QUANTITATION OF ACETAMINOPHEN IN PHARMACEUTICAL FORMULATIONS USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

YÜKSEK BASINÇLI SIVI KROMATOGRAFİSİ KULLANILARAK FARMASÖTÎK FORMÜLASYONLARDA ASETAMİNOFEN MİKTAR TAYİNİ

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ABSTRACT: *A reversed-phase high-performance liquid chromatographic method has heen developed for the determination of acetaminophen in pharmaceutical formulations. A C_{18} stationary phase is used with a methanol-water (1/2, v/v) mixture at the flow rate of 1.78 ml/min with the spectrophotometric detection at 193.3 nm. Sulphamethoxazole is used as an internal standard and analysis is completed within 5 minutes. The method showed good linearity, precision and reproducibility. The proposed method was successfully applied to the determination of acetaminophen in tablet and syrup.*

Keywords : *Acetaminophen, paracetamol, high-pressure liquid chromatography, tablet, syrup.*

ÖZET : *Bu çalışmada asetaminofenin kantitatif tayini için ters fazlı yüksek basınçlı sıvı kromatografisi yöntemi geliştirilmiştir. C_{18} stasyoner fazda, 1.78 ml/dak akış hızındaki metanol-su (1/2, v/v) karışımı kullanılarak 193.3 nm'de tayin yapılmıştır. Sülfametoksazolün internal standart olarak kullanıldığı analiz 5 dakika içinde tamamlanmaktadır. Metod iyi bir linearite, kesinlik ve tekrarlanabilirlik göstermektedir. Önerilen metod, asetaminofenin tablet ve şuruptaki analizine başarı ile uygulanmıştır.*

Anahtar kelimeler : *Asetaminofen, parasetamol, yüksek basınçlı sıvı kromatografisi, tablet, şurup.*

INTRODUCTION

Acetaminophen (paracetamol) is currently one of the most commonly used analgesic and antipyretic (1). It is often used as an alternative to aspirin and available without a prescription. Its determination in pharmaceutical dosage forms (quality control) and in biological fluids (overdose monitoring) remains great interest.

Dosage forms of acetaminophen and its combinations with other drugs have been listed in various pharmacopeias (2,3). Several methods (titrimetric, spectrophotometric and liquid chromatographic) are described in these pharmacopeias for acetaminophen in the raw material and in dosage forms. In combination with other drugs, acetaminophen has been quantitated using spectrophotometry (4,5), derivative ultraviolet spectrophotometry (6), titrimetry (7), voltammetry (8), FTIR spectrometry (9), HPLC (10-13) and capillar electrophoresis (14).

Most of these methods are not suitable for determination of acetaminophen with the presence of preservatives, colorants and flavors commonly added to liquid formulations. 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.

In this study, our objective was to develop and validate a specific, precise and reproducible method for the quantitation of acetaminophen especially in syrup which is also contain flavours (caramel or raspberry), colouring agents and common preservatives (sodium benzoate or parabens). Analytical data is presented to illustrate the usefulness of the method for the determination of the acetaminophen without pretreatment in pharmaceutical formulations using HPLC.

MATERIALS AND METHODS

Acetaminophen was of BP grade. HPLC grade methanol (Merck) and distilled, demineralized water were used. Pharmaceutical grade sulphamethoxazole was used as internal Standard.

Apparatus

Experiments were performed with a Waters liquid chromatograph (Millipore Corporation, 34 Maple Street, Milford, MA 01757), consisting of a solvent module (Waters 510 HPLC Pump) used for delivery of the mobile phase and samples, an auto sampler (Waters 717 Plus Autosampler) and a photodiode array detector (Waters 996 Photodiode Array Detector), all of

them interfaced to a PC computer. The analytical column (C_{18}) was a stainless steel column (Waters, 30 cm length x 3.9 mm i.d.) packed with reversed-phase dimethyloctadecylsilyl material (10 μm particle size).

Chromatographic Conditions

The mobile phase was prepared by adding 330 ml of methanol to 660 ml of the water. The pH of this mixture was adjusted to pH 3.0 with 10% orthophosphoric acid. The mobile phase was always filtered through a Millipore 0.45μm membrane and degassed. Isocratic elution was applied at ambient temperature and a flow rate of 1.78 ml/min and the pressure about 1800 psi. The detector was set to the wavelength of 193.3 nm.

Stock Solutions

Stock solution of paracetamol (20 mg/100 ml) was prepared in methanol. A stock solution of the internal standard sulphamethoxazole (40 mg/100 ml) was also prepared in methanol. The solutions were filtered through a 0.45 urn membrane before use, following the sonication of about 30 seconds.

