A Reliable Quantification Method for Trimethoprim in Pharmaceutical Samples by HILIC-HPLC

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Abstract

Trimethoprim is sometimes used in conjunction with sulfonamides for drug therapies Herein. A practicable method has been developed to determine trimethoprim in pharmaceutical formulations by hydrophilic interaction liquid chromatography coupled with an ultraviolet detector. The development and investigation of a new HLIC assay of trimethoprim. The separation was made in the HALO-HILIC column using a NaOAc/HAc buffer (40 mM-pH 4.75) with acetonitrile (10:90) (v/v) as mobile phase and was quantified by UV detection at 280 nm. The limit of quantification was about 0.0242 ppm and the limit of detection 0.008 ppm. The calibration curves were linear in the investigated range (0.01-3 ppm). The recovery of trimethoprim was 99-102.5%. The method was applied in pharmaceutical formulations.

Keywords: *Trimethoprim, HILIC, Tablet, syrup, UV-detection; toxicity*

Introduction

Trimethoprim is an antibiotic used exclusively in bladder therapy. For the middle ear and traveler's diarrhoea, there are other uses. In people with HIV / AIDS, pneumocyst pneumonia can be treated with sulfamethoxazole or dapsone [1, 2]. Trimethoprim class of compounds is diaminopyrimidine It has an inhibitor of sulfonamide dihydrofolate reductase, commonly prescribed synergistic antimicrobial agents mainly used in the prevention and treatment of urinary tract infections [3]. And used to treatment Pneumocystis carinii pneumonia, otitis media, shigellosis, salmonellosis, and chronic bronchitis [4]. Trimethoprim was available from 1969 Sulphametboxazol combined (Co-trimoxazole) but has been marketed in the US, Scandinavia since 1980 [5,6].

For the determination of trimethoprim in pharmaceutical or biological samples, several HPLC procedures were identified [4, 7-16]. Hydrophilic interaction liquid chromatography (HILIC) was a variant of normal stage liquid chromatography, but the separation mechanism employed in HILIC was more complex than that in NP-LC. Alpert proposed the abbreviation HILIC for the first time in 1990 [17]. Since 2003, the number of HILIC publications has significantly increased [18]. HILIC, like NP-LC, employs traditional stationary polar phases like silica, amino, or cyano [19-23]. However, the mobile phase used in the mode RP-LC is identical [18, 22, 23]. HILIC technology has thus recently begun to growing dramatically in the determination of pharmaceuticals, nucleosides, carboxylic acids, inorganic ions, dansyl-amino acids, and flavonoids by Rasheed and its co-workers [24-38]. The goal of this study was therefore to develop and validate a simple, rapid, and sensitive HILIC method in pharmaceutical samples to determine trimethoprim.

Materials and Methods

Chemicals and reagents:

In purifying solutions, Millipore filters (0.22 μm) were used. From Sigma-Aldrich obtained trimethoprim, acetonitrile (ACN) and sodium acetate as far as the chemicals are concerned. 0.1 μs/cm (System-US Millipore) of Millipore water conductivity was used. Tablets and syrups of six different commercial companies, as follows: tablets (Supreme 500 mg-Ajanta-India, Septrin 500 mg-Aspen-Germany, Metheprim 500 mg-SDI- Iraq), syrups (Septrin Paediatric Suspension

100 ml-Aspen-Germany, Bactrim 100 ml-ASIA Pharmaceutical Industries-Syrian, Bactrim 100 ml-Pioneer-Iraq).

Instrumentation and chromatography

Chromatography was performed on the Merck-Hitachi HPLC System consisting of an L-6200 gradient pump; the Rheodyne valve allows injection 20 µL and UV-visible L-4200. A HILIC HALO®2.7 column (100 mm-2.1 mm) was used. The data were collected using N2000 workstation software to empower chromatography manager. The mobile phase composed of acetonitrile with acetate buffer (40 mM-pH 4.75) (90:10) (v/v). The mobile phase was filtered through 0.2 µm Nylon filter membranes. The analysis was carried out under gradient conditions using a flow rate of 0.5 ml/min at room temperature. The chromatogram of separation and determination trimethoprim was detection at 280 nm.

Results and Discussion

Optimizing the separation of trimethoprim:

As a pharmaceutical model, trimethoprim has been selected to test the HILIC retention mechanism using HALO 2.7 column by applying the acetate buffer with ACN content as eluent. At 90% ACN and 40 mM-pH4.75 of acetate buffer, the chromatogram was obtained (Figure 1). The systemic variability of the contents of the ACN is increasing in mobile phase compounds between 50% and 95%; the concentration of eluent between 10 mM and 80 mM with a pH between 3 and 5.5.

