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HPLC Method Validation for Determination of Pentoxifylline in Pharmaceutical Dosage Forms

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Abstract: ***A selective and simple reversed phase High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for quantification of pentoxifylline in dosage forms available in local market. Firstly, different chromatographic conditions were tested. Then, the optimized method was validated. The method proven to be linear over 50% to 150% of the nominal concentration of standard pentoxifylline (R^2 0.994). The method was accurate (recovery 100.1%) and precise (RSD% <3%). The method could separate pentoxifylline of caffeine and degradation products. The method was suitable for routine analysis of pentoxifylline in tablet dosage forms.***
Keywords: Stability indicating-HPLC; Pentoxifylline; Caffeine; Validation, Pentoxifylline; Validation.

I. INTRODUCTION

Pentoxifylline belong to xanthine alkaloid family. It is a synthetic agent structurally related to the methylxanthine derivatives theophylline, caffeine, and theobromine. Pentoxifylline is classified as a vasodilator through the reduction in blood viscosity by increasing erythrocyte deformability, inhibiting platelet adhesion and aggregation, reducing plasma fibrinogen, and increasing fibrinolytic activity [1-3, 8, 9]. Pentoxifylline is reported to increase blood flow to ischemic tissues and improve tissue oxygenation in patients with peripheral vascular disease. Recenly, pentoxifylline has demonstrated to be a potential adjuvant therapy for COVID-19 [4,37,45-48]. Different techniques were used to determine pentoxifylline in human plasma or in vitro as gas chromatography (GC) [5,6,19-25], thin layer chromatography (TLC) [7, 25-30], ıhigh performance thin layer chromatography (HPTLC) [8,18,-30-37], spectrophotometric methods [9, 10], electrochemial methods [11, 12, 33-39], micellar electrokinetic chromatography (MECK) [13,39-44]. Pentoxifylline is mainly analyzed by HPLC [15-18, 25,38]. Numerous HPLC methods were performed for the determination of pentoxifylline in pharmaceutical dosage forms. Mixture of buffer and organic modifier as the mobile phase was used to separate pentoxifylline from impurities or excipients in isocratic or gradient elution mode. Hence, there was a need to develop and validate new HPLC methods for the quantification of pentoxifylline in various formulations[45,53].

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The aim of this study was to develop and validate accurate, precise and specific HPLC method using only water and organic modifier as the mobile phase in isocratic elution mode for the determination of pentoxifylline in pharmaceutical dosage forms available in the local market.

II. MATERIALS AND METHODS:

2.1. Reagents :

MeOH HPLC grade, caffeine, NaOH, HCl 37%, H_2o_2 30% were purchased from MERCK, Germany. Standard pentoxifylline was kindly gifted by Alfa compamy.

2.2. Preparation of standard pentoxifylline solutions

Stock standard solution of pentoxifylline (1.5mg/mL) was prepared by dissolving an accurate weighted quantity of standard pentoxifylline in the mobile phase. Working solutions of pentoxifylline were prepared by diluting the stock solution with the mobile phase.

2.3. HPLC apparatus and chromatographic conditions:

The study was conducted using HPLC system equipped with Jasco Pu-2089 pump, Rheodyne 7725i injector and Jasco DAD-2070 diod array detector, Data acquisition was performed using Borwin chromatography software.
The analysis was performed using a Hypersil BDS C18 (250x4.6mm, 5µm) as stationary phase. The mobile phase was composed of water and MeOH (60: 40, V:V). It was filtred through a cellulose membrane with pore size of 0.45 µm. The flow rate was 1mL/min. The volume injection was 20 µL. The detection was at □$_{max}$ □□272nm).

2.4. Method validation

As per ICH Q2AR1 recommended conditions (International Council on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use), the following parameters for the optimized method were assessed: linearity, repeatability, intermediate precision, accuracy, specificity, limit of detection (LOD) and limit of quantification (LOQ).
Linearity: the linearity of optimized method was studied by preparing standard solution of pentoxifylline at be linear over the range of 50%–150% of the nominal concentration. The peak areas were plotted against corresponding concentrations. The coefficients of determination (R^2) was used to confirm linearity.
Precision: the method repeatability was evaluated by performing 6 injection of the standard pentoxifylline solution of the same concentration during the same experimental conditions and at the same day.

