ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
25 (2001) 93–101

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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LC method for the analysis of acetylsalicylic acid, caffeine and codeine phosphate in pharmaceutical preparations

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Received 25 July 2000; received in revised form 12 September 2000; accepted 20 September 2000

Abstract

An accurate, simple, reproducible and sensitive method for the determination of acetylsalicylic acid, caffeine and codeine phosphate has been developed and validated. Acetylsalicylic acid, caffeine and codeine phosphate were separated using a μBondapack C_8 column by isocratic elution with flow rate 1.0 ml/min. The mobile phase composition was 125/125/250/0.5 (v/v) isopropyl alcohol, acetonitrile, water and o-phosphoric acid. The samples were detected at 215 nm using photo-diode array detector. The linear range of detection for acetylsalicylic acid, caffeine and codeine phosphate were between 0.40 and 1000, 0.25 and 250, and 0.48 and 96 μg/ml, respectively. The linearity, range, selectivity, system performance parameters, precision, accuracy, and ruggedness for acetylsalicylic acid, caffeine and codeine phosphate were also shown to have acceptable values.

Keywords: Acetylsalicylic acid; Caffeine; Codeine phosphate high-performance liquid chromatography; Validation

1. Introduction

Acetylsalicylic acid are widely used for analgesic and antipyretic tablet formulations [1]. Dosage forms of acetylsalicylic acid and its combinations with other drugs have been listed various pharmacopoeias [2,3]. Several methods are described in these pharmacopoeias and literatures for the quantitative determination of acetylsalicylic acid and its combinations with other drugs including titrimetry [3,4], colorimetry [5], fluorimetry [6], spectrophotometry [6–11], high-performance liquid chromatography [2,12–15] and GLC [16] in pharmaceutical preparations. Among the various analytical techniques, high-performance liquid chromatography (HPLC) constitutes the most popular chromatographic method for separating mixtures of drugs and their degradation products.

Most of these methods are not suitable for the determination of acetylsalicylic acid, caffeine and codeine phosphate in pharmaceutical formulations with the presence of other materials.

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0731-7085/01/$ - see front matter
PII: S0731-7085(00)00488-X

In this study, the objective was to develop and validate a specific, accurate, precise and reproducible quality control method of acetylsalicylic acid, caffeine and codeine phosphate as a drug substance and in pharmaceutical formulations. Analytical data is presented to illustrate the usefulness of the method for the determination of the three drugs together in tablet formulation.

This method can be used for the assay of acetylsalicylic acid, caffeine and codeine phosphate as a drug substance and also for the determination of them separately or in combination in tablet formulations. This method can not be developed as a stability indicating assay method but; if the exact contents of the pharmaceutical formulations are known, it is possible to confirm that it can be used as a stability indicating assay or not.

2. Experimental

2.1. Chemicals

Acetylsalicylic acid (Merck-100085), caffeine, benzoic acid (Merck-100130) were obtained from Merck Chemicals and codeine phosphate was received from United Pharmaceutical Works. Chromatographic grade-double distilled water, analytical grade *ortho*-phosphoric acid (Merck-100563), HPLC-grade acetonitrile (Merck-100030), HPLC-grade isopropyl alcohol (Carlo Erba Reagenti-415154) were used.

2.2. Apparatus

The assays were performed with a LC system consisting of a Waters model 515 solvent-delivery system and a Waters model 996 Photodiode-array detector (Milford, MA). Samples were injected with a Waters 717plus autosampler using a 20-μl sample loop. The system was controlled and data analyses were performed with the Millennium 2010 software. All the calculations concerning the quantitative analysis were performed with internal standardisation by measurement of peak areas.

The column, a μBondapak C_8 (5 μm, 250 mm × 4.6 mm I.D.; Waters, Milford, MA) was thermostatted at 25°C by waters temperature control module and waters column heater. A guard column (10 μm Bondapak C_{18} in disposable plastic inserts and Waters Guard-Pak holder) was used to safeguard the analytical column.