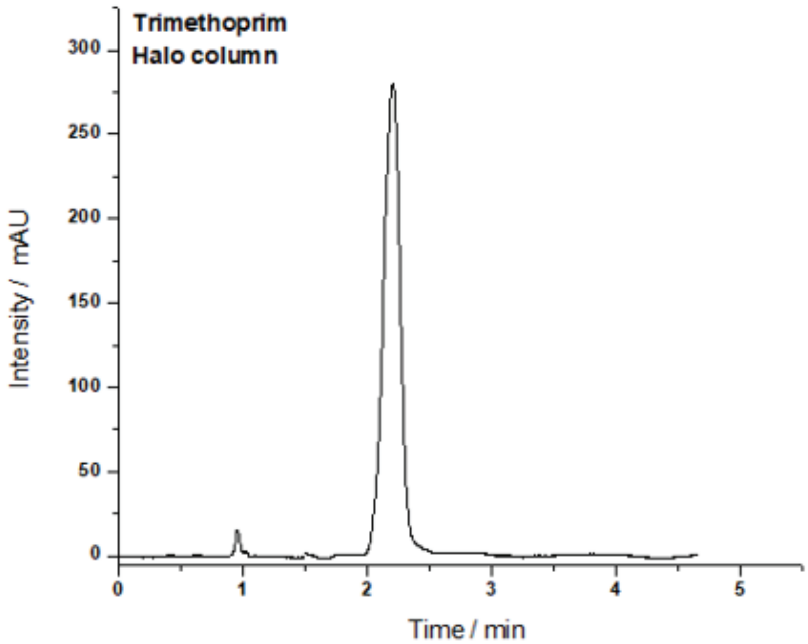

Figure 1: Chromatogram for the separations of trimethoprim using Halo column

Separation of trimethoprim with varying ACN content

Eluent ACN effect on trimethoprim retention was observed at 40 mM-4.75 pH acetate buffer. Trimethoprim hydrophilic interaction behavior appears to increase by 50% to 95% in the eluent ACN ratio, with the trimethoprim retention factor increasing. Trimethoprim hydrophilicity is the explanation for this behavior; trimethoprim's HILIC behavior is shown (Figure 2a), which was attributed to the trimethoprim log P_{OW} (-0.16) (39).

Separation of trimethoprim with varying buffer concentration

In 10-80 mM (pH 4.75) of eluent, the effect of the acetate buffer is recorded at an eluent ACN of 90 percent. The findings appear in (Figure 2b). The trimethoprim retention factor in the column should increase the rising buffer concentration in the acetate eluent. The hydrophilicity of trimethoprim is the explanation for trimethoprim behavior. The stationary process of the HILIC material is closely related.

Separation of trimethoprim with varying eluent pH

A shift in eluent pH can be used to boost the next composition of the mobile phase. The eluent pH must be modified to completely distinguish the trimethoprim in HILIC mode. At a constant buffer of 40 mM and ACN of 90%, the pH was improved from 3 to 5.5. The retention time decreases as shown in Figure 2c. This is because the amino group in trimethoprim is deprotonated.