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Then, the average, standard deviation and the percent relative standard deviation RSD% were calculated. In order to estimate the intermediate precision of the optimized method, the same concentration was analyzed at different days.

Accuracy: to evaluate the recovery of the method, known quantities of standard pentoxifylline (50%, 100%) were added to commercial pentoxifylline dosage forms. This study was performed in triplicate (n= 3 determinations). The percent of recovery for each added quantity was calculated.

Specificity; the specificity of the optimized method was assessed through injection of mixture of pentoxifylline and caffeine. Stress testing of pentoxifylline was performed to induce force degradation of this drug. The degradation products help to validate the specificity of the optimized method by assessing the resolution between the degradation products and the drug. Pentoxifylline was subjected to stress condition of hydrolysis, thermal and oxidation degradation. To perform this study, 5mL of standard pentoxifylline solution was placed in 4 volumetric flasks of 50ml. 5ml of HCl 2M was added to the first volumetric flask. 5ml of NaOH 2M was added to the second volumetric flask. 5ml of H_2O_2 30% was added to the third volumetric flask. 5ml of purified water was added to the fourth volumetric flask. The 4 solutions was placed at temperature 70° using water bath. The solutions were cooled and 5ml of NaOH and HCl solutions were added to neutralize the acidic and basic degradation solutions. The solutions were completed with the mobile phase to a final volume of 50ml.

LOD and LOQ: the LOD and LOQ of the method were estimated by analyzing different concentration of standard pentoxifylline. The mobile phase was injected at the optimized experimental conditions and then the noise was determined. The LOD was the concentration that gave a signal to noise ratio of 3:1 while LOQ was the concentration that gave a signal to noise ratio of 10:1.

2.5.Application of optimized HPLC method in commercial pentoxifylline dosage forms

To perform this study, 10 tables were weighted and then the average weight was determined. The tables were grinded and precisely weighed portion containing 50 mg of pentoxifylline was transformed into a volumetric flask of 100ml containing 20 ml of the mobile phase. The solution mixtures were then sonicated for 20 min and then completed with the mobile phase to a final volume of 100 ml. The prepared solutions were filtered and then further diluted with the mobile phase to the appropriate concentration.

III. RESULTS AND DISCUSSION:

This research aimed to develop and validate RP-HPLC method for the determination of pentoxifylline in pharmaceutical dosage forms. To achieve this aim, different mobile phases were evaluated using C18 stationary phase. The optimized conditions of the mobile phase was a mixture of water and methanol (40:60, v/v). The retention time of pentoxifylline was 14minutes (figure 1).

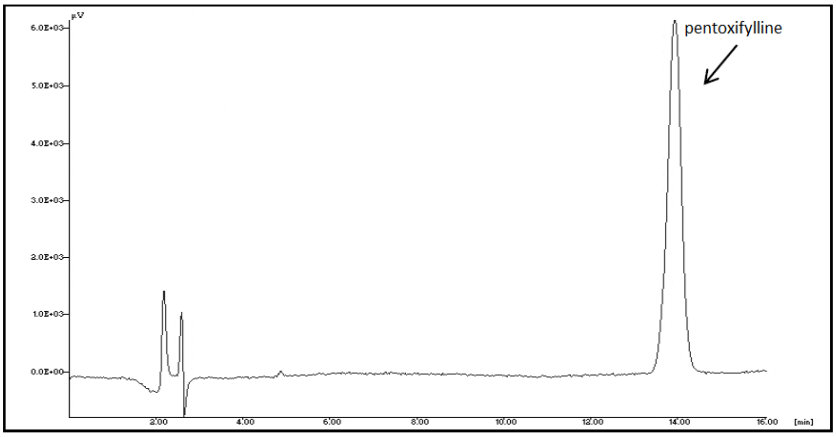

Figure 1: Chromatogram of standard pentoxifylline solution, the chromatographic conditions as in paragraph 2.4.

