to fine powder. For extraction of drugs, weighed amount of powder (6.6 mg) was taken in 25 mL volumetric flask containing little amount of methanol. This suspension was sonicated for 15 minutes in order to dissolve both the drugs and volume was made to 25 mL with methanol. It was further diluted to get concentration of 36 and 2.05 μg/mL of ASA and CAF respectively. This solution was filtered through 0.45 μm syringe filter and injected into the liquid chromatographic system for the analysis. The percentage of drug content was determined.

3. RESULTS AND DISCUSSION

3.1. Spectrophotometric analysis

The solubility of ASA and CAF was studied and 0.1N HCl and methanol were selected as the choice of solvents. ASA and CAF showed well-defined λ_{max} at 228 and 272 nm for ASA and CAF respectively in 0.1N HCl therefore these two wavelengths were considered for assay by simultaneous equation method and 244 nm as isoabsorptive point for absorbance ratio method (figure 2a, 2b and 2c). Two drugs in mixture form followed Beer-Lambert's law over the concentration range of 12-28 μg/ml and 0.69-1.6 μg/mL for ASA and CAF respectively (Figure 2d, 2e and 2f). Coefficient of correlation for ASA at 228 and 244 nm was found to be 0.9990 and 0.9997 respectively with RSD < 2.0. For caffeine, coefficient of correlation at 272 and 244 nm was found to be 0.9924 and 0.9997 respectively with RSD < 2.0. The values of correlation coefficient suggest the level of precision of the method. Table 1 gives calibration data for both the drugs in 0.1N HCl.

In methanol, ASA and CAF showed well-defined λ_{max} at 225 and 272 nm for ASA and CAF respectively therefore these two wavelengths were considered for assay using simultaneous equation method and 240 nm as iso-absorptive point for absorbance ratio method (figure 3a, 3b and 3c). Two drugs in mixture form followed Beer-Lambert's law over the concentration range of 12-28 μg/ml and 0.69-1.6 μg/mL for ASA and CAF respectively (Figure 3d, 3e and 3f). Coefficient of correlation for ASA at 225 and 240 nm was found to be 0.9999 and 0.9997 respectively with RSD < 2.0. For caffeine, coefficient of correlation at 272 and 240 nm was found to be 0.9703 and 0.9997 respectively with RSD < 2.0. The values of correlation coefficient suggest the level of precision of the method. Table 2 gives calibration data for both the drugs in methanol.

Drug content in tablet (amount found) was directly found from the above mentioned equations for both the methods. Standard deviation and RSD values were calculated and are given in table 3. Percentage estimation in tablet dosage form was in the range of 95-105 %w/w (%RSD < 2; average of four determinations) for ASA and caffeine respectively by method A and B in both 0.1N HCl and in methanol.

Table 1. Calibration data of ASA and caffeine in 0.1N HCl

Parameter	At 272 nm CAF	At 228 nm ASA	At 244 nm
Beer Lambert's law limits (µg/ml)*	0-1.6	0-28	0-28/0-1.6
Regression equation	y = 0.1358 x	y = 0.0475 x	y = 0.0151 x
Slope (m)	0.1358	0.0475	0.0151
Correlation coefficient (R^2)	0.9924	0.9990	0.9997
Interday precision (%RSD)	1.7609	0.3014	1.2666
Intraday precision (%RSD)	0.8489	0.8815	1.3317
Repeatability	97.5093±0.7288	99.8285±0.5679	95.2089±0.4188
LOD (µg/ml)	0.1316	0.6637	1.0838
LOQ (µg/ml)	0.3990	2.0113	3.2843

