ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
30 (2002) 1207–1213

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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# Simultaneous LC determination of trimethoprim and sulphamethoxazole in pharmaceutical formulations

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Received 17 April 2002; received in revised form 18 July 2002; accepted 22 July 2002

**Abstract**

In the present study, a simple, sensitive, precise and rapid reversed-phase high performance liquid chromatographic (HPLC) method with ultraviolet detection for the simultaneous analysis of trimethoprim (TMP) and sulphamethoxazole (SPM) is developed and applied to the determination in commercial pharmaceutical preparations. These compounds are well separated on a Bondapak $C_{18}$ reverse phase column using a mobile phase consisted of a mixture of methanol:water (60:40; v/v) adjusted to pH 3 with 10% orthophosphoric acid at a flow rate of 1.8 ml $min^{-1}$. The proposed method was linear in the range 2.0–10.0 μg $ml^{-1}$ for TMP and 10.0–50.0 μg $ml^{-1}$ for SPM. The limit of detection were 0.45 and 1.21 μg $ml^{-1}$ for TMP and SPM, respectively. The method which is rapid, simple and does not require any separation step, has been successfully applied to the assay of commercial tablet and oral suspension dosage forms containing TMP and SPM.


*Keywords:* Sulfonamides; Urinary tract infections; Trimethoprim; HPLC; Determination

## 1. Introduction

Sulfonamides are used primarily in the treatment of urinary tract infections; in combination with trimethoprim (TMP), they are also frequently used for the treatment of otitis, bronchitis, sinusitis and pneumoystis pneumonia. The pharmaceutical products containing sulfonamides consist, usually of one sulfonamide mixed with another drug that increases the power of sulfonamide, e.g. the sulphamethoxazole (SPM) and TMP mixture [1–3].

Several techniques have been reported in the literature for the determination of SPM individually or combination with other sulfonamides in pharmaceuticals or biological samples such as micellar liquid chromatography [4,5], high performance liquid chromatography [6–8] and spectrophotometry [9].

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0731-7085/02/$ - see front matter 
PII: S0731-7085(02)00460-0

Very few methods have been reported for the determination of TMP individually in biological fluids using by HPLC methods [10–12].

A number of reports about the analytical methods of TMP and SPM in either binary or multicompanent mixtures describe spectrophotometric [13–15], potentiometric [16] and HPLC and HPTLC [17–24] methods. Pharmacopoeial method involves the different techniques such as UV method for TMP and potentiometry for SPM [25] in British Pharmacopoeia in binary mixtures of oral solutions and tablets. United States pharmacopoeial method involves the HPLC determination of both drugs in pharmaceutical formulations [26]. But this method is time consuming and need expensive reagents. Thus, it was consider desirable to develop a simpler, faster and cheaper assay that would serve as an alternative to the current official method.

The reported methods require solid phase extraction or expensive reagents and equipments, which are not economically feasible for routine use in pharmacokinetic and pharmaceutical studies where numerous samples should be analyzed. HPLC methods are useful in the determination of drugs in pharmaceutical formulations especially to contain more than one active component. Owing to the widespread use of HPLC in routine analysis, it is important that good HPLC methods are developed and that these are thoroughly validated [27–30].

The aim of this work was to investigate the utility of HPLC in the assay of TMP and SPM in combination in pharmaceutical dosage forms without the necessity of sample pre-treatment.

This paper describes the development and validation of reliable, simple, time and money saving reversed phase HPLC assay, using UV detection, for the simultaneous determination of TMP and SPM in raw material, tablets and oral suspension. The method appears to be suitable for quality control in pharmaceutical industry due to its sensitivity, simplicity, selectivity and lack of excipients interference. This simple method allows researchers to save time and decrease cost compared with already published assays and pharmacopoeial methods.

## 2. Experimental

### 2.1. Apparatus

The chromatographic system operating in isocratic mode, consisted of the commercial components: a Waters Isocractic LC pump 510 (Waters, Milford, MA), an automatic sample injection system (Waters 717 plus Autosampler), photodiode array detector (Waters Model 996). Bondapak $C_{18}$ reverse phase column packed with 10 μm dimethyl octadecylsilyl bonded amorphous silica (300 mm × 3.9 mm) was used as the stationary phase.

### 2.2. Chemicals and reagents

SPM and TMP were kindly provided by Roche (Istanbul,Turkey) and internal standard acetylsalicylic acid was kindly supplied from Ali Raif (Istanbul, Turkey).

Chromatographic grade methanol (Merck, Germany) and analytical reagent grade orthophosphoric acid (Merck) were used. Doubly distilled water was used for preparing mobile phase solutions.

