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Estimation of Lutein from Rat Plasma by ESI-LC-MS/MS: A Pharmacokinetic Application

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Abstract: A selective, sensitive and fast High Performance Liquid Chromatography with mass spectrometric (MS) detection method was developed and validated in human plasma. Lutein was extracted from rat plasma via protein precipitate extraction (PPE) technique. An isocratic mode was used to separate interference peaks using an YMC Pack C18, 50 X 4. 6mm, 3µ column. The mobile phase composition was acetonitrile: ethanol (90:10, v/v). Linearity ranges were 2.151 ng/mL to 302.735 ng/mL. The retention time of Lutein was 0.97 min. This method was free from matrix effects and any abnormal ionization. This method was successfully applied to a pharmacokinetic study of Lutein.

Key words: Rat plasma · Lutein · Protein Precipitation Method · LCMS/MS

INTRODUCTION

Lutein [(3R,3'R,6'R)-β,ε-carotene-3,3'-diol] is a naturally occurring xanthophyll and oxycarotenoid (synonyms: vegetable lutein, vegetable luteol, Bo-Xan). Carotenoids are primarily synthesised by photosynthetic plants and micro-organisms and Lutein is one of the most abundant. It occurs with the isomeric xanthophyll, zeaxanthin [(3R,3'R)-β,β-carotene-3,3'diol], in many foods, particularly vegetables and fruits.

Lutein contains 2 cyclic end groups (one beta- and one alpha-ionone ring) and the basic C40 isoprenoid structure common to most carotenoids. Although the polyene chain double bonds present in Lutein could exist in a cis or trans conformation, the vast majority of carotenoids are in all-trans configurations. Lutein molecular formula is C40H56O2 and its molar mass is 568.87 g/mol. It is insoluble in water, but soluble in fats and lipophilic solvents.

Comprehensive exploration of literature for Lutein revealed several methods based on techniques viz. HPLC [1-5] for its determination in Lutein. So, in comparison with all other published methods, the present manuscript described a method with highest sensitivity and maximum throughput.The aim of this study was to establish a LC-MS/MS method for determining Lutein concentration in rat plasma. At the same time, method was efficient in analyzing large number of plasma samples obtained for pharmacokinetic, bioavailability or preclinical studies of Lutein.

Experimental

Chemicals and Reagents: Acetonitrile of HPLC grade was procured from JT Becker. Ethanol of HPLC grade was procured from Merck. Water HPLC grade was obtained from a Milli-Q water purification system. A reference standard of Lutein was provided by OmniActive Health Technologies Ltd India.

Instrumentation and Chromatographic Conditions: Ultra flow liquid chromatography Tandem Mass Spectrometry was used for the method development and validation. Mass Spectrometry Model API 4000, UFLC model UFLC XR equipped with a model LC-20ADXR a binary pump, SIL-20ACXR auto sampler was used to keep temperature at 5°C, CTO-20AC column oven used to keep temperature at 35°C and CBM-20Alite system controller. Detection was made at m/z 568.2/476.4 for Lutein using ESI Positive ion spray ionization mode. Analyst 1.5.1 software was used for the quantification. The stationary phase was YMC Pack C18, 50 X 4. 6mm, 3µ.

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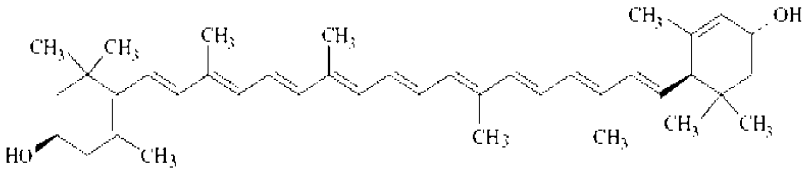

Fig. 1: Molecular Structure of Lutein

Method Optimization: The LCMS procedures were optimized with a view to develop a method of estimation for Lutein. The standard stock solution was diluted in mobile phase to a concentration containing 2 ng/mL of Lutein. Then, the stock solution is injected into the YMC Pack C18 column. Different ratio of mobile phase was tried. The optimal mobile phase was found to be acetonitrile: ethanol (90:10, v/v). The separation was carried out at ambient temperature with a flow rate of 0.600 mL. The injection volume was 10 µl and run time was 2.2 minutes. The RT of analyte was 1.83.

Preparation of Standard and Quality Control Samples: Stock solutions of Lutein were made up in ethanol at approximately 1 mg/ ml and these stock solutions were stored at 2-8°C. Working standard solutions of varying concentrations of Lutein were prepared on the day of analysis by diluting the stocks with mobile phase {Acetonitrile: Ethanol (90:10, v/v)}. Each day, before extraction, the calibration curve in rat plasma was prepared by spiking (10 µl) known amounts of Lutein into rat plasma (190 µl).

Calibration curve standards consisting of a set of eight non-zero concentrations ranging from 2.151 ng/mL to 302.735 ng/mL or Lutein were prepared. