ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
26 (2001) 857–864

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

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

Murat Kartal *

Department of Pharmacognosy, Faculty of Pharmacy, Ankara University, Tandogan, 06100, Ankara, Turkey

Received 4 April 2001; accepted 15 April 2001

Abstract

An accurate, simple, reproducible and sensitive method for the determination of paracetamol, caffeine and codeine phosphate has been developed and validated. Paracetamol, 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 420/20/30/30 (v/v/v/v) 0.01 M KH_2PO_4, methanol, acetonitrile, isopropyl alcohol and spectrophotometric detection was carried out at 215 nm. The linear range of detection for paracetamol, caffeine and codeine phosphate were between 0.400 and 1500 μg/ml; 0.075 and 90 μg/ml; 0.300 and 30 μg/ml, respectively. The method has been shown to be linear, reproducible, specific, sensitive and rugged.

Keywords: Paracetamol; Acetaminophen; Caffeine; Codeine phosphate; High-performance liquid chromatography; Validation

1. Introduction

Paracetamol (acetaminophen) is one of the most popular over-the-counter analgesic and antipyretic drugs. Paracetamol is available in different dosage forms, tablet, capsules, drops, elixirs, suspensions and suppositories. Paracetamol is generally administered as tablets containing 500 mg of active drug formulated with excipients. Dosage forms of paracetamol and its combinations with other drugs have been listed in various pharmacopoeias [1,2].

Numerous methods have been reported for the analysis of paracetamol and its combinations in pharmaceuticals or in biological fluids. Paracetamol has been determined in combination with other drugs using titrimetry [3,4], voltammetry [5], fluorimetry [6], colorimetry [6], UV-spectrophotometry [7–11], quantitative thin-layer chromatography [12], high-performance liquid chromatography [13–19] and GLC [20] in pharmaceutical preparations.

Most of these methods are used for the determination of binary paracetamol combinations like paracetamol–caffeine and paracetamol–codeine phosphate. We did not find out any LC method to determine ternary mixture of paracetamol, caffeine and codeine phosphate in our literature survey. Also, there is no an official assay method for

* Corresponding author. Fax: +90-3122131081.
E-mail address: kartal@pharmacy.ankara.edu.tr (M. Kartal).

0731-7085/01/$ - see front matter
PII: S0731-7085(01)00527-1

ternary combination of paracetamol, caffeine and codeine phosphate in the USP.

In this study, our objective was to develop and validate a specific, accurate, precise and reproducible quality control method of paracetamol, caffeine and codeine phosphate as a drug substance and their ternary combination in pharmaceutical formulations. This proposed method is highly sensitive and specific, also can be used for routine analysis of pharmaceutical formulations consisting of paracetamol, caffeine and codeine phosphate with a short preparation and analysis time. Analytical data is presented to illustrate the usefulness of the method in tablet formulations. This method can not be developed as a stability indicating assay method, because of lacking their known potential decomposition products.

2. Experimental

2.1. Chemicals

Paracetamol (acetaminophen) was used from USP reference standard (103-90-2), caffeine was obtained from Merck Chemicals (Merck-2584) and codeine phosphate was received from United Pharmaceutical Works. Chromatographic grade-double distilled water, analytical grade K_2HPO_4 (Merck-104871), HPLC-grade acetonitrile (Merck-100030), methanol (Riedel-de Haen-34860) and isopropyl alcohol (Carlo Erba Reagenti-415154) were used.

2.2. Apparatus

The method development was performed with a LC system consisting of a Waters model 515 solvent-delivery system, a Waters model 996 Photodiode-array detector (MILFORD, MA, USA) and a Waters 717plus autosampler using a 20 μl sample loop. The system was controlled and data analyses were performed with the Millennium 2010 software. The assays were performed with another LC system consisted of a JASCO model PU-980 pump and JASCO UV-975 UV/VIS detector. Samples were injected with a 7725 Rheodyne injector system with a 20 μl sample loop. The detector was set at 215 nm (0.02 a.u.f.s) and peak areas were integrated automatically by computer using Borwin software programme.