2.3. Stock and standard solutions

Acetylsalicylic acid (40.00 mg), caffeine (25.00 mg), codeine phosphate (9.60 mg), and benzoic acid (internal standard, 10.78 mg) were accurately weighed into a 10-ml volumetric flask and dissolved in the mobile phase and filled up to volume with the mobile phase.

2.4. Standard working solution

Standard working solutions were prepared individually in mobile phase for acetylsalicylic acid, caffeine, codeine phosphate, and benzoic acid (internal standard). Aliquots from each working solution were combined and diluted with mobile phase to obtain a standard solution containing 400 μg/ml acetylsalicylic acid, 50 μg/ml caffeine, 9.6 μg/ml codeine phosphate and 53.9 μg/ml benzoic acid. Studies on the stability of analytes in standard working solution showed that there were no decomposition products in the chromatogram and difference in area-ratios during analytical procedure and even after storing 1 week at + 4°C.

2.5. Pharmaceutical preparation

A commercial pharmaceutical preparation; DOLVIRAN® tablet Bayer-Türk Pharm. Ind., Turkey, Serial no: 9K206, containing acetylsalicylic acid, 400 mg; caffeine, 50 mg; and codeine phosphate, 9.6 mg was assayed.

3. Procedure

3.1. Chromatographic conditions

HPLC analysis was performed by isocratic elution with flow rate 1.0 ml/min. The mobile phase composition was isopropyl alcohol, acetonitrile, water and o-phosphoric acid 125/125/250/0.5 (v/

v). All solvents were filtered through a 0.45 μm milipore filter before use and degassed in an ultrasonic bath. Volumes of 10 μl each prepared solutions and samples were injected into the column. The chromatograms were recorded from 200 to 400 nm. Quantification was effected by measuring at the 215 nm as established from the three dimensional chromatogram. The chromatographic run time was 10 min and the column void volume was 1.735 min. Throughout the study, the suitability of the chromatographic system was monitored by calculating the capacity factor (k'), the resolution (R_S), the selectivity (α) and peak asymmetry (T).

3.2. Calibration

Mixed standard solutions containing acetylsalicylic acid (100–1000 μg/ml), caffeine (25–250 μg/ml), codeine phosphate (4.8–96 μg/ml), and with a fixed concentration of benzoic acid (internal standard, 53.9 μg/ml) were prepared in the mobile phase.

Triplicate 10 μl injections were made for each standard solution to see the reproducibility of the detector response at each concentration level. The peak area-ratio of each drug was plotted against the concentration to obtain the calibration graph. The five concentrations of each compound were subjected to regression analysis to calculate calibration equation and correlation coefficients.

3.3. Analysis of tablets

A total of 20 tablets (DOLVIRAN®) were accurately weighed and powdered in a mortar. Quantities of the powdered tablets equivalent to one tablet (acetylsalicylic acid, 400 mg; caffeine, 50 mg; and codeine phosphate, 9.6 mg) were accurately weighed and dissolved in 50 ml mobile phase in 100 ml calibrated flasks. After keeping 5 min in an ultrasonic bath, the solution was completed to volume and the 5 ml of solution filtered through 0.45 μm milipore filter (Solution A). Solution A was then diluted 1:10 with mobile phase and injected to chromatographic system The chromatograms at 215 nm (Fig. 1) and the three-dimensional (Fig. 2) of the chromatograms showed a complete resolution of all peaks.

4. Results and discussion

4.1. Method development

The mobile phase was chosen after several trials with methanol, isopropyl alcohol, acetonitrile, water in various proportions and at different pH values. A mobile phase consisting isopropyl alcohol, acetonitrile, water and o-phosphoric acid 125/125/250/0.5 (v/v) was selected to achieve maximum separation and sensitivity. Flow rates between 0.5 and 1.5 ml/min were studied.