### 2.3. Chromatographic conditions

Chromatographic analysis were carried out at ambient temperature. The compounds were separated using isocratic system with a mobile phase consisting of methanol:water (60:40; v/v) adjusted to pH 3 with 10% orthophosphoric acid. The mobile phase was prepared daily, filtered, sonicated before use and delivered at a flow rate of 1.8 ml $min^{-1}$ and the effluent was monitored at 213 nm. The mobile phase mixtures was filtered through a 0.45 μm pore nylon membrane filter (Millipore, Bedford, MA). A total of 10 μl of each solutions was injected and chromatograms were recorded.

### 2.4. Stock solutions and standards

Stock solutions were prepared by dissolving TMP and SPM in methanol to obtain a concentration of 1 and 5 mg $ml^{-1}$, respectively. The

standard solutions of TMP and SPM containing a fixed concentration (30 μg $ml^{-1}$) of acetylsalicylic acid (internal standard) were prepared in mobile phase by varying the concentrations in the range of 2.0–10.0 and 10.0–50.0 μg $ml^{-1}$, respectively. Five times 10 μl injections were made for each solution and the peak area ratio of each drug to the internal standard was plotted against the corresponding concentration to obtain calibration graph. All solutions were protected from light and were used within 24 h to avoid decomposition.

The proposed method was validated as to precision (reported as the relative standard deviation (RSD%)), linearity (evaluated by calibration equations) and accuracy. The calibration curve was characterized by its regression coefficient, slope, intercept and their RSD% values, detection and determination limits. The ruggedness and precision were checked at in the same day ($n = 5$) and different days ($n = 5$). The RSD% was calculated to check the ruggedness and precision of the methods. Accuracy was determined by recovery studies.

### 2.5. Application to pharmaceutical dosage forms

For tablets:

Ten tablets were weighed and powdered. An accurate weight of the powder equivalent to one tablet was mixed with 100 ml of methanol in a 100 ml calibrated flask, stirred for about 10 min and filtered to separate any insoluble matter. The filtrate was collected in a clean flask. Suitable aliquots were taken and diluted with mobile phase. The sample was injected to the column. The amount of TMP and SPM per tablet was calculated from related linear regression equations.

For oral suspensions:

A total of 1 ml of the suspension was taken carefully and then diluted to 100 ml with methanol. 1 ml of this solution was transferred to 100 ml volumetric flask, appropriate amount of internal standard (IS) was added and the content was diluted to volume with mobile phase. 10 μl of this solution was injected. The contents of TMP and SPM calculated from linear regression equations of the related calibration graphs.

### 2.6. Recovery studies

To study the accuracy of the proposed method and to check the interference from the excipients used in the dosage forms, recovery experiments were carried out by the standard addition method. This study was performed by addition of known amounts of TMP and SPM to a known concentration of the commercial tablets. The resulting mixtures were analyzed as described above.

## 3. Results and discussion

The working conditions for the HPLC method were established with TMP and SPM bulk drugs and then applied on the pharmaceutical dosage forms.

Various mobile phase systems were prepared and used to provide an appropriate chromatographic separation, but the proposed mobile phase comprising of methanol:water (60:40; v/v) adjusted to pH 3 with 10% orthophosphoric acid gave a better resolution and sensitivity of SPM, TMP and IS.

In HPLC methods, precision and accuracy can often be enhanced by the use of an appropriate internal standard, which also serves to correct for fluctuations in the detector response. The structure of acetyl salicylic acid is not similar to TMP or SPM. However, it was chosen as the internal standard because it showed a shorter retention time with better peak shapes and better resolution compared to other potential internal standards.

Under the experimental conditions investigated, the retention times for TMP, SPM and IS were 1.99, 3.32 and 4.78 min, respectively.

According to USP 24, method 〈621〉 [26], system suitability test are an integral part of a liquid chromatographic method. System suitability tests are used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done. System suitability tests were carried out on freshly prepared standard solution of TMP and SPM.

Resolution and selectivity factors for this system were found 1.93 and 1.90, respectively. Tailing and capacity factors were obtained as 1.06 and 2.62 for TMP and 1.18 and 4.98 for SPM, respectively. The variation in retention times among six replicate injections of TMP and SPM reference solutions were very little, giving a RSD of 0.36 and 0.39%, respectively.

