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HPLC assay of acetylsalicylic acid, paracetamol, caffeine and phenobarbital in tablets

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Abstract

This paper present a HPLC method for simultaneous determination of acetylsalicylic acid, paracetamol, caffeine and phenobarbital in tablets, using chromatographic system consisting a Bio Rad 18 01 solvent pump, Rheodine 71 25 injector and Bio Rad 18 01 UV–Vis Detector. Separation was achieved using Bio SiL HL C18, 5 μm, 250 × 4.6 mm column. Mixture of acetonitrile–water (25:75 v/v) adjusted to pH 2.5 with phosphoric acid was used as a mobile phase at a flow rate of 2.0 ml min^{-1}. UV detection was at 207 nm range 0.01 AUFS. Under the same conditions it was possible to determine the level of salicylic acid. The chromatographic parameters such as retention times, capacity factor, peak asymmetry, selectivity factor and resolution factor was determined. The validation parameters: linearity ($r > 0.998$), intra-day precision (RSD: 0.36–1.89%) and inter-day precision (RSD: 0.58–2.18%), sensitivity (LOD: 9×10^{-5}–1.7×10^{-4} mg ml^{-1} and LOQ: 2.5×10^{-4}–5.6×10^{-4} mg ml^{-1}), accuracy (recoveries: 98.35–99.14%) and reproducibility (recovery values: 98.74–102.08% for acetylsalicylic acid, 99.93–102.11% for paracetamol, 98.25–102.12% for caffeine and 98.15–102.3% for phenobarbital) (RSD: 1.21–1.85%) were found to be satisfactory. The proposed HPLC method has been applied for the determination of acetylsalicylic acid, paracetamol, caffeine and phenobarbital in Malophenum tablets. The obtained RSD values were within 0.99–1.21%. The developed method is rapid and sensitive and therefore suitable for routine control of these drugs in dosage form.

Keywords: HPLC; Acetylsalicylic acid; Paracetamol; Caffeine; Phenobarbital

1. Introduction

Multidrug pharmaceutical preparations for the therapy of pain of weaker genesis contain the different components, usually acetylsalicylic acid and paracetamol with caffeine, codeine, derivates of pyrazolones, barbiturates, vitamins, phenacetine, pentazocine which can improve the pharmacological value of these preparations. Concerning the different mechanism of action, they sometimes act as synergists which lead to a better efficiency. Since each component in the multicomponent preparation is in the fewer amounts than in the monocomponent of each one, the main metabolic organs are less loaded since any components engages the different subsystem of metabolism.

For the assay of acetylsalicylic acid, paracetamol, caffeine and phenobarbital in the mixtures, the different methods have been reported, including spectrophotometry [1], second derivative spectrophotometry [2], planar chromatography [3–5], IR spectroscopy [6] and capillary chromatography [7,8].

Considering the properties of the compounds investigated, such as mid polarity, as well as thermolability and low volatility, HPLC methods have been the most explored. They include an assay of acetylsalicylic acid, acetaminophen and ascorbic acid [9], acetylsalicylic acid, paracetamol, propyphenazone, caffeine and chlorpheniramine [10], caffeine, paracetamol, phenylpropanolamine hydrochloride, glycerilquaiacolate and chlorpheniramine [11], caffeine, 8-chlorotheophylline and diphenhydramine [12], paracetamol, pseudoephe-

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drine hydrochloride and triprolidine hydrochloride [13], paracetamol and caffeine [14], phenobarbital, phenitoin and theophylline [15] and phenobarbital, khellin and dipyrone [16].

The determinations of these drugs have been mainly performed with the fluorescent detection after postcolumn derivatisation, due to the different UV absorption characteristics of compounds, which lead to a better sensitivity of an assay.

In our previous work, we have developed the HPTLC determination of acetylsalicylic acid, paracetamol, caffeine and phenobarbital, with UV detection of corresponding spots, which provided satisfactory results [17]. Despite many reports describing the determination of analgetics, there are no data containing determination of these compounds mixed in multicomponent dosage form by an isocratic HPLC method without derivatization.

The present paper describes a sensitive and simple RP-HPLC method with UV detection without prior derivatization for direct simultaneous determination of acetylsalicylic acid, caffeine, paracetamol and phenobarbital in tablet form. A compromise wavelength for compounds investigated was 207 nm, found to be satisfactory for use of the proposed method in the routine control of multi drug pharmaceutical preparation.

2. Experimental

2.1. Chemicals

All chemicals and solvents were of analytical reagent grade. Acetonitrile and H_3PO_3 were obtained from Merck, Darmstadt, Germany. Deinoized and distilled water was used.

