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Simultaneous Analysis of Trimethoprim and Sulphamethoxazole Drug Combinations in Dosage Forms by High Performance Liquid Chromatography

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ABSTRACT

A sensitive high performance liquid chromathography (HPLC) analytical procedure was developed for the quantitive determination of trimethoprim (TMF and sulphamethoxazole (SM) in commercial dosage forms. C18 analytical column (stainless steel, 25 cm Ò 4.6 mm i.d.) was packed with 5-«m particles of the reversed phase material and used for assays. Mobile phase containing 0.025 M sodium phosphate as aqueous phase and acetonitrile with 0.4% triethylamine as organic phase. The drugs were quantified at flow-rate of 1.2 ml/min, with ultraviolet detection at 260 nm. The minimum detectable quantities in assays were 100 ng/ml for SM and 75 ng/ml for TM. The method is well suited to routine application and adequate sensitivity with precision. *Iran. Biomed. J. 4 (2 & 3): 75-78, 2000*

Keywords: Trimethoprim, Sulphamethoxazole, Co-trimoxazole, HPLC

INTRODUCTION

Co-trimoxazole [trimethoprim (TM)-sulphamethoxazole (SM)]is a broad spectrum anti-microbial agent composed of a fixed combination of a diamino-pyrimidine and a sulphon-amide [1]. It was developed by the systematic investigation of a series of compounds known to be specific enzyme inhibitors of bacterial folate synthesis. Co-trimoxazole has a wide range of activity against both Gram-positive and Gram-negative aerobic bacteria: chlamydia, actino-mycetes and Protozoa [2-5]. Many anaerobic organisms including *Bacteroides fragilis*, can be shown to be susceptible *in vitro* as well [6].

Co-trimoxazole has been shown to be effective in acute and persistent or recurrent urinary tract infections, (treatment and prophylaxis), ear, nose, throat infections, acute exacerbation of the chronic bronchitis and enteric fever [7]. A synergy or summation effect between the 2 drugs (TM and SM in a 1:5 ratio) has been demonstrated both *in vitro* and in most studies [8]. For most organisms, the optimum ratio for maximum potentiation is about 1:20 (TM : SM), which is the approximate ratio present in plasma after administration of the standard formulation. The usual recommended adult dosage is 2 standard tablets (TM 160 mg, SM 800 mg) twice a day. In children, the usual oral dose is 4 mg/kg TM, 20 mg/kg SM given twice daily[1].

A general approach for the determination of TM and SM is high performance liquid chromathography (HPLC) analysis because HPLC provides adequate sensitivity and precision for monitoring therapeutic steady state concentration [9, 10]. We report here an HPLC method capable of quantifying TM and SM simultaneously.

MATERIALS AND METHODS

Chemicals and reagents. All chemicals were of analytical grade. TM and SM were purchased from Sigma (USA) and Sobhan company (Iran). O-amino benzoic acid (OABA) and HPLC grade solvents were obtained from Merk (Germany).

Stock solution of TM was prepared by dissolving TM in the mixture of 0.02 M phosphoric acid and ethanol (1:1). Stock solution of SM was prepared

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by dissolving the compounds in a small amount of acetone and diluting to volume with ethanol. OABA was prepared in ethanol.

Chromatographic condition. The chromatographic system for UV detection of the analyte was consisted of Waters 510 pump, gradient controller of Waters and Waters 486 UV detector. The integration was carried out using the Waters 746 Data Module. The detector was operated at 260 nm. C18 analytical column (stainless steel, 25 cm × 4.6 mm i.d.) and gard column (stainless steel, 2 cm × 4.6 mm i.d.) were packed with 5 μm particles of the reversed phase material.

The mobile phase was 0.025 M sodium phosphate (pH 2.8 adjusted with 5 mM phosphoric acid) acetonitrile with 0.4% triethylamine added (80/20, v/v). The flow-rate was 1.2 ml/min. Aliquots of 2 μl were injected onto the column for the determination of TM and SM.

Calibration curves. Calibration curves were obtained by plotting the ratio of the area of each compound to the internal standard, OABA, versus their respective concentrations using five different concentrations of TM and SM were linear over all the concentration ranges studied.

