SIMULTANEOUS DETERMINATION OF ACETAMINOPHEN, ACETYLSALICYLIC ACID AND ASCORBIC ACID IN TABLET FORM USING HPLC

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SUMMARY

The purpose of the present study was to develop a simple and accurate HPLC method to measure the amount of each agent in a multidrug pharmaceutical formulation. Three drugs, acetaminophen, acetylsalicylic acid and ascorbic acid, were analyzed simultaneously. A commercial pharmaceutical effervescent tablet was examined and the amount of each of these agents successfully determined. The present method appears to be more convenient than the current procedures described in American and British Pharmacopoeias in which each drug is assayed separately.

KEY WORDS

simultaneous measurement, acetaminophen, acetylsalicylic acid, ascorbic acid, HPLC

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INTRODUCTION

A combination of acetaminophen (paracetamol), acetylsalicylic acid and ascorbic acid is used in many commercial antipyretic, analgesic preparations. Several methods have been previously described for the determination of acetaminophen and acetylsalicylic acid. Among these methods are titrimetry, chromatography, electrochemistry, spectrophotometry and high-performance liquid chromatography (HPLC) /1-4/. However, there are difficulties associated with these methods: 1) a considerable time is required for the extraction and reaction steps; 2) ascorbic acid interferes with the titrimetric methods due to the reducing properties of acetaminophen, and 3) ascorbic acid and acetylsalicylic acid may not be differentiated easily in some spectrophotometric methods /1-7/.

In order to overcome these difficulties we developed a HPLC method for the simultaneous determination of acetaminophen, acetylsalicylic acid and ascorbic acid in a pharmaceutical formulation.

MATERIALS AND METHODS

HPLC conditions

The solvent was delivered at a constant rate and pressure (1.8 ml/min and 1300 psi, respectively) by an isocratic pump (Waters 510 HPLC) and the eluent monitored at 235 nm (Waters 996 Photodiode Array Detector). The column was stainless steel (C_{18}) reverse phase column (300 mm x 3.9 mm) packed with 10 μm dimethyloctadecylsilan-bonded amorphous silica (Bondapak, Waters Associates, Milford, MA) and fitted to an autosampler (Waters 717 Plus). Separation was achieved with a mobile phase (methanol:water, 1:2, v/v) adjusted to pH 3.0 with 10% orthophosphoric acid.

Materials

Acetaminophen, acetylsalicylic acid and ascorbic acid were from Sigma; methanol and orthophosphoric acid were from Merck. All solvents used were of HPLC grade and chemicals were of analytical reagent grade. Water was demineralized and double-distilled (Aqua Nova distillation apparatus, Depertors, Sweden).

Pharmaceutical formulation

Each Afebryl® effervescent tablet (SMB Technology, Belgium) contained 200 mg acetaminophen, 300 mg acetylsalyclic acid, and 300 mg ascorbic acid.

Preparation of stock solutions

Stock solutions of acetaminophen (100 mg/100 ml), acetylsalicylic acid (150 mg/100 ml), and ascorbic acid (150 mg/100 ml) in mobile phase were prepared. The internal standard, sulfamethoxazole (200 mg/100 ml), was also dissolved in the mobile phase.

Working standard solution

Each drug stock solution (0. 1 ml) was accurately transferred into a 10 ml volumetric flask and internal standard stock solution (0.5 ml) added, and then the resulting solution was brought to 10 ml with the mobile phase.

Calibration curves

Increasing volumes (0.01, 0.02, 0.03, 0.04, and 0.05 ml) of each drug stock solution were transferred into 10 ml volumetric flasks and internal standard solution (0.04 ml) was added to each flask followed by the addition of the mobile phase. 10 µl of each solution were sequentially injected onto the column. Each calibration curve was subjected to regression analysis.

Procedure for pharmaceutical formulation

Tablets were finely powdered and the average tablet weight was calculated (n=20). Each sample, equivalent to 100, 150 and 140 mg of acetaminophen, acetylsalicylic acid, and ascorbic acid, respectively, was transferred into a 100 ml flask, dissolved with the mobile phase, sonicated for 1 min, made up to 100 ml with the mobile phase, mixed and filtered. This solution (0.01 ml) was transferred into a 10 ml volumetric flask, mixed with internal standard stock solution (0.01 ml), and the volume was made up to 10 ml with the mobile phase. This solution (10 µl) was analyzed as described above.

