ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
24 (2001) 1169–1173

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Short communication

Quantitative analysis of analgoantipyretics in dosage form using planar chromatography

J.T. Franeta, D.D. Agbaba, S.M. Eric *, S.P. Pavkov, S.D. Vladimirov, M.B. Aleksic

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, PO Box 146, Vojvode Stepe 450, 11000 Belgrade, Serbia, Yugoslavia

Received 16 May 2000; received in revised form 23 October 2000; accepted 26 October 2000

Abstract

In the therapy of pain of weaker genesis, frequently used drugs usually represent a mix of analgoantipyretics of different chemical structures, mostly derivatives of salicylic acid, pyrazolone and *p*-aminophenol as well as derivatives of propionic and acetylsalicylic acid. For the determination of these drugs, different chromatographic methods have been applied, mostly HPLC, due to the the lower polarity (pyrazolones derivatives) and thermolability, as well as nonvolatility of compounds investigated. TLC method, considering advantages which include simplicity, reasonable sensitivity, rapidity, excellent resolving power and low cost has been successfully explored for the determination of analgoantipyretic compounds.

The aim of this work was to develop a simple and rapid HPTLC method for the determination of acetylsalicylic acid, paracetamol, caffeine and phenobarbitone in dosage form. The determination of analgoantipyretics were performed on pre-coated HPTLC silica gel plates (10×20 cm^2) by development in the mobile phase dichlormethane–ethyl acetate–cyclohexane–isopropanol–0.1 M HCL–formic acid (9:8:3:1.5:0.2:0.2 v/v/v/v/v/v). Migration distances ($68.6 + 0.2$ mm, $54.1 + 0.1$ mm, $36.4 + 0.14$ mm and $85.9 + 0.11$ mm for acetylsalicylic acid, paracetamol, caffeine and phenobarbitone, respectively) with low RSD values (0.13–0.39%) showed a satisfactory reproductivity of the chromatographic system. TLC scanner was used for direct evaluation of the chromatograms in the reflectance/absorbance mode. Established calibration curves ($r > 0.999$), precision (0.3–1.02%) and detection limits, as well as recovery values (96.51–98.1%) were validated and found to be satisfactory. The method was found to be reproducible and convenient for the quantitative analysis of compounds investigated in their dosage forms.

1. Introduction

Acetylsalicylic acid, paracetamol, caffeine and phenobarbitone belong to the group of analgoan-

* Corresponding author.

0731-7085/01/$ - see front matter
PII: S0731-7085(00)00579-3

tipyretics of different chemical structures. They are usually used as different mixes in the therapy of pain of weaker genesis.

For the determination of acetylsalicylic acid, paracetamol, caffeine and phenobarbitone several instrumental methods have been applied. They include spectrophotometric [1], second derivative spectrophotometry [2], HPLC [3–7], and TLC techniques [8]. These drugs were also determined by IR spectroscopy [9] and capillary chromatography [10,11].

A conventional TLC have been used for the separation and the determination of analgoantipyretic substances in different mixtures including caffeine and acetylsalicylic acid [12], caffeine, phenacetin and acetylsalicylic acid [13], aminopyrine, caffeine, phenacetin and phenobarbital [14]. Further investigations are including HPTLC being widely used for determination of different mixtures, such as acetylsalicylic acid, acetaminophenon and caffeine [15], acetylsalicylic acid, caffeine and phenacetin [16], phenobarbitone and caffeine [17], paracetamol and mefenamic acid [18] and acetylsalicylic acid, paracetamol, caffeine, chloracetanilide and dehydroascorbic acid [19].

There are no data containing the simultaneous determination of acetylsalicylic acid, paracetamol, caffeine and phenobarbitone in mixtures.

Since instrumental planar chromatography is regarded as a reliable, fast, and accurate method for quantitative drug analysis, this work have been concentrated on HPTLC as a technique which can be used as an alternative method of drug assay.

2. Experimental

2.1. Apparatus

A TLC Scanner II with a computer system and Cats Software (V.3.15) were provided by Camag, Muttanz, Switzerland. The radiation source was a deuterium lamp. Nanomat III was used as an application device (Camag, Muttenz, Switzerland).