Standards for acetylsalicylic acid [2-(acetyloxy)benzoic acid], paracetamol [*N*-(4-hydroxyphenyl)acetamide], caffeine [3,7-dihydro-1,3,7-trimethyl-1*H*-purine-2,6-dione] and phenobarbital [5-ethyl-5-phenyl-2,4,6(1*H*,3*H*,5*H*)pyrimidinetrione] were obtained from Sigma, USA.

Malophenum tablets were obtained from 'Galen laboratory', Novi Sad.

2.2. Chromatographic equipment and conditions

HPLC analysis was performed on a chromatographic system equipped with a Bio Rad 18 01 solvent pump, Rheodine 71 25 injector, and Bio Rad 18 01 UV–Vis detector. A Value Chrom Chromatography Software data system was used to collect, integrate and analyse the chromatographic data. UV detection was carried out with set to 207 nm, at a 0.01 AUFS range.

Bio SiL HL C18, 5 μm, 250 × 4.6 mm column was used for the separation of these compounds.

2.3. Mobile phase

For the analysis, the mobile phase consisted of MeCN–water (25:75 v/v) adjusted to pH 2.5 with H_3PO_3 was used, at a flow rate of 2.0 ml min^{-1}.

2.4. Standard preparations

The stock solution containing 62.5 mg ml^{-1} of acetylsalicylic acid and paracetamol, 12.5 mg ml^{-1} of caffeine and 5 mg ml^{-1} of phenobarbital in mobile phase was used. The calibration curves were prepared by diluting the stock solution in the mobile phase to furnish solutions with final concentrations of 0.05, 0.1, 0.2, 0.25, 0.3, 0.35, and 0.4 mg ml^{-1} for acetylsalicylic acid and paracetamol, 0.01, 0.02, 0.04, 0.05, 0.06, 0.07 and 0.08 mg ml^{-1} for caffeine and 0.004, 0.008, 0.016, 0.02, 0.024, 0.028 and 0.032 mg ml^{-1} for phenobarbital.

2.5. Sample preparation

The mean weight of finely powdered Malophenum tablet containing 250 mg of acetylsalicylic acid and paracetamol, 50 mg of caffeine and 20 mg of phenobarbital was accurately transferred into 100 ml calibrated flask and 80 ml of mobile phase was added; the mixture was extracted in the ultrasonic bath for 10 min at room temperature and diluted with mobile phase to the mark. The supernatant liquid was filtered through Anotop 25, 0.02 μm filter. One millilitre of this solution was transferred to the 10 ml calibrated flask and diluted with mobile phase to the mark.

3. Results and discussion

The procedure for the simultaneous analysis of acetylsalicylic acid, paracetamol, caffeine and phenobarbital using isocratic HPLC method has been reported.

In order to avoid derivatization of compounds, our goal was to develop a simple HPLC assay to be used in routine control of these drugs in Malophenum tablets. Therefore, this work was focused on optimization of the conditions for the simple and rapid, as well as low cost and no time consuming analysis, including a selection of the proper column or mobile phase to obtain satisfactory results.

To obtain satisfactory resolution and to avoid peak tailing of compounds, an optimization of the proposed method was carried out using the different mobile phases. The use of mobile phase acetonitrile–0.05 M phosphate buffer (pH 2.5, adjusted with HCl) gave

asymmetrical peaks with tailing. Mobile phases of various compositions of acetonitrile and water were also tested. Using the system acetonitrile–water 40:60 v/v (pH 2.5 adjusted with phosphoric acid) the peaks of phenobarbital and acetylsalicylic acid were not well separated. With an increase of polarity of mobile phase, all the compounds were well separated reducing peak tailing. The best results were obtained using the mobile phase acetonitrile–water 25:75 v/v (pH 2.5 adjusted with phosphoric acid).

The most reproductive results were obtained with octadecil stacionary phase (Bio SiL HL C18, 5 μm, 250 × 4.6 mm column) on the chromatographic system consisting Bio Rad 2800 solvent pump and Bio Rad 1801 UV–Vis Detector.

The detection was performed at 207 nm in sensitivity range 0.01 AUFS. To find optimal detection conditions, the different analytical wavelength corresponding to UV maximums of acetylsalicylic acid (228 nm and 276 nm), paracetamol (240 nm), caffeine (273 nm) and phenobarbital (254 nm) were tested, no UV detection to be satisfactory for all compounds in mixture was provided.

After optimization, HPLC method was carried out on Bio SiL HL C18, 5 μm, 250 × 4.6 mm column using acetonitrile–water (25:75 v/v) adjusted to pH 2.5 with phosphoric acid as mobile phase at a flow rate of 2.0 ml min^{-1}.

Typical chromatograms obtained are illustrated in Fig. 1.