Calibration Curve

Accurately pipetted volumes of 0.5, 0.75, 1.0, 1.25, 1.5 ml of the paracetamol stock solution was placed in 10 ml volumetric flasks and 1 ml of the internal standard stock solution was added to each flask. Following the addition of mobile phase to volume, these solutions were filtered through a 0.45 μm membrane before use. 20 μl of each solution were repeatedly injected into the column. The five concentrations of the compound were subjected to regression analysis and the slope and intercept were calculated.

Pharmaceutical Formulations

Ortamol Tablet (Military Pharmaceutical Industry, Turkey) labelled to contain 500 mg of acetaminophen per tablet. Ortamol Syrup (Military Pharmaceutical Industry, Turkey) labelled to contain 2.4 g of acetaminophen per 100 ml.

Procedures for Pharmaceutical Formulations

1) Tablet: The average weight per tablet was calculated from the weight of 20 tablets. Quantities of the finely powdered tablets equivalent to 50 mg acetaminophen were accurately weighed into a 100 ml flask, and dissolved with methanol. The solution was sonicated for about 30 seconds and brought to volume with methanol, mixed and well filtered. 0.4 ml of this solution was transferred to a 10 ml volumetric flask, 1 ml of the internal standard stock solution was added and the contents were diluted to volume with mobile phase. The solution (20 μl) was

chromatographed as described before. The contents of acetaminophen were calculated from linear regression equations of the calibration graph.

2) Syrup: 1 ml of the syrup (2.4 g/100 ml) was diluted to 100 ml with methanol. 1 ml of this solution was transferred to a 10 ml volumetric flask, 1 ml of the internal standard stock solution was added and the content was diluted to volume with mobile phase. The solution (20 μl) was chromatographed as described before.

RESULTS AND DISCUSSION

Chromatographic investigations showed that acetaminophen could be resolved from coformulated excipients, preservatives, colorants and flavors, using C_{18} stationary phase and a mixture of methanol-water (1:2) adjusted to pH 3.0 using orthophosphoric acid. Sulphamethoxazole was used as internal standard. The retention times for acetaminophen and sulphamethoxazole were observed to be 2.580 and 3.713 min, respectively. A slight increase or decrease in pH of the mobile phase affected the resolution of peaks. Therefore, analyses were performed on pH 3.0.

Increasing the proportion of methanol would decrease the capacity of the peak and eluate the peak due to acetaminophen together with the peaks of colouring and flavoring agents. Decreasing the proportion of methanol would result in broaden tailed peaks i.e. distortion of the chromatographic resolution. Therefore, analysis was carried out using the 1:2 proportion of methanol-water as a mobile phase. The system was found to be suitable to separete acetaminophen from the preservative (methyl and propylparaben), colouring and flavouring agent (rasberry) and these coformulated excipients do not interfere with the analytical procedure.

Regression analysis data and reproducibility of the proposed method has obtained by five replicate injections of solutions of the acetaminophen at various concentrations in the presence of the internal standard. The ratio of the compound peak area and height to the area and height of the internal standard was calculated for each chromatogram. Regression analysis of these data at the various concentrations of the acetaminophen gave the slope, intercept and correlation coefficient for calibration curve (Table 1).

A mixture containing known amount of the acetaminophen in the presence of coformulated exicipients was used to determine the recovery of the compound. The quantitation was performed by the slope and intercept data of regression analysis for the compound. The

recovery of acetaminophen in the synthetic mixture was found to be 98.8% $\pm$ 0.83. The applicability of the proposed HPLC method was carried out by the chromatography of acetaminophen in different concentrations. The results (Table 2) show that the proposed method is sensitive with good precision (coefficient of variation is less than 1%; n = 5).

Table 1. Results of regression analysis from the chromatogram of standard solutions.

Compound	Concent. (μg/ml)	$AD/A_{I.s.} \pm SD$	Slope	Intercept	$r \pm SD$
Acetaminophen	10.0	0.47 $\pm$ 0.01	0.05	0.03	0.99$\pm$ 0.0001
	15.0	0.70 $\pm$ 0.01			
	20.0	0.95 $\pm$ 0.01			
	25.0	1.17 $\pm$ 0.01			
	30.0	1.38+0.01			

*: Data represents 5 replicate injections of standard solutions. $AD/A_{I.s.}$ is the ratio of the integrated area or height of the drug peak at a given concentration divided by the integrated area or height of internal standard (sulphamethoxazole) peak at a concentration of 40 μg/ml.
** : SD, standard deviation.

Table 2. Recovery experiments of synthetic mixtures of acetaminophen using the proposed method.

Concentration (ug/ml) Acetaminophen	Mean % recovery $\pm$ SD* Acetaminophen
10	99.3$\pm$0.98
15	97.8 + 0.57
30	99.2 $\pm$ 0.65

• Mean of 5 experiments $\pm$ standard deviation.

