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Some Applications of High Performance Liquied Chromatography -Determination of Trimethoprim

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ABSTRACT

A simple, fast and precise high performance liquid chromatographic method for the assay of trimethoprim in the presence of sulphamethoxazole has been developed. The procedure is based on using acetonitril: water: acetic acid in the ratio of 30:69.9:0.1 (v : v : v) as a mobile phase, flow rate (1ml/min.) and uv detector (254nm.).Each analysis required no longer than 10 minutes. the detector responses were linear in the range of 0.05-120µg/ml for trimethoprim with a relative standard deviation of 0.49-0.971% and a relative error of 1-2.5% .

The proposed method was also applied satisfactorily to the pharmaceutical preparation.

Key words: trimethoprim; High performance liquid chromatography; UV detector; Pharmaceutical preparation

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بعض تطبيقات تقنية كروماتوغرافيا السائل ذو الكفاءة العالية

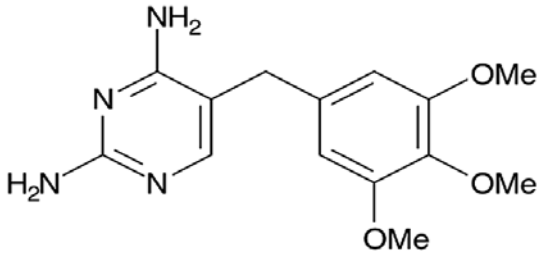
تقدير التراي ميثوبريم

الملخص

تم تطوير طريقه سهله وسريعة وذات توافق عالي لتقدير الترايميثوبريم في مستحضره الصـيدلاني باستخدام كروماتوغرافيا السـائل ذو الكفـاءة العاليـة والمـزود بكشـاف ضـوئي. اسـتخدم مـذيب الاسيتونتريل:الماء: حامض الخليك بنسبه 30 : 69.9 : 0.1 (v : v : v) كطور متحرك وبمعدل جريان 1مل/دقيقة وتم القياس عند طول موجي 254 نانوميتر وكانت العلاقة خطية تتبع قانون بير لحدود تقدير بين (0.05 و 120) مايكروغرام/مل بالنسبة للترايميثوبريم مع انحراف قياسي نسبي يتراوح بـين 0.49 و 0.97% وخطأ نسبي يتراوح بين 1 و 2.5 % .تم تطبيق الطريقة بنجاح لتقـدير الترايميثـوبريم فـي مستحضره الصيدلاني (الاقراص الدوائية).

INTRODUCTION

Trimethoprim is 5-[(3, 4, 5-Trimethoxy-benzyl) pyrimidine-2,4-diyldiamine].



$C_{14}H_{18}N_4O_3$ 290.3

Trimethroprim is dihydrofolate reductase inhibitor (British Pharmacopoeia, 2008). It is closely related to several antimalarials; in general it is potent antibacterial. Originally introduced in combination with sulphamethoxazole (Delgado and Remers, 1991).

Various methods have been reported for the determination of trimethopim in the presence of sulphamethoxazole. Some spectrophotometric methods always need previous separation (Cengic *et al.*1986), (Pawelczyk *et al* .1987), (British Pharmacopoeia, 2002). Other colorimetric methods describe the determination of trimethoprim without prior separation (Feng *et al.,* 1993) (Husain *et al.*,1995) (Mohamed and El-Shabauri,1994).

Trimethoprim was also analysed in tablet in the presence of sulphadizine using flow injection technique (Galve *et al.*, 2002).

High performance liquid chromatography (HPLC) is based on the same method of separation as classical column chromatography partition, ion exchange and gel permeation but it differs from column chromatography, in that pumped through the packed column under high pressure (Indian Drugs Review, 2000).