2.2. Reagents and materials

HPTLC plates (Silica gel 60 F_{254} 10×20 cm, Merck, Darmstadt, Germany) were used without any pretreatment. The mobile phase was dichlormethan–ethyl acetate–cyclohexane–isopropanole–0.1 M HCL–acidum fumaricum 98% (9:8:3:1.5:0.2:0.2 v/v/v/v/v/v). All chemicals and solvents were of analytical grade. Acetylsalicylic acid {*N*-[Acetylamino)carbonyl]-α-ethylbenzen acetamide}, Paracetamol {*N*-Aethyl-*p*-aminophenol}, Caffeine {3,7-Dihydro-1,3,7-trimethyl-1H-purine-2,6-dione} and Phenobarbitone {5-Ethyl-5-phenyl-2,4,6 (1H, 3H, 5H)-pyrimidinetrione} were obtained from Sigma, USA. Malophenum® tablets containing 250 mg of acetylsalicylic acid, 25 mg of paracetamol, 50 mg of caffeine and 20 mg of phenobarbitone were obtained from Galen Laboratory "Novi Sad", Novi Sad.

2.3. Standard solutions

Stock solution containing 25 mg/ml of acetylsalicylic acid and paracetamol, 5 mg/ml of caffeine and 2 mg/ml of phenobarbitone in ethanol were freshly prepared before use. Calibration solutions were prepared by diluting the stock solution such that application of 1 μl aliquots gave series of spots covering the ranges 200–1000 ng of acetylsalicylic acid and paracetamol, 40–200 ng of caffeine and 400–2000 ng of phenobarbitone.

For an assay, 250 mg of acetylsalicylic acid and paracetamol, 50 mg of caffeine and 20 mg of phenobarbitone were transferred to the 25 ml calibrated flask and dissolved with ethanol (standard solution A). An aliquot of 1 μl of solution A was spotted on the plate for the analysis of phenobarbitone.

2.5 ml of the standard solution A was then transferred to 50 ml calibrated flask and dissolved with ethanol to the mark (standard solution B). Aliquots of 1 μl of standard solution B were spotted on the plate for the assay of acetylsalicylic acid, paracetamol and caffeine.

2.4. Sample solution

A quantity of Malophenum® tablet containing 250 mg of acetylsalicylic acid, 250 mg of paracetamol, 50 mg of caffeine and 20 mg of phenobarbitone was transferred to 25 ml calibrated flask, dissolved up to the mark with ethanol and filtered (sample solution A). An aliquot of 1 μl of sample solution A was spotted on the plate for the assay of phenobarbitone.

2.5 ml of sample solution A was then transferred to 50 ml calibrated flask and dissolved to the mark with ethanol (sample solution B). Aliquots of 1 μl of sample solution B were spotted on the plate for the assay of acetylsalicylic acid, paracetamol and caffeine.

2.5. Chromatography

Certain aliquots loading of standard and sample solution were spotted on the HPTLC plate by means of Nanomat III. The chromatogram was allowed to develop to a height of about 90 mm in a chamber previously saturated with the mobile phase. The measurement of each spot was carried out in situ at 207 nm using absorbance/reflectance mode.

3. Results and discussion

The chromatograms of acetylsalicylic acid, paracetamol, caffeine and phenobarbitone are shown in Fig. 1. A mixture dichlormetane–ethyl acetate–cyclohexane–isopropanole–0.1 M HCl – acidum fumaricum (9:8:3:1.5:0.2:0.2 v/v/v/v/v/v) was used as a mobile phase. The migration distances ($\pm$SD) were 68.6 ± 0.2 mm, 54.1 ± 0.1 mm, 36.4 ± 0.14 mm and 85.9 ± 0.11 mm for acetylsalicylic acid, paracetamol, caffeine and phenobarbitone, respectively, which provided accurate densitometric reading.

The proposed mobile phase provided the separation of salicylic acid (peak 5, Fig. 1), and therefore could be used for the determination of salicylic acid as impurity and investigation of stability of the preparations containing acetylsalicylic acid. The statistical data for calibration curves of each compound are given in Table 1.