Calculations

The amount of each agent was calculated by linear regression using calibration curves prepared from standard solutions. Each value represents mean ± standard deviation (SD). The ratio of the integrated area of a drug peak at a given concentration to the integrated area of the internal standard (sulfamethoxazole) peak was also calculated ($A_D/A_{I.S.}$).

RESULTS AND DISCUSSION

In order to achieve simultaneous analysis of acetaminophen, acetylsalicylic acid, and ascorbic acid under isocratic conditions, the mobile phase composition was first optimized. The use of methanol: water (1:2, v/v) as extraction solvent yielded clear extracts with a high recovery ranging from 99.8-100% for each compound studied. The maximum absorption for acetaminophen was at 235 nm, therefore this wavelength was used throughout the analysis. Sulfamethoxazole was used as internal standard as it does not interfere with the elution patterns of the other agents. Typical chromatograms are shown of the standard solution (Fig. 1) and of a tablet containing acetaminophen, acetylsalicylic acid, and ascorbic acid (Fig. 2). Observed retention times (min) for ascorbic acid, acetaminophen, acetylsalicylic acid and sulfamethoxazole were 1.9, 2.7, 6.6 and 4.4, respectively. The total analysis lasted less than 7 min. Table 1 shows the data obtained from the regression analysis of the calibration curve performed for standard drug solutions. The average recovery data (%) for the predetermined concentrations of the three agents and for the pharmaceutical formulation are shown in Tables 2 and 3, respectively.

HPLC has been previously used to analyse multicomponent tablets containing acetaminophen and acetylsalicylic acid /1,4,7/. In the present study, in addition to these compounds, the amount of ascorbic acid in the formulation was also determined. The present data suggest that this reverse-phase HPLC methodology provides a fast (7 min), sensitive, accurate and reproducible assay for simultaneous determination of acetaminophen, acetylsalicylic acid and ascorbic acid. This should be more convenient than the current procedures described in American and British Pharmacopoeias /8,9/ in which each drug is assayed separately.