RESULTS AND DISCUSSION

The necessity of assuring the quality of polydrugs, especially those with low aqueous solubility and *in vivo* absorption, has led to the development and evaluation of new techniques that can reduce the time and cost of analysis [11]. A number of procedures such as microbiology, thin layer chromathography, and gas chromathography have been described for the determination of sulphonamides in combination with TM in biological fluids and pharmaceutical preparation [12, 13]. Microbiological and immuno-assay methods are not considered very specific and may give false positive results [14]. Thin layer chromathography has been used for the detection of sulpha drug and TM, but this method has limited application and is generally used only for screening or qualitative analysis. Gas chromathography methods have not gained wide acceptance [14]. Several HPLC methods for the determination of TM alone or in combination with a sulphonamide, have been published [14, 15]. Most of the published methods

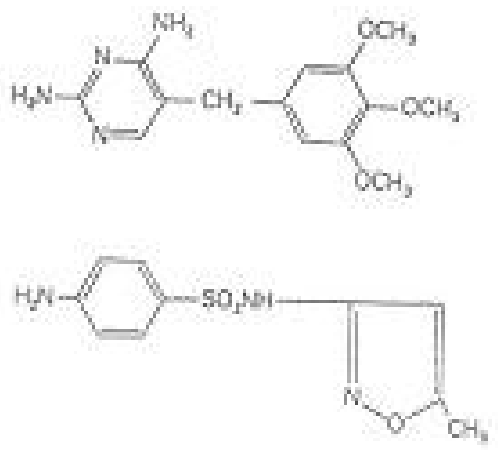

Fig. 1. Structure of trimethoprim (top), and sulphamethoxazole (bottom).

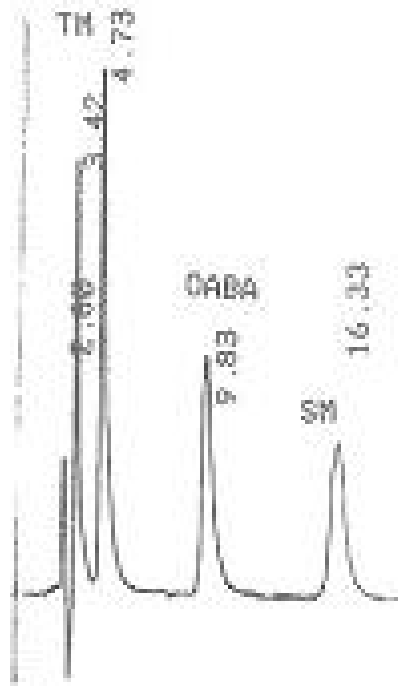

Fig. 2. HPLC chromatogram of TM, SM and OABA, chromatographic conditions: column C18 (25 cm × 4.6 mm i.d.); mobile phase, 0.025 M sodium phosphate as aqueous phase and acetonitrile with 0.4% triethylamine as organic phase (80/20); HPLC flow-rate 1.2 ml/min and ultraviolet detection (wavelength 260 nm).

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required the application of either complicated solvent-switching techniques or extensive and laborious sample treatment prior to analysis, thus rendering them unattractive for rotine laboratory use [16]. The objective of this study was to develop a simple and sensitive laboratory method for the analysis of trimethoprim and ST which could be routinely used to verify the qualitative responses obtained with dosage form (Figure 1). Reverse-phase liquid chromatog-raphy was used and deactivated C18 phase on a silica support was chosen for its good separation and low asymmetry factor of the drugs. Retention time of internal standard (OABA) was considered acceptable for the quantitative analysis. TM eluted at retention times shorter than OABA and SM (Figure 2). The TM peak was highly asymmetric. The tailing was suppressed by addition of triethylamine which had little effect on the SM peaks [9]

Table 1. Ratio of TM and SM area to the internal standard, OABA, versus their concentrations using five different concentrations of TM and SM

Trimethoprim		Sulphamethoxazole	
Concentration (µg/ml)	TM/OABA area ratio	Concentration (µg/ml)	SM/OABA area ratio
1	0.27	5	0.215
2	0.073	10	0.396
4	0.153	20	0.801
8	0.332	40	1.29
16	0.571	80	1.59
r = 0.986		r = 0.949	

Modifications of the aqueous phase and organic phase ratio of the mobile phase improve the sepration of the peaks of the drugs. TM and SM of Sobhan company and Sigma were examined with this method and gave good results. We have found a linear relationship between concentration of the test substances and the ratio of their peak areas to the internal standard peak area (Table 1).

