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Development and Validation of a Stability Indicating RP-HPLC Method for Determination of Xanthinol Nicotinate in Bulk and Sustained Release Tablet Dosage Forms

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Abstract

The present paper deals with the development and validation of a stability indicating reverse phase HPLC method for the determination of Xanthinol nicotinate on Hypersil ODS C_{18} column (250mm × 4mm, 5µm). A mobile phase consisting of methanol: 0.01mol.L^{-1} TBAHS (50:50 % v/v) was used.Doxophylline was used as the internal standard. The flow rate was 0.8mL min^{-1}.The separation was performed at room temperature. Detection was carried out at 267nm by UV detection. The developed method was statistically validated for the linearity, accuracy, specificity, LOD and LOQ.The specificity of the method was ascertained by forced degradation studies by acid and alkali degradation, oxidation, photolysis and heat degradation. The degraded products were well separated from the analyte with significant differences in their Retention time values. Beer Law is obeyed over a concentration range of 1-400µg mL^{-1} and correlation coefficient was 0.9995.

Keywords:

Xanthinol nicotinate; nicotinic acid; forced degradation; RP-HPLC

1. Introduction

Xanthinol nicotinate is a peripheral vasodilator [1]. Chemically it is 7-[2-hydroxy-3-(N-methyl-β-hydroxythylamino)-propyl]theophylline nicotinate [2]. Extensive literature review reveals that a Spectrophotometric method using Charge Transfer Reaction[3],a LC-MS method for determination of Xanthinol nicotinate in biological fuilds [4], a capillary isotachophoresis method [5] have been reported. Also some HPLC methods including a stability indicating HPLC method has been reported [6,7]. But no stability indicating RP-HPLC method has been reported so far for the determination of Xanthinol nicotinate in Sustained Release Tablet Dosage Form using methanol:0.01 mol.L^{-1} TBAHS as mobile phase. In view of these points an attempt was made to develop a simple, accurate and validated stability indicating RP-HPLC method for determination of Xanthinol nicotinate in Sustained Release Tablet Dosage Form.

2. Materials and Methods

2.1. Instruments

Quantitative HPLC was performed on a binary gradient HPLC with Shimadzu LC-10AT and LC-10AT VP Series HPLC pumps, with a 20 µL sample injection loop (manual)

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and SPD 10A VP series UV-Visible detector. The output signal was monitored and integrated using Shimadzu Class-VP Version 6.12 SP1 Software. A Hypersil ODS C_{18} column (250mm × 4mm, 5µm) was used for separation. Afcoset analytical electronic balance was used. A Thermolab thermostat was used.The System Suitability Parameters,Optimized Chromatographic Conditions and the Degradation data by the proposed chromatographic method are summarized in Table 1,Table 2 and Table 3 respectively.

Table 1. System Suitability Parameters

Parameters	Obtained values
Theoretical plates	2450
Resolution	4.49
Tailing factor	1.25
LOD(µg/mL)	0.1570
LOQ(µg/mL)	0.5234

Table 2. Optimized Chromatographic Conditions

Parameters	Conditions
Stationary phase(column)	Hypersil ODS C_{18}(250mm × 4mm,5µm)
Mobile Phase	Methanol:0.01mol.L^{-1} TBAHS(50:50 % v/v)
Flow rate	0.8mL min^{-1}
Runtime	10min.
Column Temperature(ºC)	Ambient
Volume of Injection	20µL
Detection wavelength	267nm
Internal standard	Doxophylline
Drug Retention Time(min.)	2.475,3.225
Internal standard Retention Time(min.)	4.608

Table 3. Degradation data

Conditions	Time in hrs.	% Recovery	Retention time of Drug
0.1 N HCl	½ hr.	90.71	2.492 3.233
0.1 N NaOH	½ hr.	87.82	2.508 3.233
H_2O_2 (6% v/v solution)	½ hr.	75.52	2.492 3.225
Photolysis	3 hr	98.76	2.483 3.225
Heat (80 °C)	½ hr.	95.35	2.483 3.225

2.2. Reagents and Chemicals

Methanol HPLC grade, Tetrabutyl ammonium hydrogen sulfate (TBAHS), Hydrochloric acid, Hydrogen peroxide.

2.3. Procedure

2.3.1. Selection and preparation of Mobile Phase

Different mobile phase systems like methanol: water, acetonitrile: water, methanol: 0.01 mol.L^{-1} TBAHS were tried in order to determine the best composition for separation of Xanthinol nicotinate. It was found that methanol: 0.01 mol.L^{-1} TBAHS (50:50 % v/v) gives good separation results compared to others. In this particular mobile phase the Nicotinic acid present in the Xanthinol nicotinate was separated.