3.1. Method validation:

Linearity: the calibration curve of the optimized RP-HPLC method was linear in the studied concentration range 50%-150% of the nominal concentration. The determination coefficient of the curve was 0,994.

Accuracy and precision: the optimized RP-HPLC method showed a good accuracy with a mean recovery (103.5%). The optimized RP-HPLC method was also precise en term of repeatability and intermediate precision. The RSD% of retention time and peak area were less 3% as shown in the table 1.

Table 1: Data of repeatability and intermediate precision of the optimized HPLC method.

		Interdays (3 days)		Intraday	
		Peak area	Retention time min	Peak area	Retention time min
Pentoxifylline concentration 100%	mean	66527	14.05	66318	13.92
	RSD%	1.90%	2.21%	1.07%	0.53%

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LOD and LOQ: LOD and LOQ of pentoxifylline was 0,2µg/ml and 0.4µg/ml respectively.
Specificity: firstly, the specificity of the optimized RP-HPLC method was assessed by injecting a mixture of pentoxifylline and caffeine. The resolution was satisfied between pentoxifylline and caffeine as shown in figure 2.

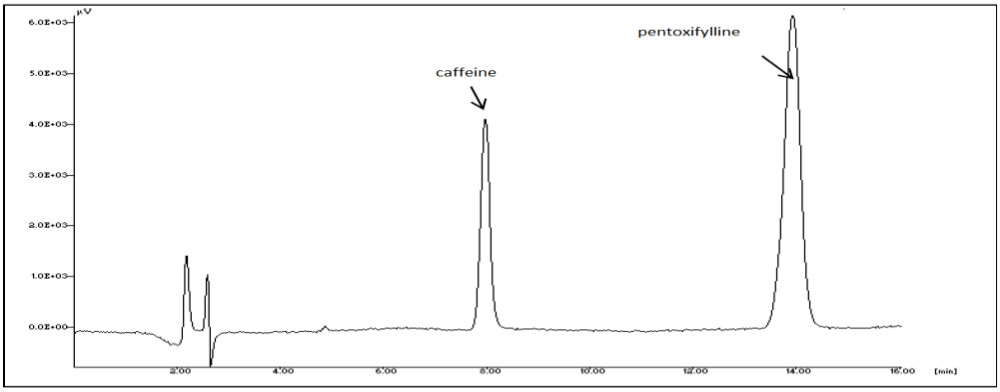

Figure 2: Chromatogram of a mixture of standard pentoxifylline and caffeine solution, the chromatographic conditions as in paragraph 2.4.

To evaluate the ability of the optimized RP-HPLC method to separate pentoxifylline from degradation products, stress condition were applied (acid hydrolysis, alkaline hydrolysis, thermal and oxidative conditions). Forced degradation results showed an appropriate specificity of the optimized method. As we can see from figure 3, pentoxifylline was stable to acid hydrolysis (HCl 2M), thermal (70·C) and oxidative conditions (H_2O_2 30%). Pentoxifylline was sensitive to alkaline hydrolysis (NaOH 2M) where the peak of pentoxifylline disappeared as a result of its degradation to major degradation product (caffeine) and to minor degradation product (theophylline).