The proposed HPLC method was applied to the determination of acetaminophen in tablets and syrups (Figure 1). Table 3 summarizes the analytical results from pharmaceutical dosage forms. This indicates that acetaminophen is stable in the pharmaceutical products produced and also the formulas of the syrup and tablet are good enough to prevent the degradation of acetaminophen.
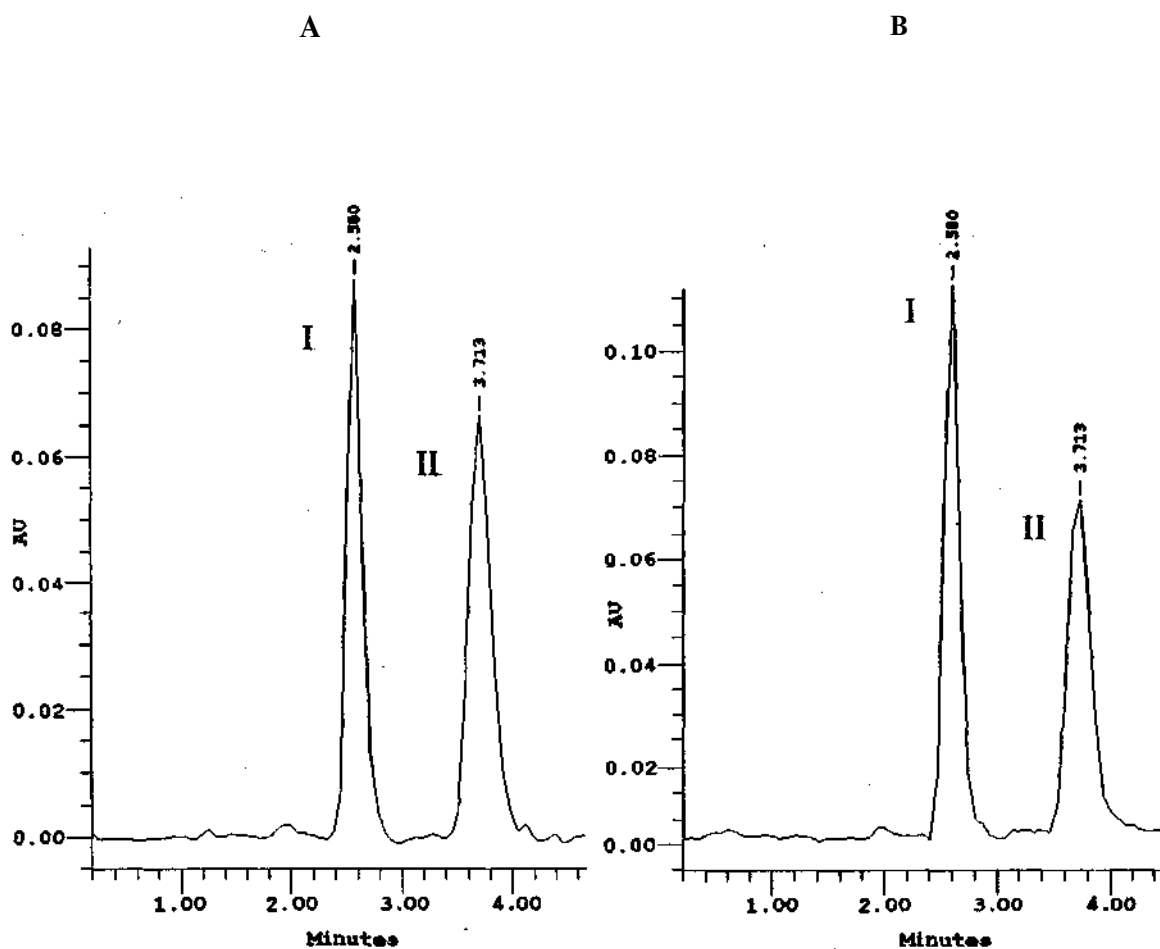
A   B

Figure 1. HPLC chromatogram of a sample of tablet (A) and a sample of syrup (B). Eluting solvent, pH: 3.0 methanol-water (1:2), flow rate 1.78 ml/min; ambient temperature; $\lambda= 193.3$ nm.

I: Acetaminophen,   II: Sulphamethoxazole.

Table 3. Analysis of acetaminophen in pharmaceutical formulations.

Pharmaceutical Dosage Form	Labelled Acetaminophen	Mean % recovery ± SD* Acetaminophen

The above **results document the usefulness of the HPLC method for the determination of** acetaminophen **in pharmaceutical formulations. The method** allows **the quantitation of** acetaminophen **in** a **short analytical time and without pretreatment in the** presence **of** coformulated **excipients. The proposed method is simple, sensitive, rapid, specific** and **could be applied** for **quality and stability monitoring of acetaminophen.**

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