0.01 mol.L^{-1} TBAHS was prepared by dissolving 3.3954 gm of TBAHS salt in 1000 mL triple distilled water. The prepared mobile phase was ultrasonicated for 30 min. and was filtered through a 0.45 µ membrane filter. Method was developed using Doxophylline as the internal standard.

2.3.2. Preparation of Standard Stock Solution

100 mg of drug and internal standard was weighed accurately and transferred to a 100 mL volumetric flask .The drug was then dissolved in 50 mL of mobile phase,shaked and finally volume was made up to mark to get a concentration of 1000 µg mL^{-1}.Both solutions were filtered through a 0.2 µ membrane filter.

2.3.3. Preparation of Calibration Curve

Appropriate aliquots of the standard stock solution of Xanthinol nicotinate (1000 µg mL^{-1}) were transferred into a series of 10 mL volumetric flasks. Each flask contained internal standard Doxophylline at a concentration of 5 µg mL^{-1}. Final volume was made up to the mark with mobile phase. Each solution was injected and a chromatogram was recorded. The chromatogram of standard Xanthinol nicotinate is shown in Fig.1.

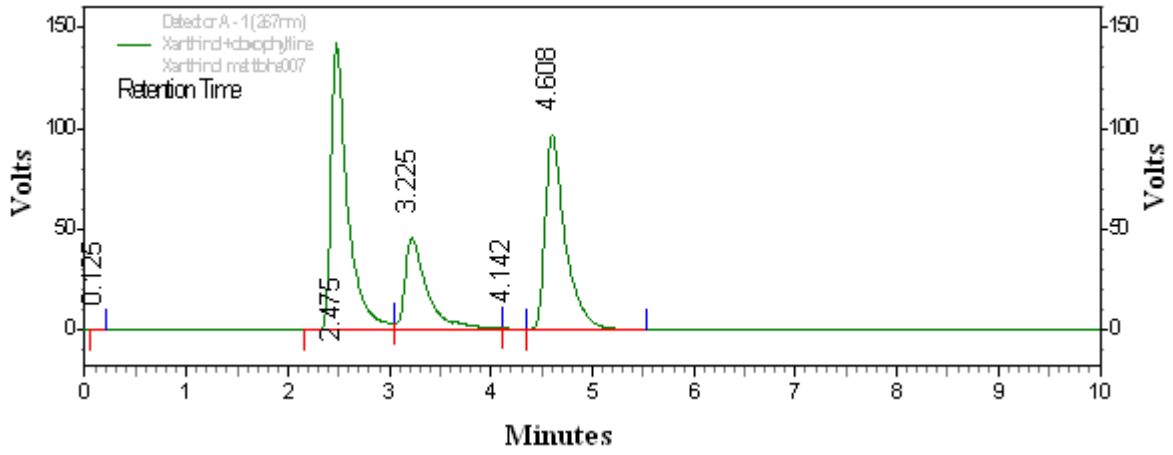

Fig.1 Xanthinol nicotinate10µg mL^{-1} (2.475, 3.225 min), Doxophylline 5 µg mL^{-1}(4.608 min) (Pure drug)

The chromatogram shows also the separation of Nicotinic acid present in the drug. The additional peak at retention time of 3.225 min was confirmed to be Nicotinic acid by injecting a known concentration of Nicotinic acid solution as shown in Fig.2. The peak for Nicotinic acid was found to be in proportion with Xanthinol throughout the linearity range. The internal

standard Doxophylline shows a retention time of 4.608 min. The Beer Lambert's Law was obeyed in a wide range of concentration from 1 to 400 µg mL^{-1} for Xanthinol nicotinate.The linearity of calibration curve was plotted for peak area ratio of drug to internal standard against concentration of drug.