*= *Average of 3 determinations*

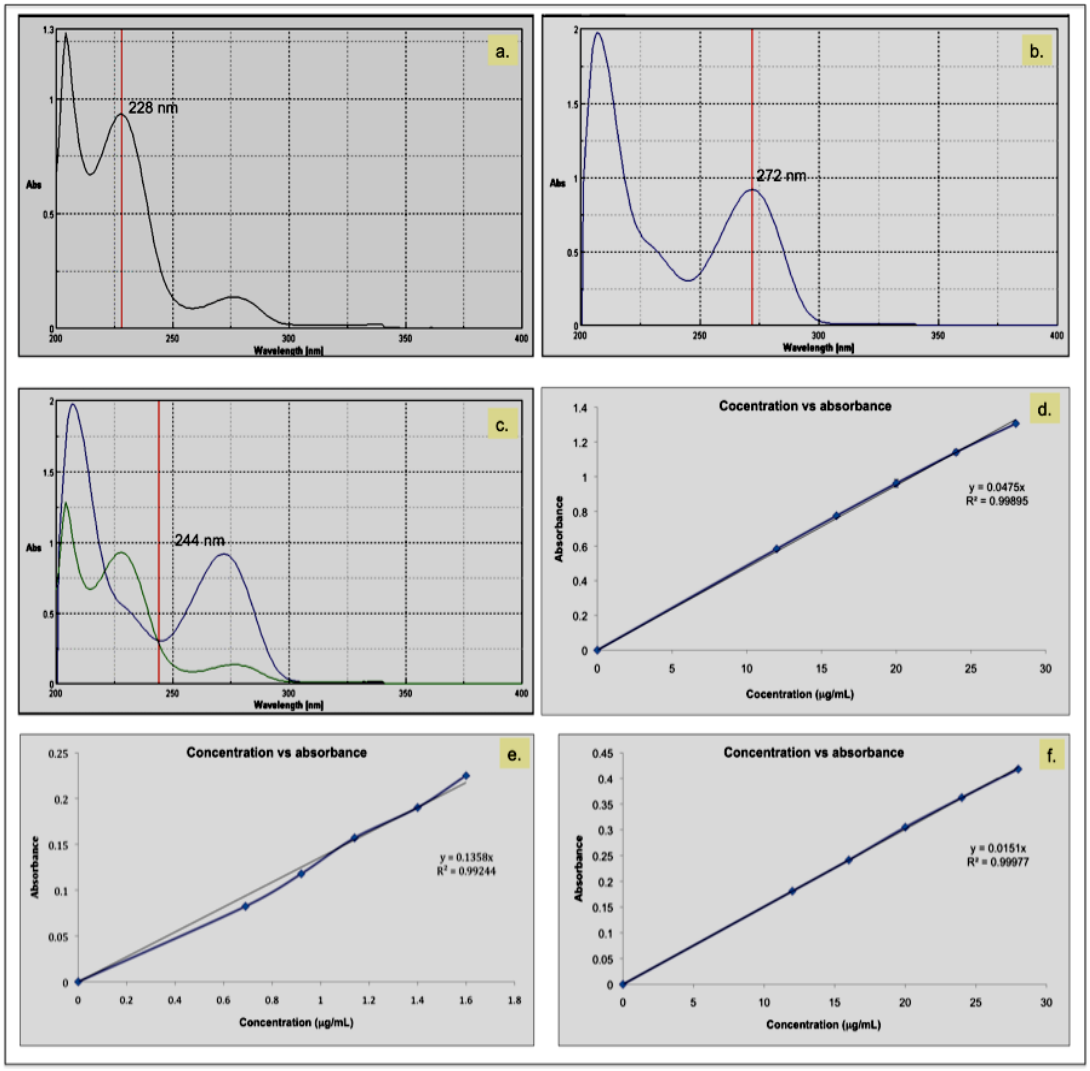


Figure 2. Spectroscopic data of ASA and caffeine in 0.1N HCl 1a. Acetylsalicylic acid spectrum; 1b. Caffeine spectrum; 1c. Overlay spectrum of ASA and CAF; 1d. Calibration curve of ASA at 228 nm; 1e. Calibration curve of CAF at 272 nm and 1f. Calibration curve at iso-absorptive point 244 nm