Separation was carried out at ambient temperature using a μBondapak C_8 (5 μm, 250 × 4.6 mm i.d.; Waters, Milford, MA, USA) column. A guard column (10 μm Bondapak C_{18} in disposable plastic inserts and Waters Guard-Pak holder) was used to safeguard the analytical column. All the calculations concerning the quantitative analysis were performed with external standardization by measurement of peak areas.

2.3. Stock and standard solutions

Paracetamol (50.00 mg), caffeine (15.00 mg), and codeine phosphate (10.00 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 paracetamol, caffeine, and codeine phosphate. Aliquots from each working solution were combined and diluted with mobile phase to yield a solution with final concentrations of 500, 30, and 10 μg/ml. Studies on the stability of analytes in standard working solution showed that there was no decomposition products in the chromatogram and difference in area-ratios during analytical procedure and even after storing for 2 days at + 4 °C.

2.5. Pharmaceutical preparation

A commercial pharmaceutical preparation; GERALGINE-K® tablet Münir Sahin Pharm. Ind, Turkey, Serial no:133/80-09, containing paracetamol, 500 mg; caffeine, 30 mg; and codeine phosphate, 10 mg.

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 0.01 M KH_2PO_4, methanol, acetonitrile and isopropyl alcohol 420/20/30/30 (v/v/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. 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), the selectivity (α) and peak asymmetry (T).

3.2. Calibration

Mixed standard solutions containing paracetamol (125–1500 μg/ml), caffeine (7.5–90 μg/ml), codeine phosphate (2.5–30 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 (GERALGINE-K®) were accurately weighed and powdered in a mortar. Quantities of the powdered tablets equivalent to one tablet (paracetamol, 500 mg; caffeine, 30 mg; and codeine phosphate, 10 mg) were accurately weighed and dissolved in 50 ml mobile phase in 100 ml calibrated flasks. After keeping for 5 min in an ultrasonic bath, the solution was completed to volume and the 5 ml of solution filtered through 0.45 μmilipore filter (Solution A). Solution A was then diluted 1:10 with mobile phase and injected to chromatographic system. The chromatogram at 215 nm showed a complete resolution of all peaks (Fig. 1).

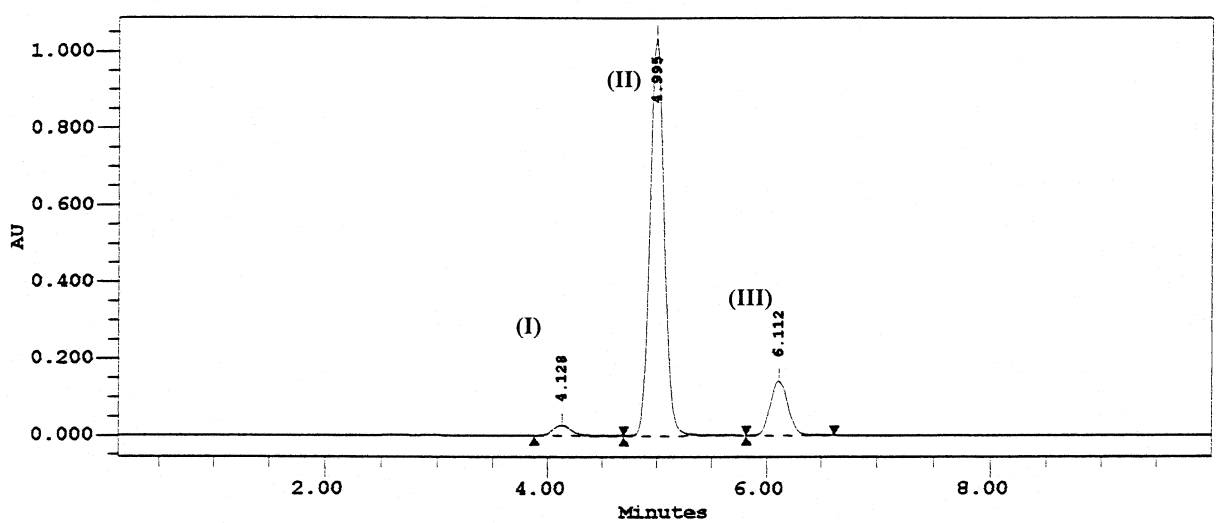

Fig. 1. Chromatogram of the codeine phosphate (I), paracetamol (II) and caffeine (III) in tablet formation (GERALGINE-K®) at 215 nm by developed LC method.