By applying this technique linear correlation was obtained between the peak area and the concentration in the range of 2.0–10.0 μg $ml^{-1}$ for TMP and 10.0–50.0 μg $ml^{-1}$ for SPM from which the related linear regression equation was calculated. Table 1, represents calibration characteristics and related parameters for TMP and SPM. The injection volume was 10 μl. The limit of detection (LOD) and limit of quantification (LOQ) of the procedure are also shown in Table 1, which were calculated according to the $3s/m$ and $10s/m$ criterios, respectively where $s$, is the standard deviation of the peak areas ($n = 5$) of the sample and $m$ is the slope of the corresponding calibration curve. Repeatability and reproducibility variabilities were characterized by RSD% and by the difference between theoretical and measured concentrations. Intra-day precision (repeatability) and accuracy of the proposed method were evaluated by assaying freshly prepared solutions at two different concentrations. Inter-day precision and accuracy of the proposed method were evaluated by assaying freshly prepared solutions for 3 different days. The results were given as the mean recovery % results (Table 2). There was no significant difference for the assay which was tested within-day and between days. All solutions are freshly prepared to ensure stability of analyte in solution. However, for stability indicating, the sample solutions injected to the column after 72 h did not show any appreciable change in assay values.

Table 1
Characteristics of TMP and SPM calibration plots

| | TMP | SPM |
|---|---|---|
| Linearity range (μg $ml^{-1}$) | 2.0–10.0 | 10.0–50.0 |
| Slope | 0.141 | 0.071 |
| Intercept | −0.054 | −0.10 |
| Correl. coeff. | 0.999 | 0.999 |
| RSD of slope | 1.08 | 0.22 |
| RSD of intercept | 0.47 | 1.03 |
| LOD | 0.45 | 1.21 |
| LOQ | 1.50 | 4.03 |

In order to demonstrate the validity and applicability of the proposed HPLC method, recovery tests were carried out by analyzing in the synthetic mixtures of TMP and SPM which reproduced different composition ratios (Table 3). When working on synthetic mixture, results encourage the use of the proposed method described for the assay of TMP and SPM in pharmaceutical dosage forms. The utility of the proposed method was verified by means of replicate estimations of pharmaceutical preparations and the results obtained were evaluated by statistically. Table 4, shows the results obtained in the analysis of tablet and oral suspension dosage forms. No potential interference may derive from their composition.

Table 2
Summary of repeatability (intra-day) and reproducibility (inter-day) precision data for TMP and SPM ($n = 5$)

| Compound concentration (μg $ml^{-1}$) | | Intra-day | Inter-day | | | |
|---|---|---|---|---|---|---|
| | | Mean recovery* %±RSD% | Recovered amount* %±RSD% | | | Mean recovery %±RSD% |
| | | | Day 1 | Day 2 | Day 3 | |
| TMP | 5.0 | 99.86±0.25 | 99.75±0.32 | 99.65±0.35 | 99.20±0.38 | 99.53±0.35 |
| | 6.0 | 99.74±0.36 | 99.72±0.29 | 99.98±0.40 | 99.90±0.12 | 99.87±0.27 |
| SPM | 25.0 | 99.82±0.35 | 99.72±0.29 | 99.60±0.33 | 99.67±0.34 | 99.66±0.32 |
| | 30.0 | 99.88±0.18 | 99.68±0.42 | 99.60±0.20 | 99.65±0.36 | 99.64±0.33 |

* Mean values represent five different sample standards for each concentration.

Table 3
Resolution of TMP and SPM in laboratory-made mixtures using by the proposed method

| Added (μg ml$^{-1}$) | | Found (μg ml$^{-1}$) | | Recovery % | | Mean recovery % | | RSD% | |
|---|---|---|---|---|---|---|---|---|---|
| TMP | SPM | TMP | SPM | TMP | SPM | TMP | SPM | TMP | SPM |
| 5 | 10 | 4.95 | 9.95 | 99.0 | 99.5 | | | | |
| 5 | 20 | 4.96 | 19.87 | 99.2 | 99.4 | | | | |
| 5 | 25 | 4.97 | 24.87 | 99.4 | 99.5 | | | | |
| 5 | 30 | 4.99 | 29.91 | 99.8 | 99.7 | | | | |
| 5 | 50 | 4.96 | 49.89 | 99.2 | 99.8 | | | | |
| | | | | | | 99.3 | 99.6 | 0.31 | 0.16 |
| 2 | 25 | 1.99 | 24.96 | 99.5 | 99.8 | | | | |
| 4 | 25 | 3.97 | 24.96 | 99.8 | 99.6 | | | | |
| 5 | 25 | 5.01 | 24.89 | 100.2 | 99.6 | | | | |
| 7 | 25 | 6.98 | 25.02 | 99.7 | 100.1 | | | | |
| 10 | 25 | 9.96 | 24.99 | 99.6 | 100.0 | | | | |
| | | | | | | 99.7 | 99.8 | 0.34 | 0.27 |