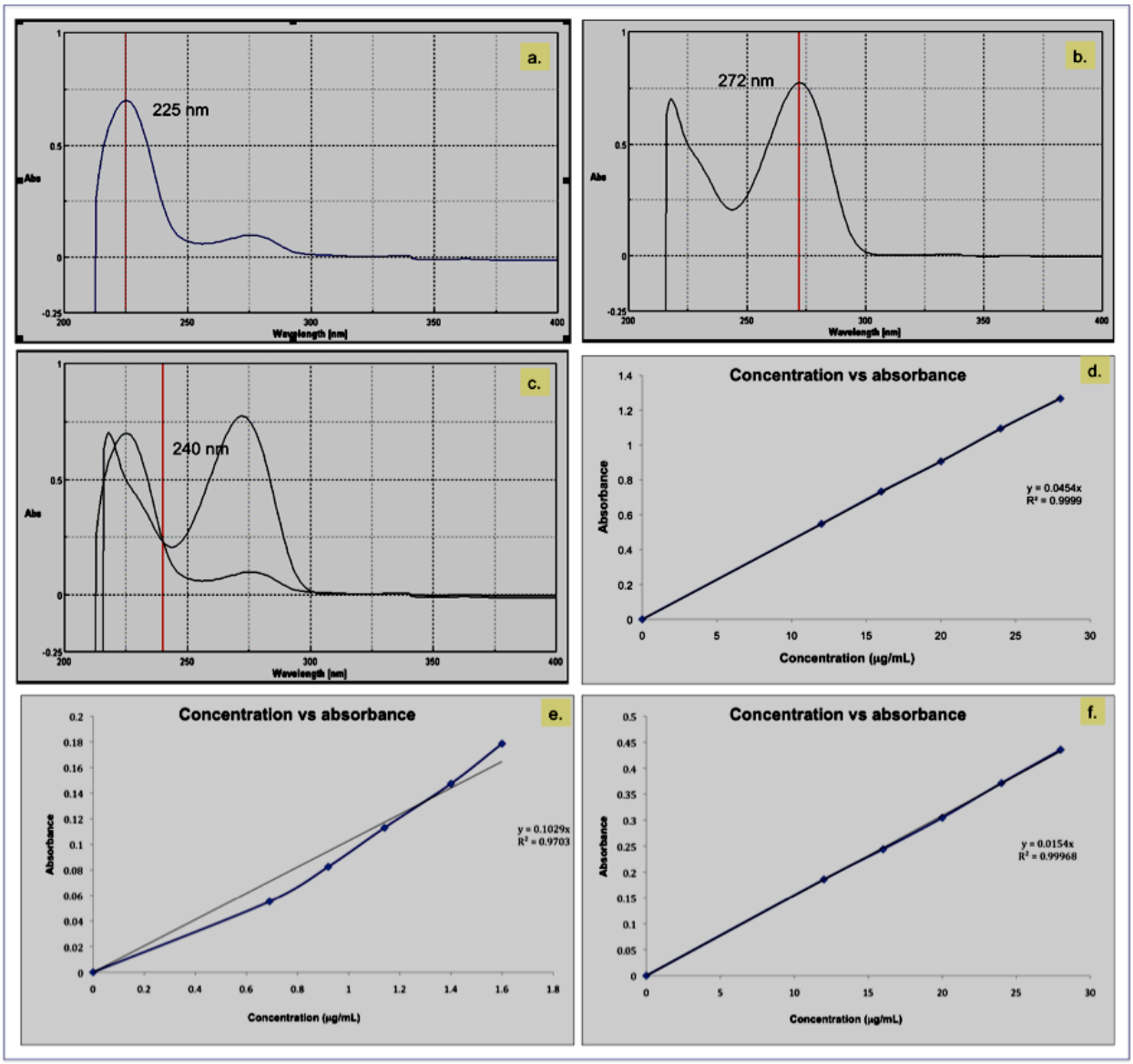


Figure 3. Spectroscopic data of ASA and caffeine in methanol 1a. Acetylsalicylic acid spectrum; 1b. Caffeine spectrum; 1c. Overlay spectrum of ASA and CAF; 1d. Calibration curve of ASA at 225 nm; 1e. Calibration curve of CAF at 272 nm and 1f. Calibration curve at iso-absorptive point 240 nm

Table 2. Calibration data of ASA and caffeine in methanol

Parameter	At 272 nm CAF	At 225 nm ASA	At 240 nm
Beer Lambert's law limits (µg/ml)*	0-1.6	0-28	0-28/0-1.6
Regression equation	y = 0.1029 x	y = 0.0454 x	y = 0.0154 x
Slope (m)	0.1029	0.0454	0.0154
Correlation coefficient (R^2)	0.9703	0.9999	0.9997
Interday precision (%RSD)	1.7609	0.1800	1.2314
Intraday precision (%RSD)	1.2666	0.1975	1.3098
Repeatability	106.029±0.3864	95.1580±0.8749	100.2497±0.4188
LOD (µg/ml)	0.2861	1.3825	1.7991
LOQ (µg/ml)	0.8671	4.1895	5.4519