Trimethoprim in pharmaceuticals was analyzed using HPLC in the presence of sulphamethoxazole or sulphadoxine or sulphadiazine, the separation was achieved on a reversed phase column (C8) utilizing acetonitrile-0.05M phosphoric acid (1:3) as the mobile phase with spectrophotometric detection at 230 nm (Helbo and Thomsen, 1977).

Reversed phase HPLC with UV detection for simultaneous determination of trimethoprim(2-10 µg/ml) and sulphamethoxazol (10-50 µg/ml) were used in conjunction with Bondapak C18 column. The mobile phase consist of a mixture of methanol: water, the pH of the mobile phase was adjusted to be 3 with 10% of orthophosphoric acid, the flow rate was 1.8 ml/min.(Akay and Ozkan, 2002).

Trimethoprim has been analyzed in serum or urine with low detection limit 0.01mg/ml using high performance liquid chromatographic technique coupled with electrochemical detector while the detection limit of the same technique coupled with UV-detector was not less that 0.1 mg/ml (Moffat *et al.*, 2005).

Other different HPLC procedures were used for the determination of trimethoprim in dosage forms; these procedures contain many prior extraction steps (Carini, 1994) and (Abounassif *et al.*, 1992).

More complicated technique was used with the combination of HPLC in the determination of trimethoprim and other antibiotics after many extraction steps (Renew and Huang, 2004).

The purpose of this investigation was to determine trimethoprim in the tablets in the presence of sulphamethoxazole without prior extraction steps using HPLC technique with UV detection at 254nm.

EXPERIMENTAL

Apparatus:

A shimadzu LC-2010 HPLC system with C8 stainless steal column (25cmx4.6mm) was used in the analysis. The mobile phase consisted of (30:69.9:0.1 v : v :v) acetonitrile: water: acetic acid.

The operating conditions for HPLC were:

Ambient temperature, flow rate (1ml/min.), detector wavelength (254nm), injection volume 20 µl.

Reagents:

All chemicals used were of analytical HPLC grade, trimethoprim standard powder material was provided from the state company for drug industries and medical appliances Sammara-Iraq.

-Trimethoprim stock solution (500 µg/ml):

0.1gm of trimethoprim was dissolved in 200 ml of distilled water in a volumetric flask 200 ml.

- Acetic acid (HPLC grade).
- Acetonitrile (HPLC grade).
- water used was doubled distilled and filtered using membrane filter.
- Mobile phase was prepared by mixing 1ml acetic acid with 300ml acetonitrile and the volume is completed to one liter by distilled water.

Procedure and calibration graph:

Trimethoprim standard solutions prepared in the concentration of 0.05-120 µg/ml. Twenty µl of each standard solution was injected to HPLC column (auto injector) and the peak area at 254 nm was measured. For optimization of conditions and subsequent experiments 20 µg/ml solution of trimethoprim was used.

Choice of the appropriate mobile phase ratio:

Different polarities were tried first as mobile phase to elute the trimethoprim. Different volume ratios percentage of acetonitrile, water and acetic acid were tried and it was found experimentally that acetonitrile: water: acetic acid (30:69.9:0.1) gave the optimum results. Table (1) shows the capacity ratio (K^{-}) using different composition of mobile. The capacity ratio shows the efficiency of the column and should conveniently be within the range of 1 to 5(Al-Abachi *et al.*, 2003).

Table 1**:** types of mobile phase experimented with their identical capacity ratio.

Mobile phase	**Ratio**	**Capacity ratio***
Acetonitril:water	70:30	1.5
Acetonitril:water	30:70	5.2
Acetonitril:water:acetic acid	30:69.9+0.1	3.5

* K^{-} : is calculated by t_R-t_M/t_M where t_R is the retention time of the drug ; t_M is the dead time (Skoog *et al.*,1994).

From the results in table 1 the last composition of mobile phase is selected for the following applications where the capacity factor within the range with less cost in organic solvent.