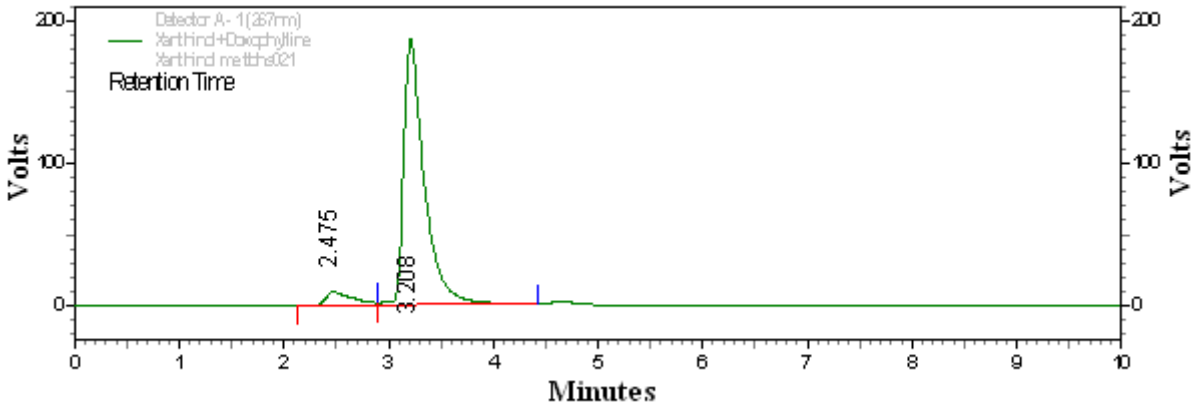

Fig.2 Nicotinic acid 10 µg mL^{-1} (Pure drug)

2.3.4. Procedure for Analysis of Sustained Release Tablet Dosage Form

20 tablets were weighed accurately; the average weight was determined and then ground to a fine powder. Powder equivalent to 100 mg of Xanthinol nicotinate was dissolved in 50 mL of mobile phase and ultrasonicated for 45 minutes. Then the volume was made up to the mark with the mobile phase and filtered through 0.22 µ membrane filter. The tablet solution was further diluted with the mobile phase to obtain sample solutions within the Beer's Law range. Sample solution of concentration 10 µg mL^{-1} and 20 µg mL^{-1} along with internal standard 5 µg mL^{-1} were prepared.

The sample solutions were injected and chromatograms were obtained. A representative chromatogram of Xanthinol nicotinate in formulation has been given in Fig.3. The amount of drug present in the sample solution was determined using the calibration curve of standard Xanthinol nicotinate. The results are given in Table 4.

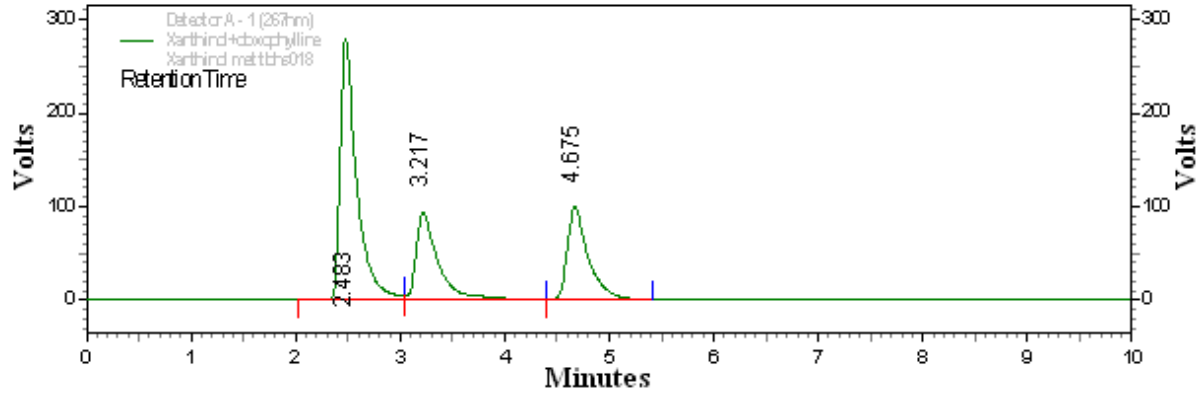

Fig.3 Xanthinol nicotinate 20 µg mL^{-1} (Formulation)

Table 4. Analysis of Commercial Formulation

Brand Name	Label Claim	% of label Claim Estimated*	Standard Deviation	%RSD
Complamina Retard SR Tablet	500 mg	105.14	0.3464	0.3294

*Average of six determinations

2.3.5. Precision

The precision of the method was ascertained separately from the peak area ratios obtained by actual determination of eight replicates of a fixed amount of drug and internal standard. The percent relative standard deviation was calculated.

2.3.6. Recovery Studies

To check the accuracy of the proposed method, recovery studies were carried out at 80,100 and 120 % of the test concentration as per ICH guidelines [8].The recovery study was performed 3 times at each level. The results of recovery study are given in Table 5.