**= Average of 3 determinations*

Table 3. Analysis of tablet formulation

Solvent system used	Method	Tablet components	Labeled claim (mg/tab)	Amount found* (mg/tab)	% Purity	SD*	%RSD*
0.1N HCl	A	ASA	350	349.40	99.8285	0.7275	0.7288
		CAF	20	19.5019	97.5093	0.5537	0.5679
	B	ASA	350	333.2314	95.2090	0.3987	0.4188
		CAF	20	21.2469	106.2349	0.4747	0.4469
Methanol	A	ASA	350	333.0531	95.1580	0.8325	0.8749
		CAF	20	21.2058	106.0290	0.4097	0.3864
	B	ASA	350	350.8740	100.2497	0.9745	0.9721
		CAF	20	19.7156	98.5778	0.4189	0.4249

*= *Average of 4 determinations; Method A, Simultaneous estimation method; Method B, Absorbance ratio method*

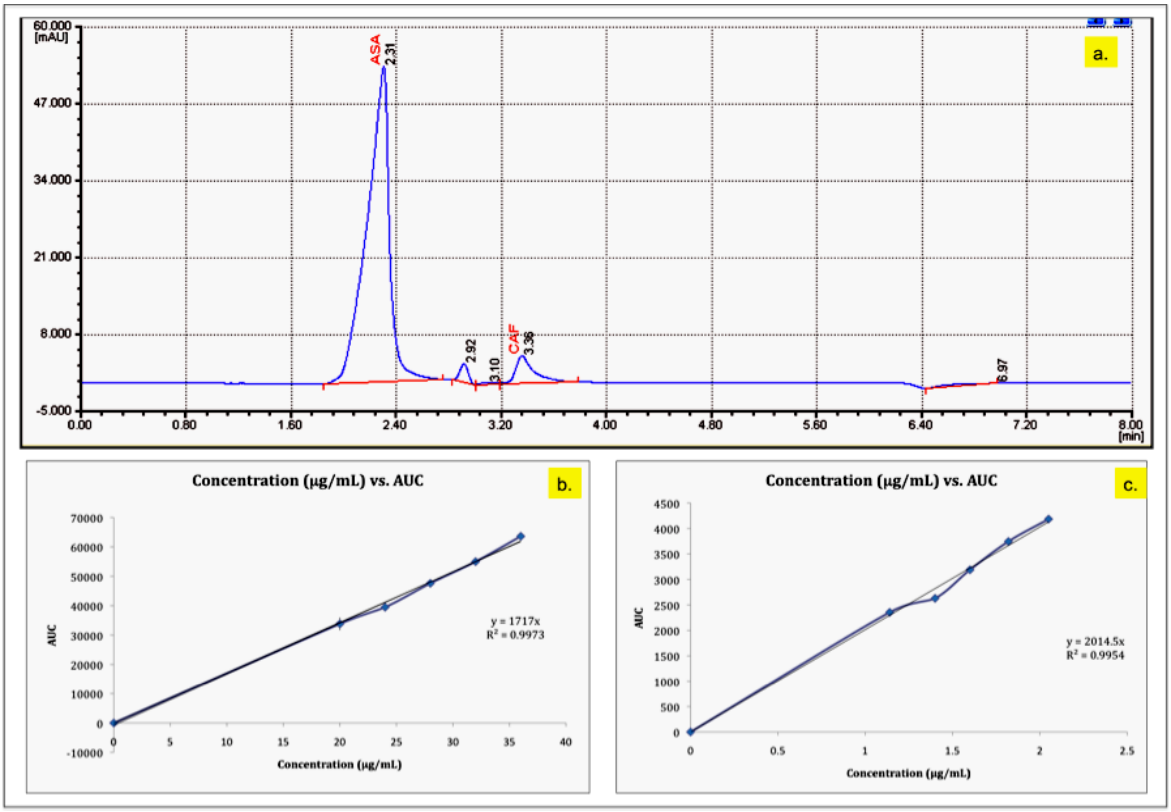


Figure 4. Chromatographic data of ASA and caffeine in methanol 1a. Chromatogram of ASA and CAF in mixture (36 and 2.05 µg/mL, respectively) at 240 nm; 1b. Calibration curve of ASA; 1c. Calibration curve of CAF

The method using 0.1N HCl and methanol was validated according to International Conference on Harmonization (ICH) 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 and 2. Limit of detection (LOD) and limit of quantitation (LOQ) were determined using the formula and are mentioned in table 1 and 2.