Choice of the appropriate flow rate of mobile phase:

a flow rate 0.5, 1, and 1.5 ml/min. of mobile phase was checked and 1ml/minutes has been selected which seems to give higher resolution from the peak of sulphamethoxazole (Fig.1) where the resolution and retention time are affected by each other and both of them affected by flow rate(Skoog *et al.*, 1994).

The retention time of trimethoprim standard was 3.3 minute as shown in (Fig.2)

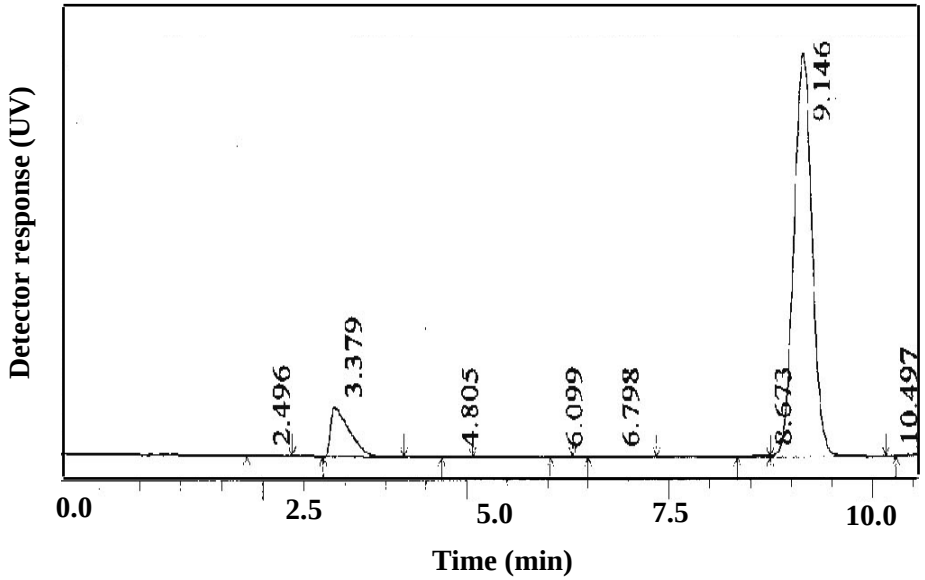


Fig. 1: Resolution of trimethoprim and sulphamethoxazole. Retention time of trimethoprim is 3.3 min. Retention time of sulphamethoxazole is 9.1.

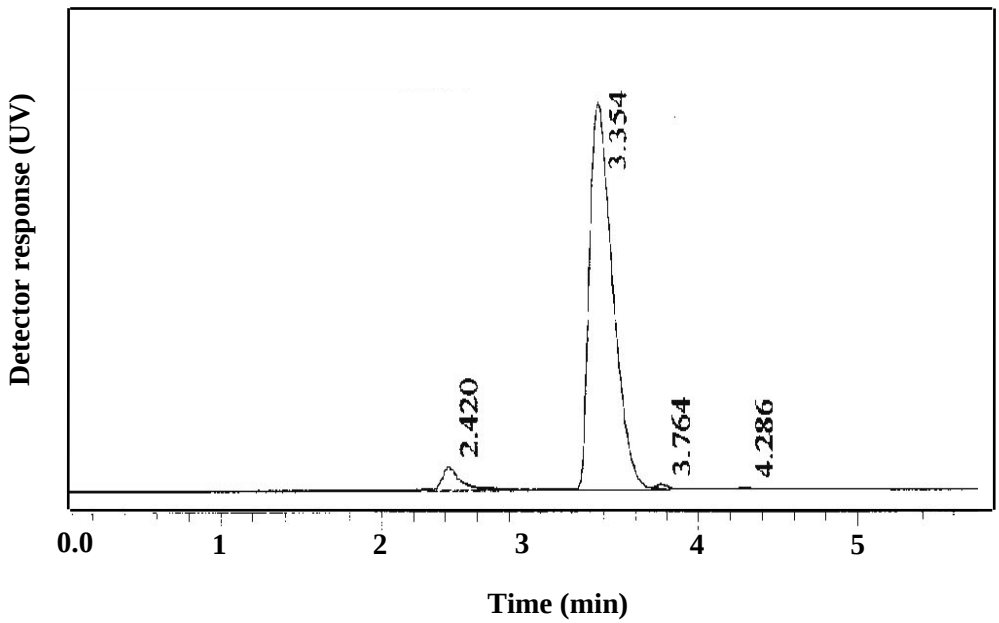


Fig. 2: Retention time of 20 µg trimethoprim.

