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Simultaneous determination of pentoxifylline and its impurities in tablet dosage forms by RP-HPLC

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ABSTRACT

A reversed-phase high-performance liquid chromatographic (RP-HPLC) method with UV detection was proposed for separation of pentoxifylline and its impurities from tablet dosage forms. The best separation was achieved on a LiChrosorb C18, 250 mm x 4.6 mm, 5 µm column at a detector wavelength of 274 nm. Isocratic regimen usage with 80:20:1:3 (v/v/v/v) mixture of water, methanol, o-phosphoric acid and tetrahydrofuran as a mobile phase at a flow rate of 1 ml/min enabled acceptable resolution of pentoxifylline, in large excess, from possible impurities, in a short elution time (15 min). Analytical parameters linearity, accuracy, precision and selectivity were determinated by validation procedure in accordance with ICH requirements and found to be satisfactory. Overall, the proposed method was found to be simple, rapid, precise and accurate for quality control of pentoxifylline and its impurities in dosage forms and in raw materials.

Key words: HPLC, impurities, pentoxifylline, tablets, validation.

INTRODUCTION

Pentoxifylline (Ptx), a tri-substituted xanthine derivative (3,7-Dimethyl-1-(5-oxohexyl)-3,7-dihydro-1*H*-purine-2,6-dione), is a hemorheologic agent used for the treatment of peripheral arterial disease and intermittent claudication [1]. Ptx improves blood flow through the peripheral circulation by decreasing blood viscosity, inhibiting platelet aggregation, enhancing erythrocyte flexibility and diminishing fibrinogen concentration [2]. Apart from these well known hemorheological properties, it has been found to exert a wide range of immunological activities. It has been reported that Ptx disturbs polarization and migration of human leucocytes [3]. Pentoxifylline also diminishes leucocyte-endothelium interaction and have a therapeutic role in preventing ischemia reperfusion injury in microsurgical operations [4]. It prevents atherosclerosis in diabetes mellitus [5] and is a nonselective phosphodiesterase inhibitor that decreases tumor necrosis factor gene transcription [6]. It also helps to prevent strokes and can be used in managing sickle cell disease and improves blood flow to the brain. It was also established that that Ptx increase the motility and longevity of fresh and freeze-thawed spermatozoa [7].

Several methods for its determination in pharmaceuticals have been reported, including high performance liquid chromatography [8, 9], spectrophotometry [10, 11], high performance thin layer chromatography [12], gas chromatography [13, 14] micellar electrokinetic chromatography [15] and electrochemical methods [16-19]. All these methods are adapted to decide rather closed problems. Some of them have a validation but it is opened the question about the quality control of pentoxifylline in different media thus needed for generic drug formulation producers.
Recently the European Union (EU) regulates the use of generic drug formulations and has been set up in each member state to determinate and to detect the residues of the drugs and its related substances in different products

and blood plasma. To the EU requirements have been added the US FDA recommendations about drug preparations testing.

In this paper we therefore focused on finding optimal conditions for simultaneous separation and determination of pentoxifylline and its potential impurities in pharmaceutical dosage forms. The related substances of pentoxifylline we examined were theobromine and caffeine, both mentioned in the European Pharmacopoeia (EP) as impurity A and impurity F, respectively.

MATERIALS AND METHODS

Chemicals and reagents

Pentoxifylline reference standard (RS) was supplied from Sigma-Aldrich (Germany). Impurities A and F were obtained from Merck (Darmstadt, Germany). HPLC grade methanol, tetrahydrofuran and orthophosphoric acid were produced from Merck. Pentoxypharm tablets containing 100 mg pentoxifylline were commercially obtained.

Instrumentation and chromatographic conditions

Chromatography was carried out isocratically, on modular HPLC system LC-10A Shimadzu (Japan) arranged with a LC-10A pump, solvent degasser DGU-3A, Rheodyne injector with 20 µl loop, column oven CTO-10A, SPD-M10A diode array detector and communication bus module CBM-10A. Compounds were separated on a LiChrosorb C18, 250 mm x 4.6 mm, 5 µm column. The mobile phase was 80:20:1:3 (*v/v/v/v*) mixture of water, methanol, o-phosphoric acid and tetrahydrofuran. Isocratic elution was carried out at a flow rate of 1 ml/min at ambient temperature. UV-detection was performed at 274 nm.