The developed method was validated for linearity, precision, LOQ and LOD, accuracy, reproducibility and % recovery by standard addition. The recovery of added standard was calculated at different concentration levels (50, 100 and 150%). From the total amount of drug found the percentage recovery was calculated which was between 90-110 %w/w (RSD < 2.0; average of 3 determinations) for both the drugs.

3.2. Chromatographic analysis

LC based analytical methods are widely and routinely used in quality control of drugs and formulations thereof. As

mentioned above, the LC method for simultaneous estimation of ASA and CAF is not available; therefore herein our objective was to establish a simple and efficient LC method. Mobile phase was selected to obtain a rapid and simple assay method for simultaneous estimation of ASA and CAF with a reasonable run time, suitable retention time and sharpness of the peak. Under experimental conditions, the chromatogram of ASA and CAF (figure 4a) showed well separated peaks at retention times of around 2.30 and 3.36 min. The developed method has several key features such as cheaper mobile phase (methanol), routinely used C18 column and shorter run time (8 min). The developed method comprising methanol as a mobile phase was found to be highly reproducible. With the use of non-buffered mobile phase, problems associated with buffers *viz*. time required in its preparation, pH adjustments, chocking of tubings and proper washing of the system after its use has been avoided.

ASA and CAF showed well-resolved peaks at iso-absorptive wavelength of 240 nm (figure 4a). Two drugs in mixture form followed Beer-Lambert's law over the concentration range of 20-36 μg/ml and 1.14-2.05 μg/mL for ASA and CAF respectively (figure 4b and 4c). Coefficient of correlation for ASA and CAF was found to be 0.9973 and 0.9954 respectively with RSD < 2.0. The values of correlation coefficient suggest the level of precision of the method. Table 4 gives calibration data for both the drugs in methanol.

Drug content in tablet (amount found) was directly calculated from calibration equations. Standard deviations, RSD was calculated and is given in table 4. Percentage estimation in tablet dosage form was 95.6682 and 99.3318 (%RSD < 2; average of six determinations) for ASA and caffeine respectively.

The developed method was validated and 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 4. The developed method was validated for linearity, precision, LOQ and LOD, accuracy and reproducibility (table 4). LOD and LOQ were determined by using the formula and are mentioned in table 4. Characteristic quantities of chromatogram were determined and are given in table 5.

Table 4. Calibration data of ASA and caffeine using LC method in methanol at 240 nm

Parameter	At 240 nm	
	CAF	ASA
Beer Lambert's law limits (μg/ml)*	0-2.05	0-36
Regression equation	y = 2014.5 x	y = 1717 x
Slope (m)	2014.5	1717
Correlation coefficient (R^2)	0.9954	0.9973
Interday precision (%RSD)	1.6219	1.1118
Intraday precision (%RSD)	1.2666	1.1862
Repeatability#	99.3318±0.4122	95.6682±1.3937
LOD (μg/ml)	0.2657	1.7064
LOQ (μg/ml)	0.8052	5.1710

*= *Average of 3 determinations;* #= *Average of 6 determinations*

Table 5. Characteristic quantities of the chromatogram of ASA and CAF

Sr. No.	Parameters	ASA	CAF
1	Peak height (H)	6.3 cm	0.6 cm
2	Retention time (t_R)	2.3 min	3.36 min
3	Dead time (t_m)	1.8 min	3.1 min
4	Dwelling time (t'_R)	0.5 min	0.26 min
5	Base width of peak (W_B),	2.4 cm	1.6 cm
6	Peak width at half-height (W_H)	0.5 cm	0.5 cm
7	Peak area (A)	3.15 cm^2	1.1 cm^2
8	Tailing factor (T) or asymmetry factor (A_f)	1.0	2.0
9	Capacity factor (k')	0.2777	0.0838
10	Retention volume (V_R)	1.7250 mL/min	2.52 mL/min
11	Number of theoretical plates (N)	4000	

Thus, it can be concluded that the developed analytical methods are simple, accurate, sensitive and precise. Hence, these can be applied successfully in simultaneous estimation of acetylsalicylic acid and caffeine in bulk and in marketed formulations.