Calibration graph:

Employing the condition described under the operating conditions calibration graph for trimethoprim was obtained. Fig.3 shows that a linear calibration graphs over the concentration range of 0.05-120 µg/ml with good correlation coefficient. Table(2) shows some information about the calibration graph.

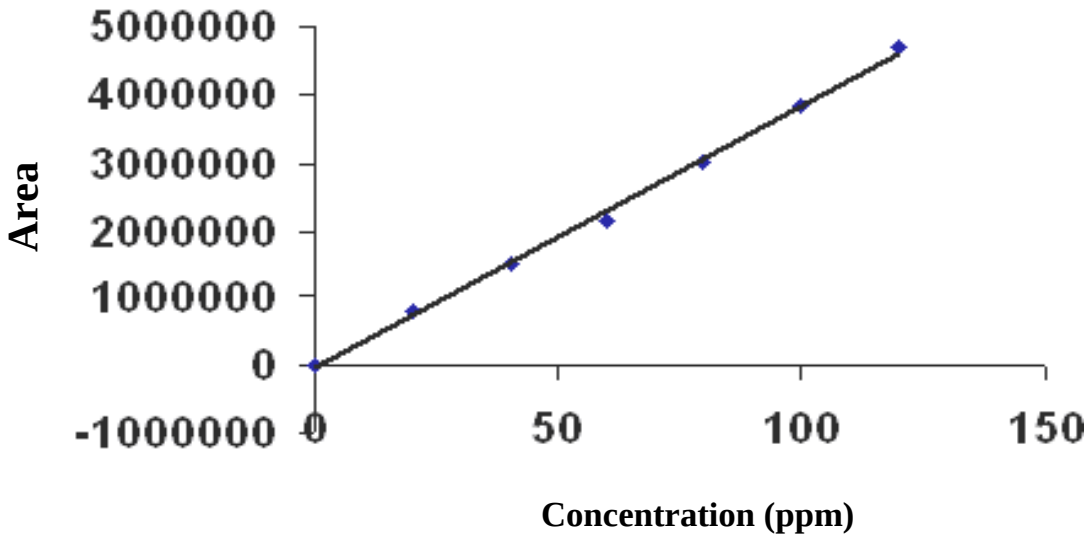


Fig.3:Calibration graph

Table 2: Some information about calibration graph

Linearity range (µg/ml)	**Slope***	**Correlation coefficient**
0.05 – 120	38798	0.9985

Accuracy and precision:

To determine the accuracy and precision of the method, trimethoprim was determined at three different concentrations. The results obtained are given in Table (3).

Table 3: Accuracy and precision of the method.

Amount of trimethoprim (µg/ml)		**Recovery%****	**Reletive standard deviation (RSD) %****
Taken	Found		
80	81	101.25	± 0.49
100	101	101.00	± 0.77
120	123	102.25	± 0.97

** Average of five determinations

* slope : is the ratio between the measured quantity in the analytical technique to the concentration of the substance to be determined, a large change in the measured quantity to the small change in concentration indicate a good sensitivity (high slope). (Kenneth,1982).

Table (3) indicates a satisfactory precision and accuracy could be obtained with the proposed method.