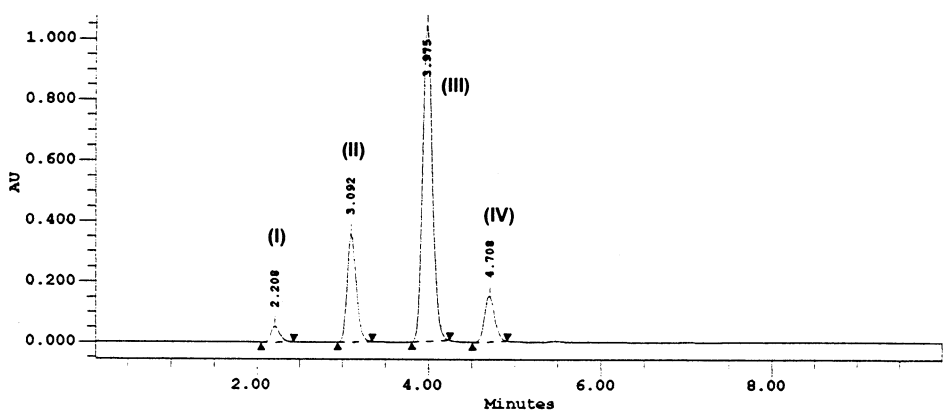

Fig. 1. Chromatogram of the mixture of codeine phosphate (I), caffeine (II), acetylsalicyclic acid (III) and benzoic acid (IV, internal standard) by developed LC method.