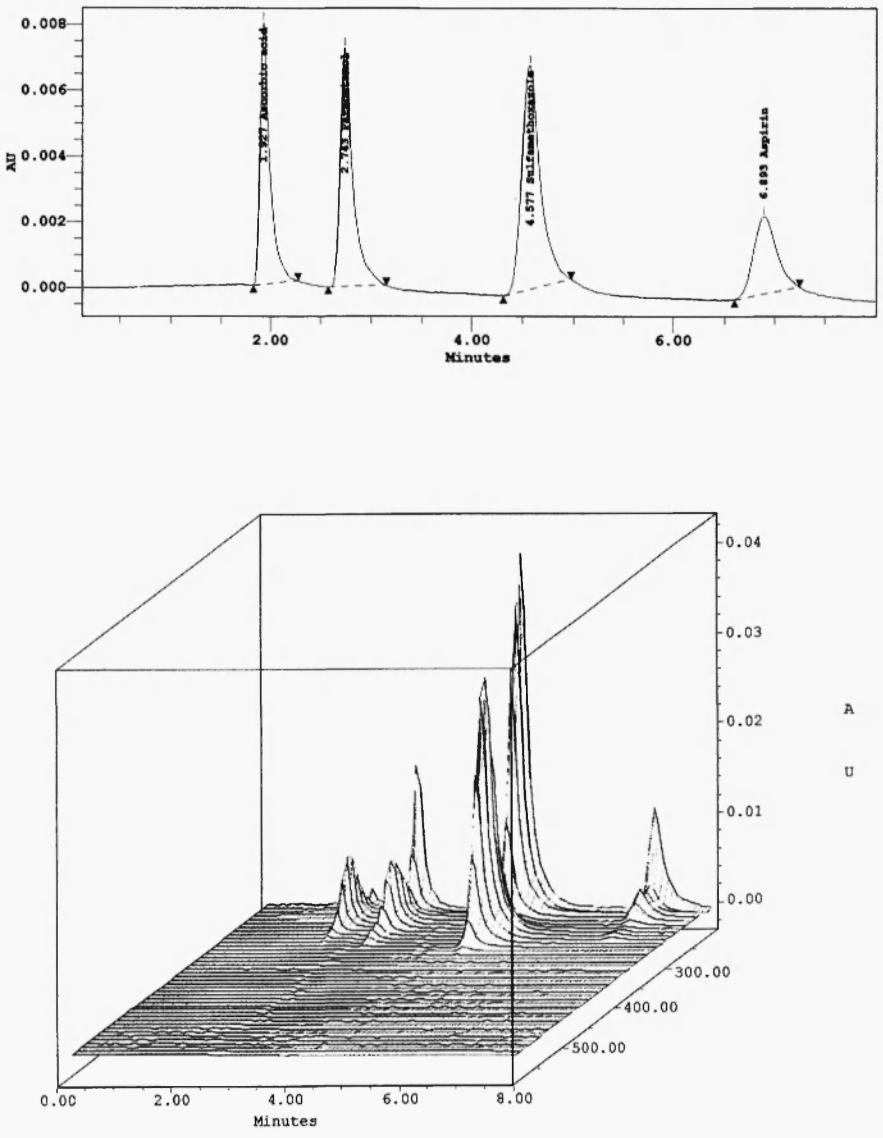

Fig. 1: A typical chromatogram and three-dimensional chromatogram of a standard solution containing ascorbic acid (I, 1.5 μg/ml), acetaminophen (II, 1 μg/ml), sulfamethoxazole (III, 2 μg/ml) and acetylsalicylic acid (IV, 1.5 μg/ml). Eluting solvent methanol:water (1:2), pH 3.0, flow rate 1.8 ml/min, room temperature, λ: 235 nm.