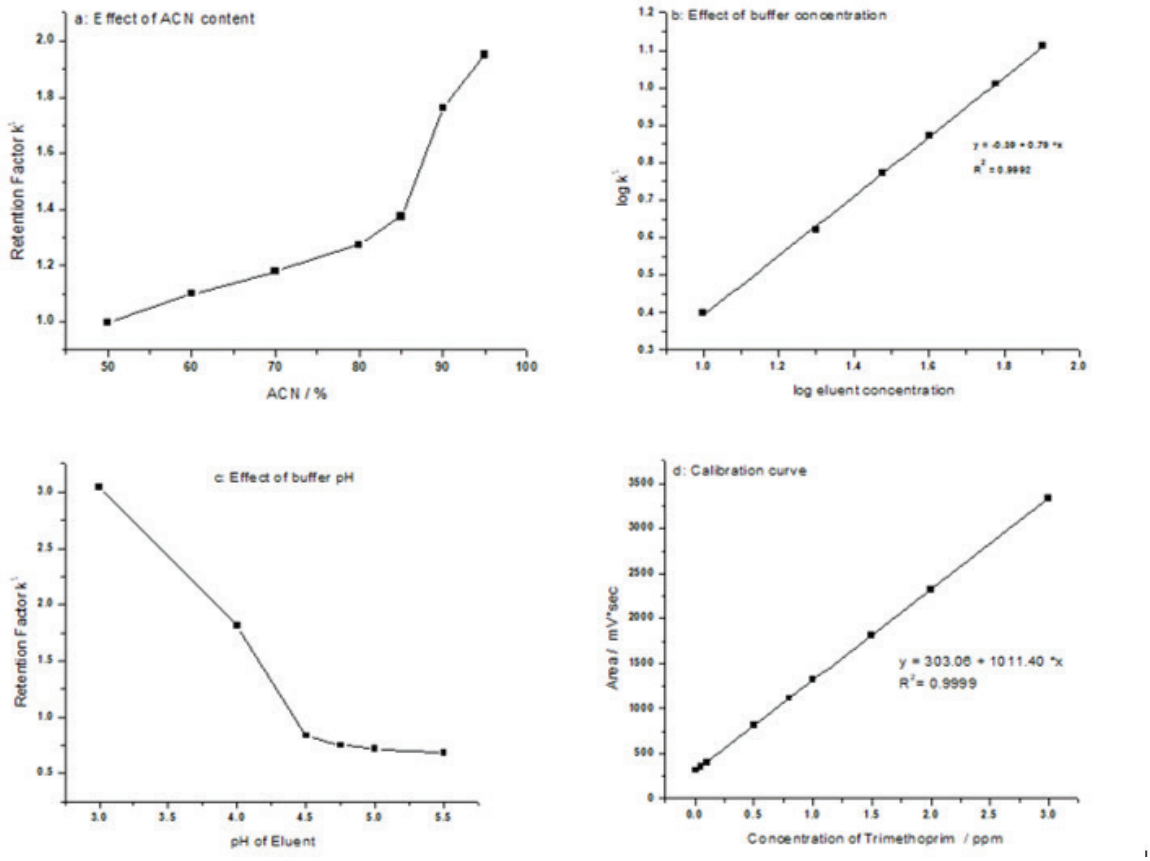

Figure 2: Study separation mechanism of trimethoprim (a) EffectiofdACN4content (b) Effectiofibuffertconcentration (c) EffectiofibuffertpH (d) The calibration curve.

Calibration graph:

The calibration trimethoprim curve is developed by plotting the trimethoprim concentration against the peak area and showing the concentration range (0.01-3 ppm) as Figure 2d shows.

Statistical data information:

A detailed evaluation under HILIC conditions and a monitor of statistics in Table 1 were used for the corresponding calibration curve of trimethoprim. Precision and accuracy were evaluated on the same day and different days with RSD% and Rec.% were evaluated. The relatively small defaults and high recuperation values indicate that the proposed method is successful (Table 2).

Table 1: The results for standard trimethoprim curve are checked using the HALO column.

Function	HALO method
Linearitya (ppm)	0.01-3
Regressiona equation	y = 303.06+ 1011.40* x
R2	0.9999
LODi(ppm)	0.008
LOQf(ppm)	0.0242

Table 2: Trimethoprim statistical results on the same day as on various days.

	Same-Day Analysis n=4				Day-to-Day Analysis n=4			
Trimeth. Takenf (ppm)	**Trimeth. Foundf (ppm)**	**% Rec.**	**% Erel.**	**%RSD**	**Trimeth. Foundf (ppm)**	**% Rec. f**	**% Erel. f**	**%RSDf**
0.5	0.510	102.00	2.00	0.67	0.498	99.60	- 0.40	0.87
1.5	1.492	99.46	-0.54	0.45	1.496	99.73	-0.27	0.76
2.0	1.980	99.00	- 1.00	0.55	2.05	102.50	2.5	0.61

Determination of trimethoprim in drug samples:

In evaluating trimethoprim in six of the pharmaceutical forms, the approach developed is used successfully, with the results described in Table 3.

Table 3: Pharmaceutical appliance of the proposed method of trimethoprim determination.

Name of drug	Company	Present (mg)	Get it (mg)	%Rec.	%RSD n=4	% Erel.
Septrin Paediatric Suspension 100 ml- Syrup	Aspen-Germany	40	39.87	99.67	0.23	-0.33
Bactrim 100 ml- Syrup	ASIA Pharmaceutical Industries-Syrian	40	40.10	100.25	0.42	0.25
Bactrim 100 ml- Syrup	Pioneer-Iraq	40	39.77	99.42	0.66	-0.58
Supreme 500 mg-Tablet	Ajanta-India	80	80.23	100.23	0.55	0.23
Septrin 500 mg-Tablet	Aspen-Germany	80	79.80	99.75	0.44	-0.25
Metheprim500 mg-Tablets	SDI- Iraq	80	79.60	99.37	0.76	-0.63

Such findings have been compared to the results produced about those obtained in the United States Pharmacopeia protocol [40] to determine the competence and efficiency of the HILIC approach. The results of the t-test and F-test variance-ratio (Table 4) which were confidential to 95% were used for data analysis. The measured values of t and F did not surpass the theoretical values so that the accuracy of the determination of trimethoprim in six pharmaceutical types does not substantially vary in both methods.

Table 4: The comparison of the proposed method with the standard method for trimethoprim analysis by investigating t- and F-statistical tests.

Name of drug	Halo method	Standard Method (40)	t-Test (theor.)	F-Test (theor.)
Septrin Paediatric Suspension 100 ml-Syrup	99.67	99.88	0.5722 (2.2281)	0.4058 (5.0503)
Bactrim 100 ml- Syrup	100.25	100.66		
Bactrim 100 ml- Syrup	99.42	99.22		
Supreme 500 mg-Tablet	100.23	100.65		
Septrin 500 mg-Tablet	99.75	99.43		
Metheprim500 mg-Tablets	99.37	99.87		

Conclusions

A new validated HILIC method was developed to determine trimethoprim in syrups and tablets. The HILIC method has demonstrated compliance with the ICH Harmonized Tripartite Guideline. To evaluate low ppm trimethoprim ranges, a HILIC method was developed. The suggested method was simple, fast, and sensitive enough. HILIC interaction with trimethoprim is seen in the stationary HALO phase. It is due to the Octanol-water partition coefficient value of trimethoprim.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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