Analytical application:

Methoprim tablet containing 80mg trimethoprim and 400mg sulphamethoxazole have been analyzed in the following way:

Weigh and finally powder 10 tablets; take a weight of this powder accurately equivalent to 0.01g weight of trimethoprim; dissolve in mobile phase to prepare 100 µg/ml of trimethoprim solution; (20, and 80µg/ml can be prepared by dilution with mobile phase) transfer a portion of each solution to special vail and give the order to inject 20 µl of the solution to the column under 1 ml / min. flow rate of mobile phase.

The result obtained are given in Table (4) and Figs (4-6).

Table 4: Analytical application of the method

Drug	**Amount of trimethoprim taken (µg/ml)**	**Area**	**Recovery %**
Methoprim (SDI) 80mg trimethoprim and 400mg sulphamethoxazole/tablet	20	772150.9	100
	80	3046062.0	101.2
	100	3944187.61	102.5

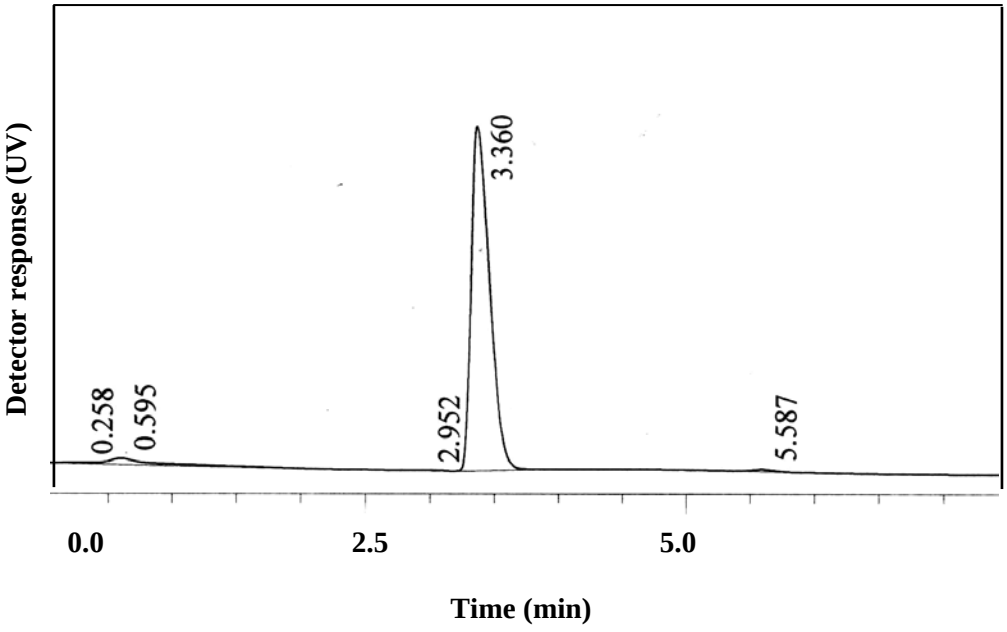


Fig. 4 : Chromatogram of 20 microgram of trimethoprim