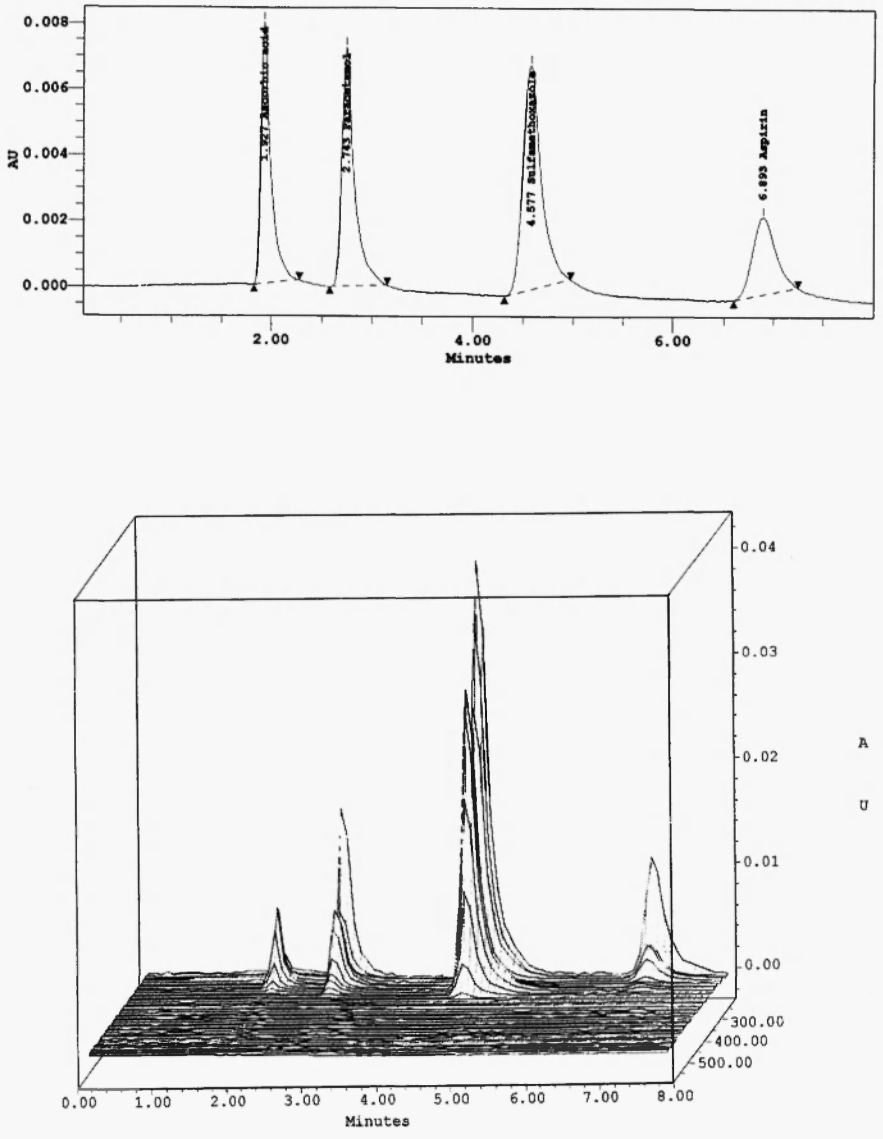

Fig. 2: A typical chromatogram and three-dimensional chromatogram of a sample tablet (Afebryl® effervescent tablet). I: ascorbic acid, II: acetaminophen, III sulfamethoxazole, IV: acetylsalicylic acid. Eluting solvent methanol:water (1:2), pH 3.0, flow rate 1.8 ml/min, room temperature, λ: 235 nm.

TABLE 1

Regression analysis results of known concentrations of acetaminophen, acetylsalicylic acid, ascorbic acid (n=5)

Compound	Concent.	A_D/A_{LS}.* ± SD**		Slope		Intercept		r ± SD**	
	(μg/ml)	Area	Height	Area	Height	Area	Height	Area	Height
Acetaminophen	0.5	0.2594 ± 0.004	0.3519 ± 0.005	0.01012	0.0305	0.04613	0.06733	0.9998 ± 0.0004	0.9975 ± 0.0005
	1.0	0.5267 ± 0.004	0.7183 ± 0.006						
	2.0	1.046 ± 0.009	0.2975 ± 0.007						
	3.0	1.529 ± 0.006	1.9345 ± 0.005						
	4.0	2.063 ± 0.001	2.7706 ± 0.004						
Ascorbic acid	0.75	0.1770 ± 0.007	0.2922 ± 00278	0.0405	0.0305	0.05513	0.04613	0.9968 ± 0.0005	0.9980 ± 0.005
	1.5	0.3567 ± 0.043	0.4726 ± 0.006						
	3.0	0.3568 ± 0.021	1.5900 ± 0.005						
	4.5	1.1047 ± 0.034	1.6193 ± 0.002						
	6.0	1.7868 ± 0.017	2.5000 ± 0.004						
Acetylsalicylic Acid	0.75	0.0652 ± 0.005	0.0557 ± 0.003	0.0604	0.0402	0.02450		0.9994±0.0004	0.9996±0.0046
	1.5	0.1022 ± 0.005	0.1240 ± 0.002						
	3.0	0.3957 ± 0.003	1.2976 ± 0.004						
	4.5	0.5341 ± 0.004	0.3925 ± 0.004						
	6.0	0.6110 ± 0.005	0.5419 ± 0.008						

*A_D/A_{LS}. is the ratio of the integrated area of the drug peak at a given concentration to the integrated area of internal standard (sulfamethoxazole, 8 μg/ml) peak.

** SD: standard deviation.

TABLE 2

Recovery data of acetaminophen, acetylsalicylic acid and ascorbic acid using standard solutions (n=5)

Compound	Concentration (μg/ml)	Amount found (μg/ml)	% Recovery ± SD
Ascorbic acid	6	5.98	99.67 ± 0.44
Acetaminophen	4	4.00	100.00 ± 0.65
Acetylsalicylic acid	6	5.99	99.83 ± 0.60

TABLE 3

Analysis of acetaminophen, acetylsalicylic acid and ascorbic acid in a pharmaceutical formulation (Afebryl®)

Compound	Label (mg/tablet)	Amount found (%) ± SD
Ascorbic acid	300	99.86 ± 0.09
Acetaminophen	200	100.20 ± 0.25
Acetylsalicylic acid	300	100.60 ± 0.02

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