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

Compound	λ	Equation	R^2	LOQ μg/ml	LOD μg/ml
Paracetamol	215	$Y = 16\,343.87X + 1\,159\,689$	0.9935	0.400	0.150
Caffeine	215	$Y = 37\,057X - 17\,066.2$	0.9984	0.075	0.023
Codeine Phosphate	215	$Y = 34\,860.63X + 13\,306.49$	0.9981	0.300	0.100

X, concentration (μg/ml); ***Y***, area.

Table 2
System performance parameters of codeine phosphate, caffeine and paracetamol compound

	t_r ($n = 9$, mean)	Area ($n = 9$, mean)	k'	R	α	T
Codeine Phosphate	4.169 (0.24)	369 674.11 (0.19)	1.40	3.200 (0.70)	1.357 (0.27)	1.254
Paracetamol	5.038 (0.32)	9 865 317.88 (0.10)	1.90			1.167
Caffeine	6.176 (0.37)	1 054 670.50 (0.27)	2.56	4.271 (0.95)	1.344 (0.21)	1.134

R.S.D.% values are given in the parenthesis.

4. Results and discussion

4.1. Method development

The mobile phase was chosen after several trials with methanol, isopropyl alcohol, acetonitrile, water and buffer solutions in various proportions and at different pH values. A mobile phase consisting 0.01 M KH_2PO_4, methanol, acetonitrile and isopropyl alcohol 420/20/30/30 (v/v/v/v) was selected to achieve maximum separation and sensitivity.

Flow rates between 0.5 and 1.5 ml/min were studied. 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, paracetamol, and caffeine were observed to be 4.128, 4.995, and 6.112 min, respectively. Total time of analysis was less than 8 min.

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

4.2. Linearity

Table 1 presents the equation of the regression line, determination coefficient, R.S.D. values of the slope and intercept for each compound. Excellent linearity was obtained for compounds between peak-area ratios and concentrations of 125–1500 μg/ml with $r^2 = 0.9935$, 7.5–90 μg/ml with $r^2 = 0.9984$ and 2.5–30 μg/ml with $r^2 = 0.9981$ for paracetamol, caffeine and codeine phosphate, respectively.

4.3. Limits of detection and quantification

Limits of detection (LOD) were established at a signal-to-noise ratio (S/N) of 3. Limits of quantification (LOQ) were established at a signal-to-noise ratio (S/N) of 9. LOD and LOQ were experimentally verified by six injections of paracetamol, caffeine, codeine phosphate at the LOD and LOQ concentrations. The limit of detection was calculated to be 0.150, 0.023 and 0.100 μg/ml and the limit of quantification was calculated to be 0.400, 0.075 and 0.300 μg/ml for paracetamol, caffeine and codeine phosphate, respectively (Table 1).

4.4. Suitability of the method

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 3.10 and the selectivity were not less than 1.30.

Table 3
Precision of the developed method at the LOQ level ($n = 9$)

Compound	λ	Peak Area ($n = 9$, mean)	RSD%
Paracetamol	215	7099.17	2.22
Caffeine	215	2709.92	2.96
Codeine Phosphate	215	9773.5	1.39

R.S.D.%, (S.D./Mean) × 100.

Capacity factors (k') were found to be 1.40, 1.90 and 2.56 for codeine phosphate, paracetamol and caffeine, respectively.

4.5. Precision

The precision of the method (within-day variations of replicate determinations) was checked by injecting nine times of paracetamol, caffeine, codeine phosphate at the LOQ level. The precision of the method, expressed as the relative standard deviations (R.S.D.%) at the LOQ level were 2.22, 2.96 and 1.39% for paracetamol, caffeine and codeine phosphate, respectively, (Table 3).