Table 5. Recovery Studies

Type of Recovery, %	Amount Added Pure Drug (µg mL^{-1})	Amount Present Formulation (µg mL^{-1})	Recovery, %*	RSD, %†
80	8	10	99.2	0.2520
100	10	10	99.96	0.3513
120	12	10	99.96	0.4622

*Average of three determinations
† is the Relative Standard Deviation

2.3.7. LOD and LOQ

The LOD and LOQ were separately determined based on the S/N Ratio. For LOD the S/N ratio is 3:1 and for LOQ the ratio is 10:1.

2.3.8. Specificity

The specificity of the HPLC method was determined by complete separation of Xanthinol nicotinate in presence of its degradation products. The forced degradation of the drug was carried out with 0.1N HCl, 0.1N NaOH, 6% Hydrogen peroxide, Photolysis and Heat. The representative chromatograms of untreated drug and drug with degraded products in 0.1N HCl, 6% Hydrogen peroxide and Heat degradation at 80°C are given in Fig.4, 5, 6 and 7 respectively. The degradation data along with percent recovery of the drug by the proposed method are given in Table 3.

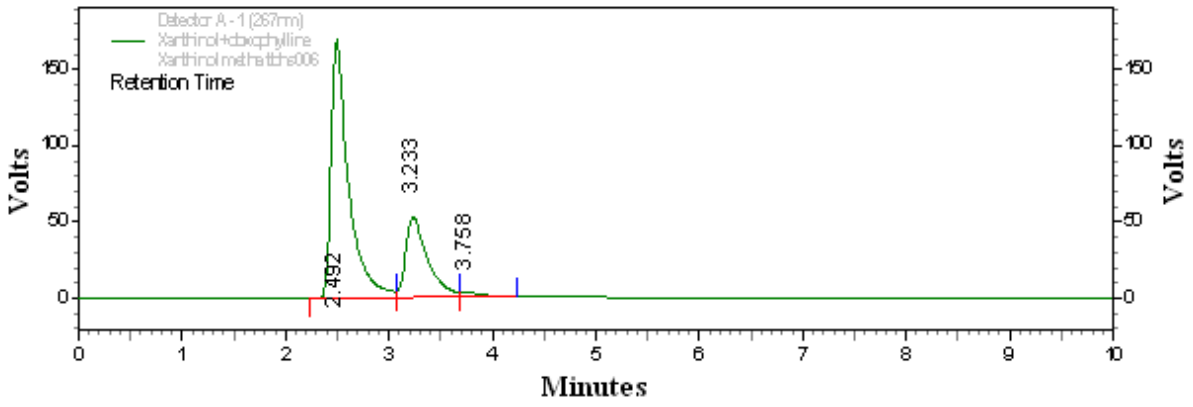

Fig.4 Untreated Xanthinol nicotinate 10 µg mL^{-1}

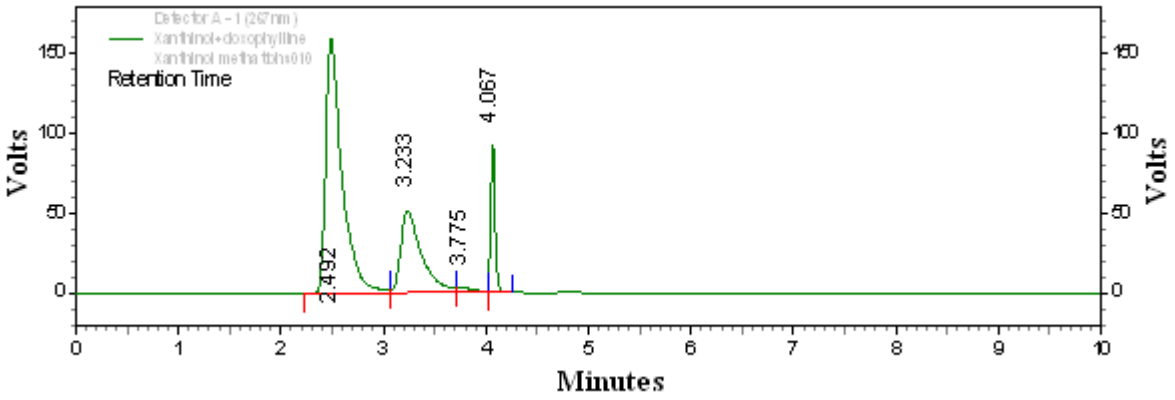

Fig.5 Xanthinol nicotinate 10 µg mL^{-1} in 0.1 N HCl

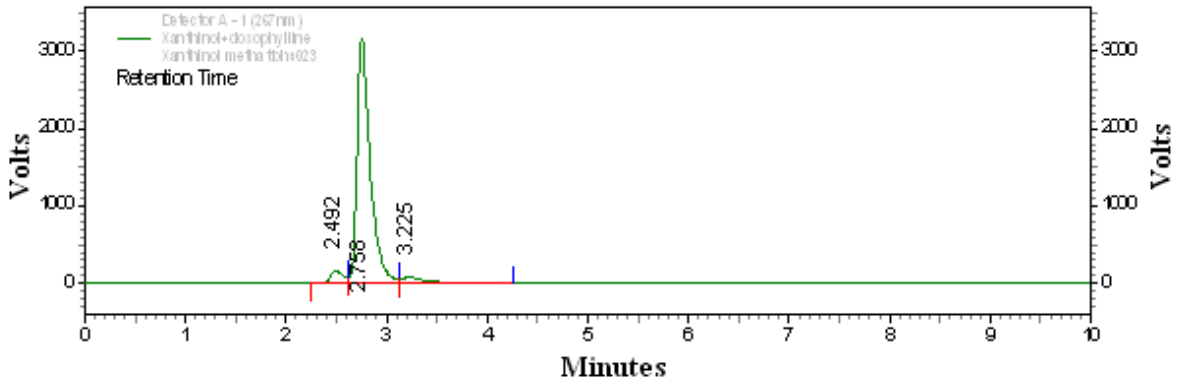

Fig.6 Xanthinol nicotinate 10 µg mL^{-1} in 6%H_2O_2

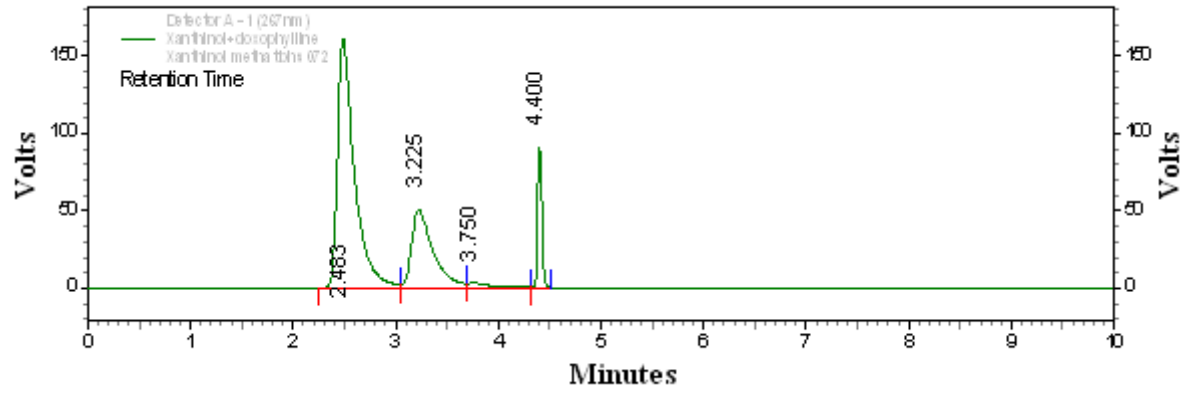

Fig.7 Xanthinol nicotinate 10 µg mL^{-1} at 80°C

3. Results and Discussion

Xanthinol nicotinate is a peripheral vasodilator drug used mainly for the treatment of peripheral vascular disease and disordered cerebral function. Literature survey shows that no stability indicating RP-HPLC method has been reported for Xanthinol nicotinate in sustained release tablet dosage form using methanol: 0.01 $mol.L^{-1}$ TBAHS (50:50 % v/v) as the mobile phase. So an attempt was made to develop a simple and accurate RP-HPLC method to determine Xanthinol nicotinate in presence of its degradation products.

A critical evaluation of the method was performed by statistical analysis of data where the slopes, intercepts and correlation coefficient are found to be 0.1008, 0.228 and 0.9995 repectively. The % recovery was found to be 105.14. The good % recovery in sustained release formulation suggests that the excipients present in the formulation have no interference in the determination.

The %RSD was also less than 2% showing high degree of precision of the proposed method. The developed method was also capable of determining Xanthinol nicotinate in presence of its degradation products.

4. Conclusion

It can be concluded that the developed RP-HPLC method is stability indicating simple, accurate, and precise and can be employed successfully for the determination of Xanthinol nicotinate in bulk and sustained release tablet dosage form.

Acknowledgements

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