5. REFERENCES

1. Panchal HJ, Suhagia BN, Patel NJ, Rathod IS, Patel BH. *Chromatographia*, 2009; **69**: 91-95.
2. Gandhimathi M, Ravi TK, Abraham A, Thomas R. *J Pharm Biomed Anal*, 2003; **32**: 1145-1148.
3. Anandakumar K, Ayyappan T, Raman VR, Vetrichelvan T, Sankar ASK, Nagavalli D. *Indian J Pharm Sci*, 2007; **69**: 597-599.
4. Rajput SJ, George RK, Ruikar DB. *Indian J Pharm Sci*, 2008; **70**: 450-454.
5. Lipton R, Diamond S, Reed M, Diamond M, Stewart W. *Headache*, 2001; **41**: 638-645.
6. Rasmussen B, Jensen R, Olesen J, *J Epidemiol Community Health*, 1992; **46**: 443-446.
7. Michel, P. (2000). 'Socioeconomic costs of headache'. In The headaches. 33 eds J. Olesen, P. Tfelt-Hansen and K. Welch. Philadelphia: Lippincott Williams & Wilkins.
8. Khoshayand MR, Abdollahi H, Shariatpanahi M, Saadatfard A, Mohammadi A. *Spectrochimica Acta Part A*, 2008; **70**: 491-499.
9. Vichare V, Mujgond P, Tambe V, Dhole SN. *Int J PharmTech Res,* 2010; **2**: 2512-2516.
10. Fiebich B, Lieb K, Hüll M, Aicher B, van Ryn J, Engelhardt G. *Neuropharmacology*, 2000; **39**: 2205-2213.
11. Camann W, Murray R, Mushlin P, Lambert D. *Anesth Analg*, 1990; **70**: 181-184.

4. ACKNOWLEDGEMENT

Authors are grateful to the Principal, Modern College of Pharmacy (for Ladies), Moshi, Pune for providing the necessary facilities to carry out this work.

12. Ward N, Whitney C, Avery D, Dunner D. *Pain*, 1991; **44**: 151-155.
13. Yücel A, Özyalcin S, Talu G, Yücel E, Erdine S. *Rg Anesth Pain Med*, 1999; **2**: 51-54.
14. Myers D, Shaikh Z, Zullo T. *Headache*, 1997; **37**: 654-658.
15. Karim MM, Jeon CW, Lee HS, Alam SM, Lee SH, Choi JH, Jin SO, Das AK. *J Fluoresc*, 2006; **16**: 713-721.
16. Dalibor S, Isabel N, Petr S, Hana S, Conce M, Montenegro B, Araujo A. *J Sep Sci*, 2004; **27**: 529-536.
17. Vincenzo P, Roberto M, Maria AR, Salvatore F. *Electrophoresis*, 2004; **25**: 615-621.
18. Wei W, Xiaodong Y, Huangxin J. *J Chromatogr Sci*, 2004; **42**: 155-160.
19. Sena M, Poppi R. *J Pharm Biomed Anal*, 2004; **34**: 27-34.
20. Mohammad M, Chi W, Hyun S, Seikh M, Sang H, Jong H, Seung O, Ajoy K. *J Fluoresc*, 2006; **16**: 713-721.
21. Dominguez VA, Garcia RJ, Ortega BP, Molina DA. *Anal Lett*, 2002; **35**: 2433-2447.
22. Franeta J, Agbaba D, Eric S, Pavkov S, Aleksic M, Vladimirov S. *Farmaco*, 2002; **57**: 709-713.
23. Hashem HA-A. *Chromatographia*, 2010; **71:** 31-35.
24. Bouhsain Z, Garrigues S, De GM. *Fresenius J Anal Chem*, 1997; **357**: 973-976.
25. Bharate SS, Kolhe SR, Bharate SB. *J Adv Sci Res*, 2011; **2**: 34-41.