4.6. Accuracy

A standard working solution containing of the paracetamol, caffeine and codeine phosphate, to give final concentrations, respectively, 500, 30, 10 μg/ml was prepared. The prepared mixture of standards was injected nine times as a test sample. From the respective area counts, the concentrations of the paracetamol, caffeine and codeine phosphate were calculated using the detector responses. The accuracy, defined in terms of % deviation of the calculated concentrations from the actual concentrations are listed in Table 4.

4.7. Ruggedness

The ruggedness of the HPLC method was evaluated by carrying out the analysis using standard 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 after 48 h time period. The R.S.D. of less than 0.355% for areas and 0.369% for retention times

Table 4
Accuracy of the developed method

Compound	Spiked concentration μg/ml	Measured concentration (μg/ml) mean ± S.D.	% R.S.D.	% Deviation
Paracetamol	500	532.65 ± 0.630	0.118	6.53
Caffeine	30	28.92 ± 0.076	0.262	3.60
Codeine phosphate	10	10.25 ± 0.064	0.619	2.50

%S.D. = ((Spiked concentration − mean measured concentration) × 100)/spiked concentration.

Table 5
Day to day variability according to area

	3 May 2000			5 May 2000		
	Paracetamol	Caffeine	Codeine phosphate	Paracetamol	Caffeine	Codeine phosphate
Area	9 865 317.88	1 054 670.50	369 674.11	9 826 149.11	1 052 849.92	367 301.78
S.D.	10 310.25	2812.62	710.31	11 753.04	3733.97	415.59
R.S.D.%	0.105	0.267	0.192	0.120	0.355	0.113

Mean values of nine determinations.

Table 6
Day to day variability according to retention time

	3 May 2000			5 May 2000		
	Paracetamol	Caffeine	Codeine phosphate	Paracetamol	Caffeine	Codeine phosphate
Area	5.038	6.176	4.169	5.092	6.251	4.292
S.D.	0.016	0.023	0.010	0.014	0.021	0.008
RSD%	0.323	0.369	0.237	0.282	0.343	0.177

Mean values of nine determinations.

Table 7
Assay results of commercial product (GERALGINE-K® tablet)

HPLC Method for GERALGINE-K®	Paracetamol (labelled 500 mg)	Caffeine (labelled 30 mg)	Codeine phosphate (labelled 10 mg)
Amount found ± S.D.	490.44 ± 7.55	31.95 ± 0.96	9.12 ± 0.17
RSD%	1.54	3.00	1.89

Mean values of five determinations.

were obtained (Tables 5 and 6). The comparable detector responses obtained on different days are indicated that the method is capable of producing results with high precision on different days.

Similarly, the ruggedness of the method was tested by injecting the standard working solution into a different HPLC unit. The high degrees of reproducibility of detector response and retention times indicate that the method is fairly rugged.

4.8. Analysis of pharmaceutical formulations

The validity of the proposed method for pharmaceutical preparations were studied by assaying GERALGINE-K® tablet (labelled to contain paracetamol, 500 mg; caffeine, 30 mg; and codeine phosphate, 10 mg as active substances) and the results were shown in Table 7.

Recovery studies in this method were performed on the synthetic mixtures prepared by adding accurately weighed amounts of drugs (Table 8). Mean recoveries and RSDs were found to be 101, 68 and 4.90% for paracetamol, 99.57 and 2.74% for caffeine, 99.15 and 3.95% for codeine phosphate, respectively.

5. Conclusion

HPLC methods are commonly used for separation and determination of compounds in finished pharmaceutical products. The developed method is suitable for the identification and quantification of the paracetamol, caffeine, codeine phosphate as a drug substance and in tablet formulations. High percentage of recovery shows that the compounds are completely extracted from tablet formulations and the results indicate that the developed method can be used to quantify paracetamol, caffeine and codeine phosphate in ternary combination without interference from other ingredients. In conclusion, the developed HPLC method has been successfully used on a routine basis and allows the quantitation in pharmaceutical formulations using the same dilution and the same injection volume in a short analytical time. This method is sensitive, simple, uses a fast, easy extraction procedure and possesses excellent linearity and precision characteristics. It is possible to use this method as an official method for ternary combination of paracetamol, caffeine and codeine phosphate in pharmaceutical formulations.