The analytic procedure was linear for both drugs over concentration range which adequated covers levels commonly achieved during routine therapy. The method is well suited for routine application in the analytical laboratory because of the relatively simple procedure which allows the simultaneous determination of the two drugs. The assay provides adequate sensitivity and precision for monitoring therapeutic steady state concentrations as well as the subtherapeutic concentrations which are encountered following the administration of a single solid oral dose of co-trimoxazole for bio-availability assessment.

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REFERENCES

1. Wormser, G.P., Keusch, G.T. and Heel, R. (1982) Co-trimoxazole (trimethoprim-sulfamethoxazole): An updated review of its antibacterial activity and clinical efficacy. *Drugs 24: 459-518.*
2. Chin, T.W., Vandenbrouke, A. and Fong, I.W. (1995) Pharmacokinetics of trimethoprim-sulphamethoxazole in critically ill and non-critically ill AIDS patients. *Antimicrob Agents Chemother. 39 (1): 28-33.*
3. Eliopolos, G.M. and Wennersten, C.B. (1997) *In vitro* activity of trimethoprim alone compared with trimethoprim-sulphamethoxazole and other anti-microbials against bacterial species associated with upper respiratory tract infection. *Diagn. Microbiol. Infect. Dis. 29 (1): 33-38.*
4. Reeves, D. (1982) Good antimicrobial prescribing: sulphonamides and trimethoprim. *Lancet 14: 370-373.*
5. Richards, R.M. and Xing, J.Z. (1995) Mechanism of sulphadiazine enhancement of trimethoprim activity against sulphadiazine-resistant *Enterococcus faecalis. J. Antimicrob. Chemother. 36 (4): 607-618 .*
6. Hale, E., Habtegarbr, E. and Mcqueen, R. (1994) Co-trimoxazole for the treatment of listeriosis and its successful use in a patients with AIDS. *J. Infect. 28 (1): 110-113.*
7. Kumar, A.S. (1996) Treatment of anaphylaxis following oral Co-trimoxazole. *Indian Pediatr. 33 (3): 249-250.*
8. Richards, R.M., Taylor, R.B. and Zhu, Z.Y. (1996) Mechanism for synergism between sulphonamides and trimethoprim clarified. *J. Pharm. Pharmacol. 48 (9): 981-984.*
9. Behzadian Nejad, G., Rezaee, A. and Kebriaeezadeh, A. (1998) High-performance liquid chromatographic determination of trimethoprim in mouse liver. *Pharm. Pharmacol. Commun. 4: 439-441.*

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[DOR: 20.1001.1.1028852.2000.4.2.4.3]

10. Gochin, R., Kanfer, I. and Haigh, J.M. (1981) Simultaneous determination of trimethoprim-sulphamethoxazol and N-Acetyl sulphamethoxazole in serum and urine by high performance liquid chromatography. *J. Chromatogr. 223: 139-145.*
11. Bonazzi, D., Andrisano, V., Pietra, A.M. and Cavrini, V. (1994) Analysis of trimethoprim and sulfonamide drug combination in dosage forms. *Farmaco. 49 (6): 381-386.*
12. Hormazabal, V. (1992) Simultaneous determination of sulphadiazine and trimethoprim in plasma and tissues of cultured fish for residual and pharmacokinetic studies. *J. Chromatogr. 583:201-207.*
13. Nordholm, L. and Dalgard, L.A. (1982) Assay of trimethoprim in plasma and urine by high performance liquid chromatography. *J. Chromatogr. 233: 427-431.*
14. Agarwal, V.K. (1992) High performance liquid chromatographic methods for the determination of sulfonamides in tissue, milk and eggs. *J. Chromatogr. 624: 411-423.*
15. Horowitz, W. (1981) Analytical methods for sulfonamides in food and feed. II performance characteristics of sulfonamide methods. *J. Assoc. Off. Anal. Chem. 64 (4): 814-824.*
16. Nachilobe, P., Cassidy, R.M. and Fesser, A.C.E. (1993) Determination of trimethoprim in bovine serum high performance liquid chromatography with confirmation by thermospray liquid chromathography-mass spectrometry. *J. Chromathogr 616: 243-252.*

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