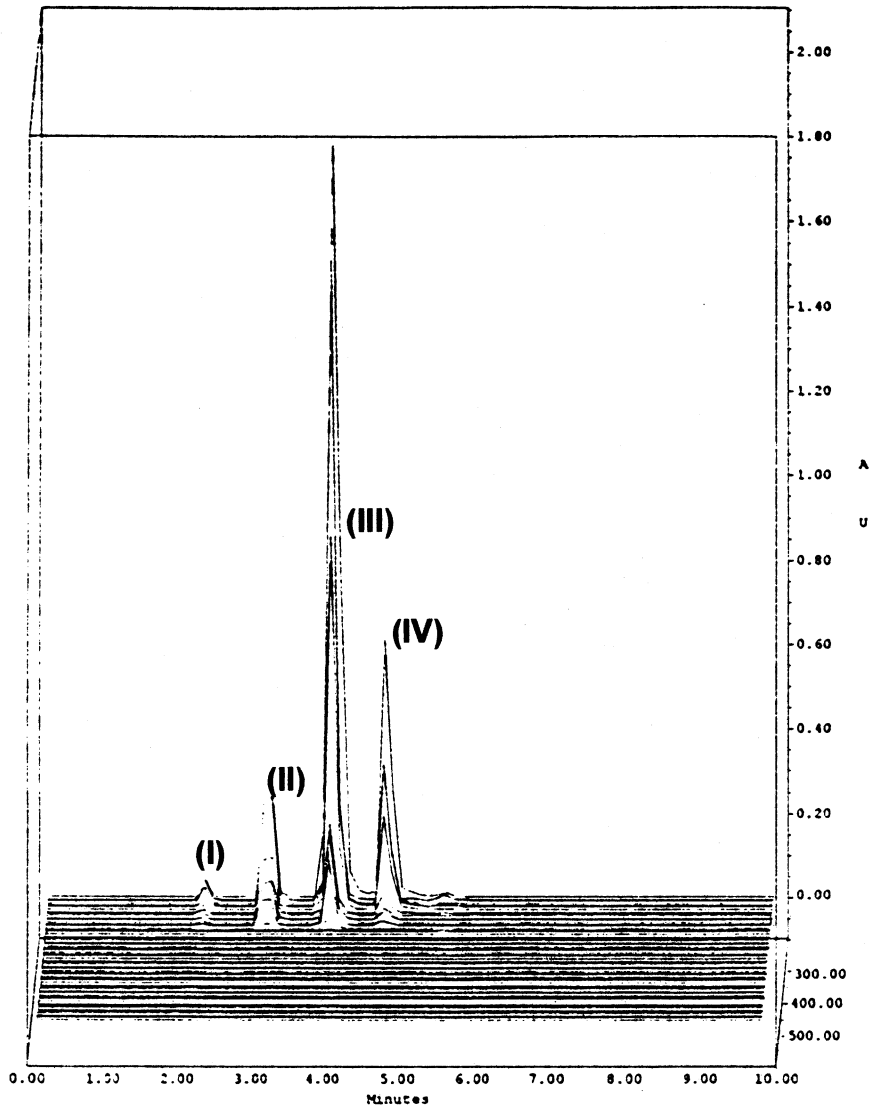

Fig. 2. Three dimensional chromatogram of the mixture of codeine phosphate (I), caffeine (II), acetylsalicyclic acid (III) and benzoic acid (IV, internal standard) by developed LC method.

A flow rate of 1.0 ml/min gave an optimal signal-to noise ratio with a reasonable separation time. Using reversed-phase C_8 column, the retention times for codeine phosphate, caffeine, acetylsalicylic acid and benzoic acid (internal standard) were observed to be 2.224, 3.114, 4.001 and 4.735 min, respectively. Total time of analysis was less than 6 min.

The maximum absorbtion of acetylsalicylic acid, caffeine and codeine phosphate together were found to be at 215 nm and this wavelength was chosen for the analysis.

4.2. Linearity

The peak-area ratios obtained for the three analyses were averaged at each concentration. Table 1 presents the equation of the regression

line, determination coefficient, RSD values of the slope and intercept for each compound. Excellent linearity was obtained for compounds between peak-area ratios and concentrations of 100–1000 μg/ml with $r^2 = 0.9993$, 25–250 μg/ml with $r^2 = 0.9986$ and 4.8–96 μg/ml with $r^2 = 0.9989$ for acetylsalicylic acid, caffeine and codeine phosphate, respectively.

4.3. Limits of detection and limits of quantification

Limits of detection (LOD) were established at a signal-to-noise ratio (S/N) of 3. The limits of quantification (LOQ) were estimated using two criteria; the first is the S/N ratio not less than 9, and the second is the percent relative standard deviation (RSD%) not more than 5% for six replicate injections of the LOQ solution. LOD and LOQ were experimentally verified by six injections of acetylsalicylic acid, caffeine, codeine phosphate at the LOD and LOQ concentrations. The limit of detection was calculated to be 0.080, 0.100 and 0.096 μg/ml and the limit of quantification was calculated to be 0.40, 0.25 and 0.48 μg/ml for acetylsalicylic acid, caffeine and codeine phosphate, respectively (Table 1).

4.4. Suitability of the method

The system performance parameters of the proposed chromatographic method were tested by injecting a standard working solution. The chromatographic parameters such as resolution, selectivity and peak asymmetry were satisfactory for these compounds (Table 2).

The calculated resolution values between each peak-pair were not less than 3.30 and the selectivity were not less than 1.30. Capacity factors (k') were found to be 0.28, 0.80, 1.31 and 1.73 for codeine phosphate, caffeine, acetylsalicylic acid and benzoic acid (internal standard), respectively. Capacity factor (k') values seem to be relatively small according to high column void volume(t_0 value was 1.735 min) but; peaks are well-resolved from other peaks and the void volume.

Table 1
Linearity results, limit of detection (LOD) and limit of quantification (LOQ)[a]

Compound	λ	Equation	R^2	Slope (RSD%)	Intercept (RSD%)	LOQ (μg/ml)	(LOD (μg/ml)
Acetylsalicylic Acid	215	$Y = 0.015512X + 0.04863$	0.9993	0.049	4.044	0.40	0.080
Caffeine	215	$Y = 0.042024X - 0.02978$	0.9986	0.270	5.606	0.25	0.100
Codeine phosphate	215	$Y = 0.030634X - 0.02942$	0.9989	0.323	2.287	0.48	0.096

[a] X, concentration (μg/ml); Y, area/area$_{\text{Internal Standard (Int. Sta.)}}$.