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

	Paracetamol			Caffeine			Codeine Phosphate		
Mixture number	Added (μg)	Found (μg)	Recovery (%)	Added (μg)	Found (μg)	Recovery (%)	Added (μg)	Found (μg)	Recovery (%)
1	1500	1452.84	96.86	90	89.14	99.04	30	29.74	99.13
2	1000	1060.92	106.09	60	62.29	103.82	20	20.70	103.50
3	750	750.50	100.07	45	44.34	98.53	15	14.43	96.20
4	500	533.11	106.62	30	28.92	96.40	10	10.250	102.50
5	250	240.49	96.20	15	15.01	100.07	2.5	2.36	94.40
			$X = 101.68$, %R.S.D. = 4.90			$X = 99.57$, %R.S.D. = 2.74			X = 99.15, %R.S.D. = 3.95

References

[1] J.E.F. Reynolds, Martindale: The Extra Pharmacopoeia, 31 edn, Pharmaceutical Press, London, 1996, pp. 27–28.
[2] The United States Pharmacopeia, 24th revision, U.S. Pharmacopeial Convention, Rockville, MD, 2000, pp. 17–39.
[3] British Pharmacopoeia CD 1998, version 2, The Stationery Office Ltd., Norwich, 1998.
[4] European Pharmacopoeia 1997, third edition, Convention on the Elaboration of a European Pharmacopoeia (European Treaty Series No.50), Strasbourg, 1996, pp. 748–749.
[5] O.V. Lau, S.F. Luk, Y.M. Cheung, Analyst 114 (1989) 1047–1051.
[6] H.A. El-Obeid, A.A. Al-Badr, Analytical Profiles of Drug Substances, Am. Pharm. Assoc. 14 (1985) 551–596.
[7] H-K. Chan, D.J.W. Grant, Int. J. Pharm. 57 (1989) 117–124.
[8] N. Erk, F. Onur, Anal. Lett. 30 (6) (1997) 1201–1210.
[9] M.C. Pascual-Marti, M. Llobat-Estelles, M.I. Roig-Marco, Pharmazie 55 (5) (2000) 362–363.
[10] H.N. Doğan, Pharmazie 51 (10) (1996) 773–774.
[11] O. Atay, F. Dinçol, FABAD J. Pharm. Sci. 20 (1995) 13–19.
[12] P. Kahela, E. Laine, M. Anttila, Drug Dev. Ind. Pharm. 13 (1987) 213–224.
[13] S. Süzen, C. Akay, S. Tartilmis, R.S. Erdöl, A. Önal, S. Cevheroglu, J. Faculty of Pharm. Ankara Uni. 27 (2) (1998) 93–100.
[14] W.R. Sisco, C.T. Rittenhouse, L.A. Everhart, A.M. McLaughlin, J. Chromatogr. 354 (1986) 355–366.
[15] M. Hossain, J.W. Ayres, Int. J. Pharm. 133 (1996) 223–235.
[16] L. Carnevale, J. Pharm. Sci. 72 (2) (1983) 196–198.
[17] R. Thomis, E. Roets, J. Hoogmartens, J. Pharm. Sci. 73 (12) (1984) 1830–1833.
[18] V.D. Gupta, J.T. Jacob, Drug Dev. Ind. Pharm. 13 (1) (1987) 113–126.
[19] H.M. Ali, M.M.A. Homeida, J. Ford, C.A. Truman, C.J.C. Roberts, A.A. Badwan, Int. J. Pharm. 42 (1988) 155–159.
[20] J.J. Bergh, A.P. Lötter, Drug Dev. Ind. Pharm. 10 (1) (1984) 127–136.