Reference solutions

Reference stock solutions of theobromine (0.1 mg/ml) (Impurity A), caffeine (0.4 mg/ml) (Impurity F) and pentoxifylline (0.2 mg/ml) were prepared in the mobile phase and filtered through 0.45-µm membrane filter.

Calibration solutions

Calibration solutions for pentoxifylline were prepared by diluting the reference stock solution to furnish concentrations in the range 16-80 µg/ml. Calibration solutions for theobromine were prepared by diluting the reference stock solution to obtain concentrations in the range 2-16 µg/ml.

Calibration solutions for caffeine were prepared by diluting the stock reference stock solution to achieve concentrations in the range 4-20 µg/ml.

Working solutions

Working standard solutions for analysis of related substances contained 1.6 mg/ml pentoxifylline, 8.00 µg/ml impurity A and 10.00 µg/ml impurity F. Working standard solution for the test assay contained 32.00 µg/ml pentoxifylline.

Sample preparation

Pentoxyfarm tablets were used for the investigation. One tablet contains 100 mg active substance. Sample solutions were prepared by first preparing stock solutions. Twenty tablets were weighed and finely powdered. An amount of the powder equivalent to 100.0 mg pentoxifylline for assay and 400.0 mg for analysis of related substances were weighed into 250.0 ml volumetric flasks and approximately 150 ml mobile phase was added to each. The samples were sonicated for 20 min and the solutions were then diluted to volume with mobile phase, mixed well, and filtered. For assay, 4.00 ml stock solution was diluted to 50.00 ml with mobile phase to give a solution containing 32 µg/ml pentoxifylline. The solution used for analysis of related substances contained 1.6 mg/ml pentoxifylline.

RESULTS AND DISCUSSION

From the chromatogram shown in Fig. No1, it is evident, that under the chosen chromatographic conditions, theobromine and caffeine were completely separated (Rs = 1.77), which indicated that the method is selective and could be used for their simultaneously identification, quantification and purity tests.

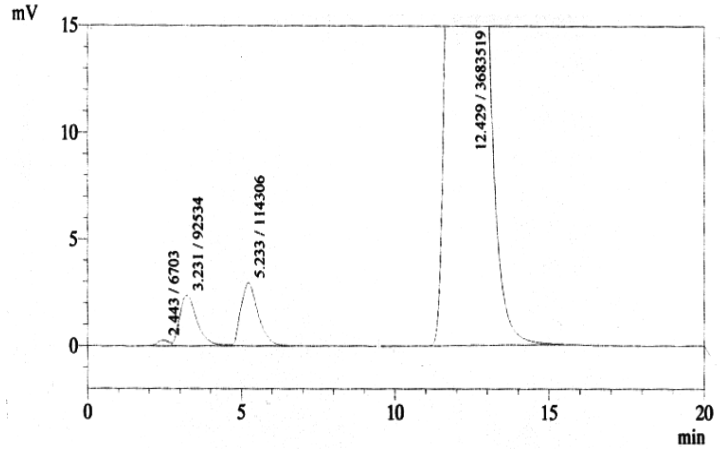

Fig. No 1. Chromatogram obtained from system suitability study

Retention times, number of theoretical plates and tailing factors obtained by used of the HPLC method under the optimum chromatographic conditions are listed in Table No 1.

Table No 1. Chromatographic data from HPLC method (system-suitability test)

Parameter	Pentoxifylline	Theobromine	Caffeine
Retention time (min)	12.43	3.23	5.23
Tailing factor	0.92	0.85	0.90
Theoretical plates	2514	1250	1453

The validation study allowed the evaluation of the method for its suitability for routine analysis in related substances tests procedure. The proposed method was validated with respect to selectivity, linearity, precision and accuracy to show it could be used for simultaneous determination of pentoxifylline and its impurities both in the bulk drug and in pharmaceutical formulations. The specificity of the HPLC method was confirmed by injecting blank samples, placebos, reference solutions and in respect of supplements. No other peaks were observed at the retention times of pentoxifylline and its impurities A and F, indicating that interfering substances were not present. Pentoxifylline was identified by comparison of the retention times of the peaks attributed to pentoxifylline from the sample solution and from reference solution. Quantification was achieved by a single standard method.