The retention times were 7.78 min for acetylsalicylic acid, 4.86 min for caffeine, 2.97 min for paracetamole, 9.89 min for phenobarbital and 11.66 min for salicylic acid.

System suitability tests were performed and chromatographic parameters calculated from experimental data, such as capacity factor (k'), peak asymmetry

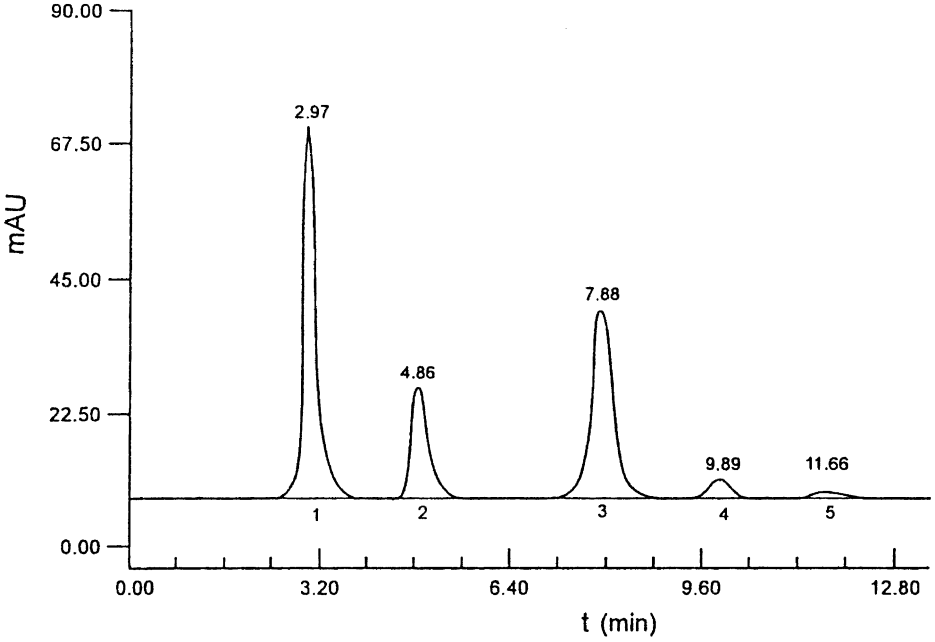

Fig. 1. The chromatograms of acetylsalicylic acid (t_R −7.88), paracetamol (t_R −2.97), caffeine (t_R −4.86), phenobarbital (t_R −9.89) and salicylic acid (t_R −11.66) obtained by HPLC (column: Bio SiL HL C18, 5 μm, 250 × 4.6 mm; mobile phase–acetonitrile: water (25:75 v/v) adjusted to pH 2.5 with phosphoric acid).

Table 1
Chromatographic parameters obtained in HPLC assay of analgoantipyretics

Component	Capacity factor (k')	Peak asymmetry (A)	Selectivity factor (α)	Resolution factor (Rs)
Acetylsalicylic acid	7.78	0.28		
Caffeine	2.97	0.22		
Paracetamol	4.86	0.16		
Phenobarbital	9.89	0.26		
Caffeine–paracetamol			1.64	3.33
Acetylsalicylic acid–caffeine			1.60	4.80
Phenobarbital–acetylsalicylic acid			1.27	3.64
Salicylic acid–phenobarbital			1.18	3.65

Table 2
Precision of HPLC method

Comp.	Concentrations (mg ml^{-1})	n	Intra-day precision		Inter-day precision	
			Peak area	RSD (%)	Peak area	RSD (%)
Acetylsalicylic acid	0.2	10	11.746311	1.31	11.982756	1.78
	0.25	10	15.468241	1.48	16.247892	2.05
	0.3	10	19.363271	0.93	19.012542	1.22
Paracetamol	0.2	10	16.606869	1.09	16.789541	1.15
	0.25	10	20.497361	0.79	20.541478	1.05
	0.3	10	24.382964	0.61	25.124571	0.88
Caffeine	0.04	10	6.249716	1.52	7.157843	1.78
	0.05	10	7.680291	1.89	7.958745	2.18
	0.06	10	9.499796	0.36	9.845698	0.58
Phenobarbital	0.016	10	1.450659	0.58	1.558985	1.12
	0.02	10	1.813707	1.05	1.651273	1.75
	0.024	10	2.205103	0.66	2.254789	1.47

factor (A), selectivity factor (α) and resolution factor (Rs) are given in Table 1. The capacity factor, calculated as a relation of the time of injection of sample to column ($1 < k' < 10$), selectivity factor ($\alpha > 1$) and resolution factor (Rs > 1) were found to be satisfactory. The values obtained for peak asimmetry were significantly lower than theoretical values ($1 < A < 1.2$).