Table 2
System performance parameters of codeine phosphate, caffeine, acetylsalicylic acid and benzoic acid (internal standard)[a]

Compound	t_r ($n = 9$, mean)	Area ($n = 9$, mean)	k'	R	α	T
Codeine phosphate	2.224 (0.25)	379940.6 (0.45)	0.28		2.823 (1.28)	1.41
				4.653 (1.14)		
Caffeine	3.114 (0.18)	2791567.9 (0.17)	0.80		1.643 (0.39)	1.30
				4.352 (0.89)		
Acetylsalicylic acid	4.001 (0.21)	8966708.2 (0.12)	1.31		1.324 (0.09)	1.24
				3.359 (0.40)		
Benzoic acid (internal standard)	4.735 (0.17)	1405933.2 (0.14)	1.73			1.18

[a] RSD% values are given in the parenthesis.

Table 3
Precision of the developed method at the limit of quantification (LOQ) level ($n = 9$)[a]

Compound	λ	Peak area ($n = 9$, mean)	RSD%
Acetylsalicylic acid	215	79068.22	2.32
Caffeine	215	30501.66	0.45
Codeine phosphate	215	20611.55	3.03

[a] RSD% (standard deviation/mean) × 100.

4.5. Precision

The precision of the method (within-day variations of replicate determinations) was checked by injecting 9 times of acetylsalicylic acid, caffeine, codeine phosphate at the LOQ level. Table 3 gives the precision of the method, expressed as the RSD at the LOQ level were 2.32, 0.45 and 3.03% for acetylsalicylic acid, caffeine and codeine phosphate, respectively.

4.6. Accuracy

A standard working solution containing of the acetylsalicylic acid, caffeine and codeine phosphate, and benzoic acid (internal standard) to give final concentrations respectively 400, 50, 9.6, and 53.9 μg/ml was prepared. The prepared mixture of standards was injected 9 times as a test sample. From the respective area counts, the concentrations of the acetylsalicylic acid, caffeine and codeine phosphate were calculated using the detector responses. The accuracy, defined in terms of percent deviation of the calculated concentrations from the actual concentrations are listed in Table 4. The results are obtained within the acceptable range of $\pm 5\%$, the method is deemed to be accurate.

4.7. Ruggedness

The ruggedness of the HPLC method was evaluated by carrying out the analysis using standard

Table 4
Accuracy of the developed method[a]

Compound	Area/Area$_{\text{Int. Sta.}}$	Spiked concentration (μg/ml)	Measured concentration (μg/ml), mean ± S.D.	% RSD	% Deviation
Acetylsalicylic acid	6.378 ± 0.0036	400	408.01 ± 0.235	0.058	2.003
Caffeine	1.986 ± 0.0011	50	47.96 ± 0.027	0.056	4.080
Codeine phosphate	0.270 ± 0.0010	9.6	9.79 ± 0.033	0.335	1.979

[a] % Deviation = (Spiked Concentration − Mean Measured Concentration) × 100/Spiked Concentration.

Table 5
Day to day variability according to area/area$_{\text{Int. Sta.}}$[a]

	May 3, 2000			May 5, 2000			May 10, 2000		
	ASA	Caffeine	Codeine	ASA	Caffeine	Codeine	ASA	Caffeine	Codeine
Area-ratio	6.378	1.986	0.270	6.301	1.978	0.268	6.260	1.999	0.272
S.D.	0.0036	0.0011	0.0010	0.0090	0.0033	0.0005	0.0129	0.0049	0.0006
RSD%	0.06	0.06	0.37	0.15	0.17	0.19	0.21	0.25	0.25

[a] Mean values of nine determinations.