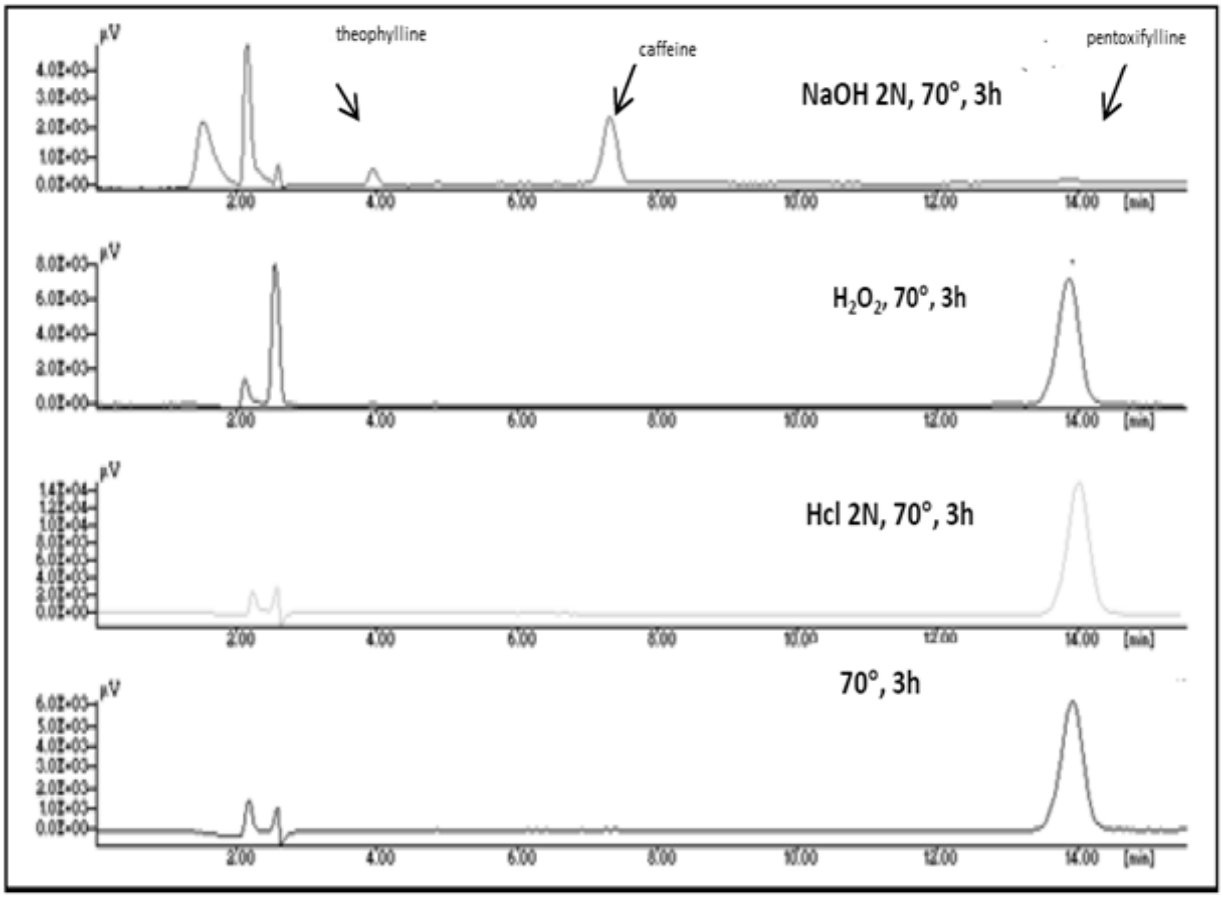

Figure 3: stability study of pentoxifylline (acid hydrolysis, alkaline hydrolysis, thermal and oxidative conditions), the chromatographic conditions as in paragraph 2.4.

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3.2. Assay of pentoxifylline in extended release tablets

The optimized RP-HPLC was applied to assay the pentoxifylline in the extended release tablets available (400mg) in the Syrian market. The percent of pentoxifylline content was in compliance with USP specification where all the values were in the range of 95-105% as shown in table 2.

Table 2: percent of pentoxifylline content in its extended release tablets

	Extended release pentoxifylline tablets 400mg			
Company	**A**		**B**	
Lot	**A2**	**A1**	**B2**	**B1**
Content %	101.12±0.833	100.37±0.856	102.49±0.86	99.43±0.782

IV. CONCLUSION

A new RP-HPLC for the determination of pentoxifylline was validated for linearity, accuracy, precision, LOD, LOQ and specificity. The optimized RP-HPLC was successfully applied to determine the content of pentoxifylline in its extended release tablets.

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