The validity of the liquid chromatographic assay was established through a study of linearity, sensitivity, intra-day and inter-day precision, accuracy and reproducibility.

The linearity was established with a series of working solutions prepared by diluting the stock solution with mobile phase to the final concentrations. Each concentration was injected in triplicate and the mean value of peak area was taken for the calibration curve. A linear response in peak area ratios was observed over the concentration range 0.05–0.4 mg ml^{-1} for acetylsalicylic acid, 0.05–0.4 mg ml^{-1} for paracetamol, 0.01–0.08 mg ml^{-1} for caffeine and 0.004–0.032 mg ml^{-1} for phenobarbital. The mean of five different calibration graphs yielded the following equations: $y = 63363900x - 318110$ ($r = 0.9993$) for acetylsalicylic acid, $y = 78436100x + 904175$ ($r = 0.9994$) for paracetamol, $y = 155666000x - 29951$ ($r = 0.9993$) for caffeine and $y = 894999000x + 10982$ ($r = 0.9987$) for phenobarbital.

The limits of detection (LOD) and quantification (LOQ) were determined by fitting interday back-calculated standard deviations of each calibration standard. The LOD is defined as the lowest determinable quantity that indicates the presence of an analyte at a given statistical level of confidence (3 SD), and the LOQ is defined as the lowest measured quantity above which the analyte can be quantified at a given statistical level of confidence (10 SD).

The limits of detection, calculated statistically, were found to be 7.5×10^{-4} mg ml^{-1} for acetylsalicylic acid and phenobarbital, 1.7×10^{-4} mg ml^{-1} for caffeine and 9×10^{-5} mg ml^{-1} for paracetamol.

The experimental limit of detection for acetylsalicylic acid and paracetamol was found to be lower than calculated one (1.0×10^{-5} mg ml^{-1}).

The limits of quantification were found to be 2.5×10^{-4} mg ml^{-1} for acetylsalicylic acid and phenobarbital, 2.9×10^{-4} mg ml^{-1} for paracetamol and 5.6×10^{-4} mg ml^{-1} for caffeine.

The experimental limit of quantification for acetylsalicylic acid and paracetamol was found to be lower (5×10^{-5} mg ml^{-1}).

The intra-day precision of the method was determined by preparing the standards of acetylsalicylic acid, paracetamol, caffeine and phenobarbital at three different concentrations and values for each compound were determined by 10 repeated analyses. Inter-day precision was checked with the same concentrations as intra-day assay, and the determination of each compound was repeated day by day during 5 days. The results are given in Table 2. The method was found to be precise with RSD values within 0.36–1.89% for intra-day, and with RSD values within 0.58–2.18% for inter-day assay.

The accuracy of HPLC method was confirmed by determining the high recovery values for acetylsalicylic acid (98.50%), paracetamol (98.35%), caffeine (99.15%) and phenobarbital (99.02%). The recoveries are obtained by determination of these drugs in laboratory-prepared dosage formulations containing 80, 100 and 120% of active substances.

The applicability of the method for the simultaneous determination of acetylsalicylic acid, paracetamol, caffeine and phenobarbital was verified by the determination of these compounds in Malophenum tablets. The

Table 3
The determination of analgoantipyretics in tablet

Comp.	Taken (mg)	*n*	Found (mg)	RSD (%)	Recovery (%)
Acetylsalicylic acid	250	10	244.93	1.18	98.74–102.08
Paracetamol	250	10	246.83	0.99	99.93–102.11
Caffeine	50	10	49.59	1.21	98.25–102.12
Phenobarbital	20	10	19.78	1.12	98.15–103.3

results are given in Table 3. RSD values obtained within 0.99–1.21% were found to be satisfactory.

The determination of these compounds in 10 single Malophenum tablets was carried out to confirm the reproducibility of the proposed HPLC method. The high recovery (98.25–102.12%) and RSD values within 1.21–1.85% confirm the suitability of the proposed method for the routine determination of these compounds in tablets. The content of salicylic acid in tablet was found to be lower than 0.1% of acetylsalicylic acid, which is in accordance with USP XXIV.

Compared with the results obtained in our previous work on HPTLC determination of acetylsalicylic acid, caffeine, paracetamol and phenobarbital in Malophenum tablets [17], it was shown that the proposed HPLC method provides better sensitivity of the assay, as well as significantly shorter analysis time.

4. Conclusion

HPLC method is simple, rapid and sensitive and therefore suitable for the routine analysis of acetylsalicylic acid, paracetamol, caffeine and phenobarbital in tablets.

The proposed method could be used for the determination of salicylic acid as impurity in dosage forms containing acetylsalicylic acid.

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