Calibration and linearity

Response (peak area) was proportional to concentration over the ranges tested. We prepared a series of six calibration solutions with a concentration range shown in Table No 2. Calibration plot data slope (a), intercept (b), and correlation coefficient (r) are listed in Table No 2.

Table No 2. Validation data for the calibration plots

Drugs	Pentoxifylline	Theobromine	Caffeine
Concentration range (µg/ml)	16-80	2-16	4-20
Slope	25345.8	37110.1	46058.4
Intercept	685.4	-917.9	7712.8
Correlation coefficient (r)	0.9997	0.9998	0.9996

Precision

The precision of the analytical system was investigated by performing six consecutive replicate injections of the same standard solution. The standard deviation (S_d) and relative standard deviation (RSD) obtained are listed in Table No 3.

Table No 3. Values of S_d and RSD as confirmation of precision

Compound	Mean (µg/ml)	S_d	RSD (%)
Pentoxifylline	31.69	0.298	0.94
Theobromine	7.81	0.101	1.29
Caffeine	9.81	0.098	1.00

Accuracy

The accuracy of the method was investigated by determination of both impurities in the presence of pentoxifylline. A solution containing pentoxifylline (C = 1.6 mg/ml) with no detectable impurities was spiked with the reference substances at appropriate concentrations. The recovery and relative standard deviations (RSD) obtained (Table No 4) confirmed the satisfactory accuracy of the method.

Table No 4. Results from study of accuracy

Compound	Recovery (%)	S_d	RSD (%)
Pentoxifylline	99.12	0.419	0.42
Theobromine	98.93	0.385	0.39
Caffeine	99.02	0.637	0.64

Limit of quantification and limit of detection

The limit of detection (LOD) was calculated to be three times the standard deviation of baseline noise from analysis of each compound. The limit of quantification (LOQ) was measured as the lowest amount of analyte that could be reproducibly quantified above the baseline noise, i.e. for each duplicate injection resulted in an RSD≤2%. LOD and LOQ are listed in Table No 5.

Table No 5. Limits of detection and quantification

Compound	LOD (μg/ml)	LOQ (μg/ml)
Pentoxifylline	0.50	2.00
Theobromine	0.10	0.50
Caffeine	0.25	1.00

The obtained data from this analytical study were compared with European pharmacopoeia LC method for related substances test of pentoxyfylline substance (Table No 6). EP method is based on linear gradient regimen with two phases and 45 min elution time, different column containing base-deactivated octylsilyl silica gel for chromatography R (5 μm) and column temperature about 30 ^{0}C.

Table No 6. Obtained data from proposed method (Method I) and EP LC method

Parameter	Method I	EP method
Selectivity	positive	positive
Precision	0.94 %	commensurable
Accuracy	± 0.42 %	± 1.0 %
LOD	0.5 μg/ml	0.2 μg/ml
LOQ	2 μg/ml	2 μg/ml
Linearity interval	16 – 80 μg/ml	commensurable
Relative retention against Impurity A	0.26	0.30
Relative retention against Impurity F	0.42	0.40
LOD of Impurity A	0.1 μg/ml	0.2 μg/ml
LOD of Impurity F	0.25 μg/ml	0.2 μg/ml
Run time	15 min	45 min

The tested procedures by above HPLC methods for pentoxifylline RS and in drug preparation are for identification, related substances and assays. For identification all of studied methods are suitable. LOD values are comparatively of the order of μg range. EP method is not validated for pentoxifylline in drug preparation or in other matrix, but there is a very good accuracy. Assays test depends prior to precision, accuracy, linearity and system suitability test. Some results obtained from different methods are similar but doubtless the favorite method is proposed method I because of better suitability and simplicity. In the cases when analysts will be obtained relative comparatively results the estimation is based on the studies for selectivity, linearity and resolution. These studies lead not only to an exact assessment but they make analysis more ensure and adequate to real state.

CONCLUSION

HPLC procedure for quality control of pentoxifylline was performed at different chromatographic conditions. The method was validated in respect of purposes of pharmaceutical practices. The developed RP-HPLC method was suitable for simultaneous qualitative and quantitative determination of pentoxifylline and its related substances in Pentoxypharm tablets and in raw materials.

Acknowledgments

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