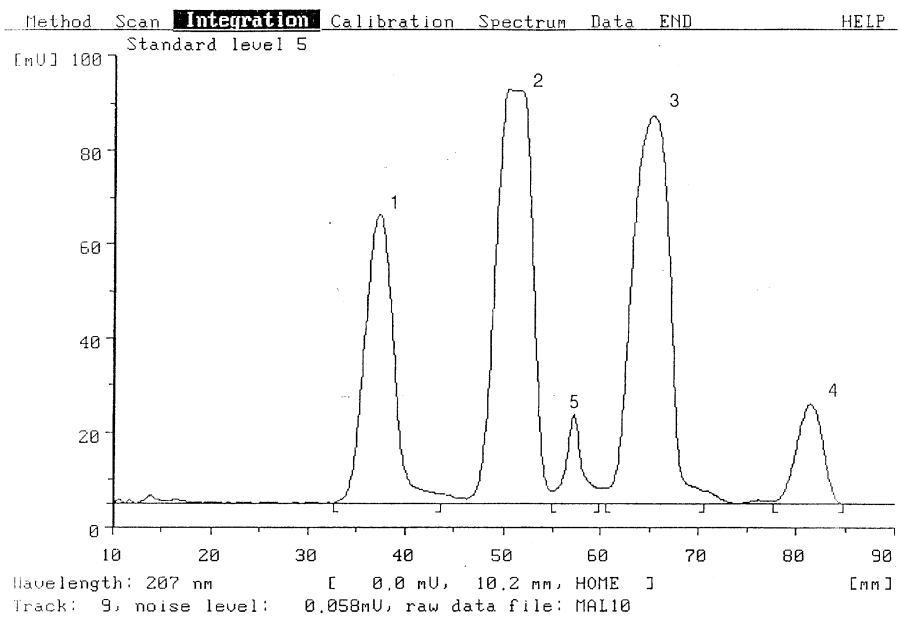

Fig. 1. Typical chromatogram of (1) acetylsalicylic acid, (2) paracetamol, (3) caffeine, (4) phenobarbitone and (5) salicylic acid obtained by HPTLC method.

Table 1
Statistical data for calibration curves and detection limits of acetylsalicylic acid, paracetamol, caffeine and phenobarbitone (Calibration function $y = a + bx$)

Compound	Range (ng)	n	Area (mean)	Residues	a	b	r
Acetylsalicylic acid	200	10	185	8.3871	779.41	20.73	0.9992
	400	10	332	−0.4946			
	500	10	405	−5.4355			
	700	10	554	−12.3172			
	1000	10	810	9.8602			
Paracetamol	200	10	595	21.4839	2544.68	64.58	0.9995
	400	10	1052	−30.4517			
	500	10	1351	14.0807			
	700	10	1826	−19.8549			
	1000	10	2624	14.7419			
Caffeine	40	10	392	3.4839	8648.39	42.58	0.9993
	80	10	741	6.5484			
	100	10	914	6.5806			
	140	10	1220	−33.3549			
	200	10	1789	16.7419			
Phenobarbitone	400	10	583	26.3077	1216.24	77.215	0.9993
	800	10	1031	−19.5385			
	1000	10	1261	−36.461			
	1400	10	1821	29.6923			
	2000	10	2501	22.2654			

The calibration plots (peak area against amount of substance applied) over the ranges tested were determined by linear regression; the correlation coefficients were $r > 0.999$. The limit of detection (LOD) for salicylic acid was determined by fitting the inter-day, back-calculated standard deviations of each calibration standard. The y-intercept was then equal to S_0, (the estimated standard deviation at zero concentration). The LOD was defined at $3S_0$. The LOD and LOQ for salicylic acid were found to be 2.15 and 7.15 mg, respectively (equivalent to impurity levels of 0.0007–0.00023%).

The suitability of HPTLC method for quantitative determination of compounds was further approved through next validation specifications: precision, accuracy and repeatability. The precision of the method was determined by running replicate samples of three concentrations of each compound; the relative standard deviations were 0.58–0.95% for acetylsalicylic acid, 0.48–0.63% for paracetamol, 0.33–1.02% for caffeine and 0.30–1.01% for phenobarbitone (Table 2).

The accuracy of the method was proven by determination of the drugs in laboratory-prepared dosage formulations containing defined quantities of the active substance. The recoveries obtained were 98.50%, 98.35%, 99.14% and 99.02% for acetylsalicylic acid, paracetamol, caffeine and phenobarbitone, respectively.