Table 6
Day to day variability according to retention time[a]

	May 3, 2000			May 5, 2000			May 10, 2000		
	ASA	Caffeine	Codeine	ASA	Caffeine	Codeine	ASA	Caffeine	Codeine
Retention	4.001	3.114	2.224	4.129	3.110	2.207	4.093	3.084	2.193
S.D.	0.0085	0.0056	0.0057	0.0048	0.0049	0.0049	0.0051	0.0050	0.0049
RSD%	0.21	0.18	0.25	0.12	0.16	0.22	0.12	0.16	0.22

[a] Mean values of nine determinations.

Table 7
Assay results of commercial product (DOLVIRAN® tablet)[a]

HPLC method for DOLVIRAN®	Acetylsalicylic acid (labelled 400 mg)	Caffeine (labelled 50 mg)	Codeine phosphate (labelled 9.6 mg)
Amount Found ± SD	402.64 ± 2.34	49.43 ± 2.35	9.74 ± 0.18
RSD%	1.80	4.75	0.58

[a] Mean values of five determinations.

working solution, same chromatographic system and the same column on different days. Small differences in area-ratios and good constancy in retention times were observed for repetitive 2 and 7 days time period (Tables 5 and 6). The RSD of less than 0.370% for areas and 0.250% for retention times were obtained. The comparable detector responses obtained on different days are indicated that the method is capable of producing results with high precision on different days.

4.8. Analysis of pharmaceutical formulations

The validity of the proposed method for pharmaceutical preparations were studied by assaying DOLVIRAN® tablet (labelled to contain acetylsalicylic acid, 400 mg; caffeine, 50 mg; and codeine phosphate, 9.6 mg) and the results were shown in Table 7. The RSD% values of the quantitative analysis in tablet formulation were found to be 1.80 for acetylsalicylic acid, 4.75 for caffeine and, 0.58 for codeine phosphate.

Recovery studies in this method were performed on the synthetic mixtures prepared by adding accurately weighed amounts of drugs (Table 8). Mean recoveries and relative standard deviations were found to be 99.54 and 2.21% for acetylsalicylic acid, 99.02 and 1.92% for caffeine, 99.16 and 2.51% for codeine phosphate in the synthetic mixtures prepared in the laboratory.

5. Conclusion

The method described is suitable for the identification and quantification of the acetylsalicylic acid, caffeine, codeine phosphate as a drug substance and in tablet formulations. HPLC with photo-diode array detection is the technique commonly used for separation and determination of compounds in finished pharmaceutical products. High percentage of recovery shows that the compounds are completely extracted from tablet formulations and free from the interference of the excipients. In conclusion, the developed HPLC method allows the quantitation of three compounds in pharmaceutical formulations using the same dilution and the same injection volume in a short analytical time.

Acknowledgements

The authors would like to thank to Mr. Tufan Çelik for his technical assistance.

Table 8
Recovery results for acetylsalicylic acid, caffeine and codeine phosphate in synthetic mixtures by HPLC

Mixture no.	Acetylsalicylic acid			Caffeine			Codeine phosphate		
	Added (μg)	Found (μg)	Recovery (%)	Added (μg)	Found (μg)	Recovery (%)	Added (μg)	Found (μg)	Recovery (%)
1	800.00	802.69	100.34	150.00	153.48	102.32	38.40	37.19	96.85
2	600.00	616.39	102.73	100.00	97.56	97.56	19.20	18.91	98.49
3	400.00	396.56	99.14	50.00	49.25	98.50	9.60	9.77	101.77
4	200.00	197.36	98.68	25.00	24.50	98.00	7.68	7.82	101.82
5	100.00	96.79	96.79	10.00	9.87	98.70	1.92	1.86	96.88
			$\bar{X} = 99.54$			$\bar{X} = 99.02$			$\bar{X} = 99.16$
			RSD = 2.21			RSD = 1.92			RSD = 2.51

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