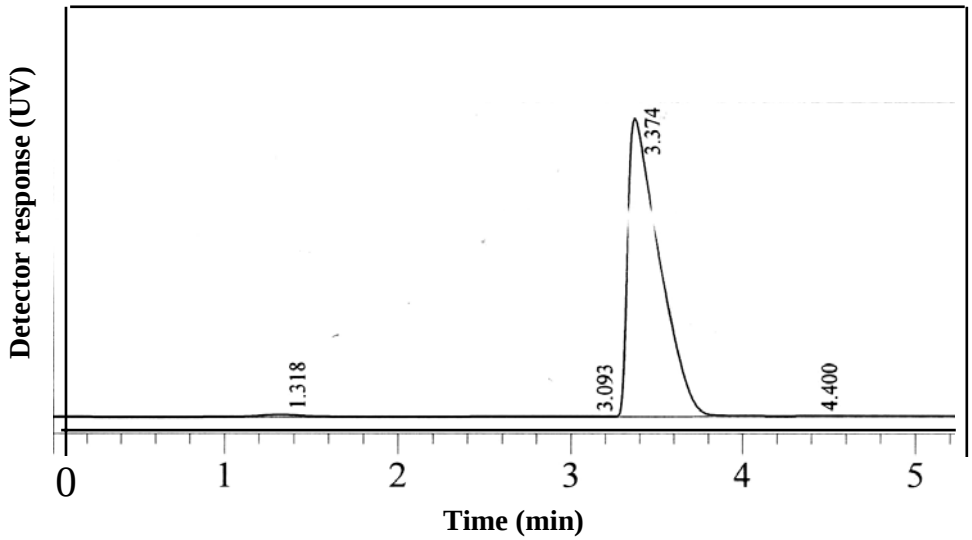


Fig. 5: Chromatogram of 80 microgram of trimethoprim

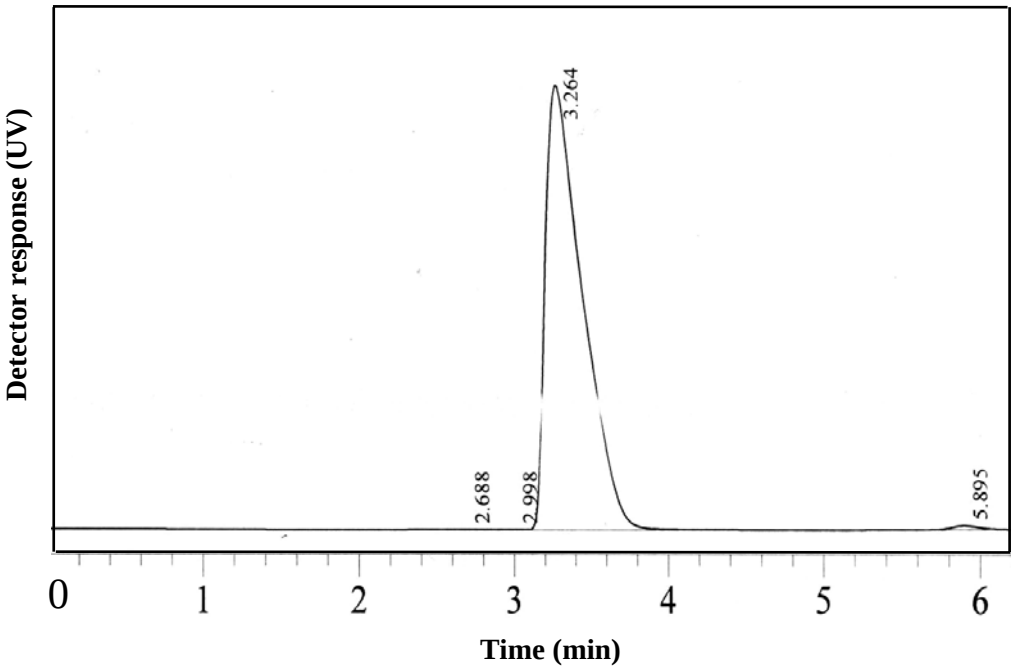


Fig. 6 : Chromatogram of 100 microgram of trimethoprim

Table 5: Comparison of the methods.

Method	Mobile phase	Linearity range (µg/ml)	Detector	Column	Notes	
(Akay and Ozkan, 2002)	Methanol: water	10-50	UV	C18	H_3PO_4 was used to adjust the pH	Applicable for tablet and oral suspension
Present method	Acetonitril: water: acetic acid	0.05-120	UV	C8	pH independ-ent	Applicable for tablet only

CONCLUSION

As the developed method for the determination of trimethoprim is precise and accurate, it is also selective were trimethoprim has been determined in the presence of five fold of sulphamethoxazole without any previous separation. The proposed method may be used for determination of trimethoprim in biological fluids because of the low concentration level .

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