The repeatability of the analytical system was determined using five sample concentration of each compound within the calibration curves. RSD values were 0.98–2.49% for acetylsalicylic acid, 0.38–1.17% for paracetamol, 0.66–1.47% for caffeine and 0.21–1.12% for phenobarbitone and found to be satisfactory. The proposed method was applied to the determination of compounds investigated in commercial pharmaceutical dosage form. The results of quantitative determination of acetylsalicylic acid, paracetamol, caffeine and phenobarbitone in Malophenum® tablets are presented in Table 3.

The repetition of the whole procedure, including sample-preparation of tablets, was performed four times, and given RSD values obtained from

Table 2
Precision of HPTLC determination of acetylsalicylic acid, paracetamol, caffeine and phenobarbitone

Compound	Amount per spot (ng)	*n*	Peak area	RSD (%)
Acetylsalicylic acid	200	10	211.9	0.95
	500	7	441.8	0.58
	1000	5	833.0	0.24
Paracetamol	200	10	594.8	0.48
	500	7	1383.2	0.58
	1000	5	2624.2	0.63
Caffeine	40	10	411.8	1.02
	100	7	940.4	0.76
	160	5	1237.2	0.33
Phenobarbitone	400	10	572.6	0.75
	1000	7	1284.3	0.30
	1400	5	1795.4	1.21

Table 3
HPTLC determination of acetylsalicylic acid, paracetamol, caffeine and phenobarbitone in Malophenum® tabletes

Compound	Taken (mg)	*n*	Found		SD	RSD (%)
			mg	(%)		
Acetylsalicylic acid	250	9	243.7	97.48	2.47	1.01
Paracetamol	250	9	245.26	98.10	3.45	1.41
Caffeine	50	9	49.07	98.14	0.54	1.10
Phenobarbitone	20	9	19.30	96.50	0.24	1.26

nine measurements (Table 3) represent the mean value of procedures and found to be satisfactory. To conclude, the method is simple, reproducible, accurate and can be used as a more effective alternative to other chromatographic techniques.

References

[1] B.W. Glombitza, P.C. Schmidt, J. Pharm. Sci. 83 (1994) 751–757.
[2] Z. Kokot, K. Burda, J. Pharm. Biomed. Anal. 18 (1998) 871–875.
[3] A.M. Di-Pietra, R. Gatti, V. Andrisano, V. Cavrini, J. Chromatogr. 729 (1996) 355–361.
[4] C. Barbas, A. Garcia, L. Saavedera, M. Castro, J. Chromatogr. A 870 (2000) 97–103.
[5] Y.R. Ku, K.C. Wen, L.K. Ho, Y.S. Chang, J. Pharm. Biomed. Anal. 20 (1999) 351–356.
[6] E. Dinc, J. Pharm. Biomed. Anal. 21 (1999) 723–730.
[7] G. Indrayanto, A. Sunarto, Y. Adriani, J. Pharm. Biomed. Anal. 13 (1995) 1555–1559.
[8] V.M. Shinde, N.M. Tendolkar, B.S. Desai, J. Planar Chromatogr. 7 (1994) 50–53.
[9] Z. Bouhsain, S. Garrignes, M. de-la-Guardia, Fresenius – J. Anal. Chem. 357 (1997) 973–976.
[10] S. Booukerd, M. Lauwers, M.R. Detaevdernier, Y. Michote, J. Chromatogr. 695 (1995) 97–102.
[11] K.J. Lee, G.S. Heo, N.J. Kim, D.C. Moon, J. Chromatogr. 608 (1992) 243–250.
[12] J.W. Elder, J. Chem. Educ. 72 (1995) 1049.
[13] Q. Zhang, L. Wu, A. Liu, J. Chin. Chromatogr. 8 (1990) 193–194.
[14] D. Guo, Chin. J. Pharm. Anal. 5 (1985) 306–307.
[15] J. Sherma, C.D. Rolfe, J. Planar Chromatogr. 5 (1992) 197–199.
[16] J. Sherma, S. Stellmacher, T.J. White, J. Liquid Chromatogr. 8 (1985) 2961–2967.
[17] V.W. Kamble, M.V. Garad, V.G. Dongre, J. Planar Chromatogr. 9 (1996) 280–281.
[18] A.P. Argekar, J.G. Sawant, J. Planar Chromatogr. 12 (1999) 361–364.
[19] J. Krzek, M. Starek, J. Planar Chromatogr. 12 (1999) 356–360.