Table 4
Results of the determination and the recovery analysis of TMP and SPM in pharmaceutical dosage forms

| | Tablets (mg tablets$^{-1}$) | | Oral Suspension (mg 5 ml$^{-1}$) | | Official method [26] | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Tablets (mg tablets$^{-1}$) | | Oral suspension (mg 5 ml$^{-1}$) | |
| | TMP | SPM | TMP | SPM | TMP | SPM | TMP | SPM |
| Labelled claim (mg) | 80.00 | 400.00 | 40.00 | 200.00 | 80.00 | 400.00 | 40.00 | 200.00 |
| Mean of amount found (mg)* | 79.75 | 398.42 | 39.14 | 198.84 | 79.51 | 398.11 | 39.28 | 199.14 |
| RSD% | 0.67 | 0.34 | 0.99 | 0.36 | 0.69 | 0.48 | 0.81 | 0.51 |
| $t$-test of significant** | 0.49 | 0.77 | 0.84 | 0.60 | $t_{theoretical}$ | :2.31 | | |
| $F$-test of significant** | 0.95 | 0.52 | 0.58 | 0.51 | $F_{theoretical}$ | :6.39 | | |
| Added (mg) | 5.0 | 25.0 | 5.0 | 25.0 | | | | |
| Amount found (mg)* | 4.96 | 24.91 | 4.91 | 24.84 | | | | |
| Recovery % | 99.20 | 99.64 | 98.22 | 99.36 | | | | |
| RSD% | 0.90 | 0.21 | 0.77 | 0.28 | | | | |

* Mean value of the five determinations.
** Tabulated $t$ and $F$ value for $P$: 0.05.

Fig. 1 shows the chromatogram obtained after injection 10 μl of tablet solutions, respectively.

TMP and SPM pharmaceutical dosage forms were also determined with the official procedure, which involves a high performance liquid chromatographic method [26]. The results obtained for the formulations are listed in Table 4 and compared with the official HPLC method [26]. Both methods showed similar accuracy and precision. Statistical analysis of the results obtained by both methods using Student $t$-test and the variance ratio $F$-test shows no significant difference between the two methods regarding accuracy and precision. At 95% confidence level, the calculated $t$ and $F$ values were less than that of theoretical $t$ and $F$ values showing that there is no any significative differences between the proposed and reference method. The develop HPLC method is simpler, faster and cheaper than the other methods. This method could be used of the simultaneous determination of TMP and SPM in pharmaceutical dosage forms.

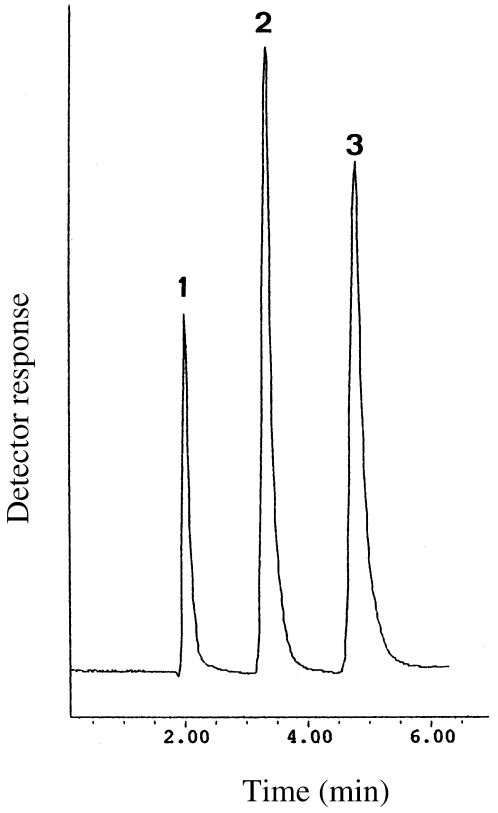


Fig. 1. Chromatogram obtained from tablet dosage forms containing 4 μg $ml^{-1}$ TMP (1); 20 μg $ml^{-1}$ SPM (2) and 30 μg $ml^{-1}$ IS (3).

In order to check the accuracy and precision of the developed method, we also carried out a recovery study. The results of the recovery tests were presented in Table 4. It can be concluded from Table 4 that the proposed method is sufficiently accurate and precise to be applied to pharmaceutical dosage forms within a short analysis time ( $< 5$ min). The proposed method is very simple, cheap, rapid and it does not involve use of any complex instrument or complicated sample preparation. The high percentage recovery indicates that the method is not affected by the interference due to the excipients used of in the formulations. Therefore, the method can be useful in routine quality control analysis of TMP and SPM.

## 4. Conclusion

The validated HPLC method has the advantage of simplicity, precision, rapidity and reliability. The proposed method gives a good resolution between SPM, TMP and IS. Compared with other reported methods, the proposed method has the advantages of simplicity, reproducibility, sensitivity and requires less expensive reagent than the other methods. The developed method offers a short analysis time of TMP and SPM which is a prerequisite in routine analysis of pharmaceutical preparations. Thus the proposed method is suitable for the screening of formulated samples.

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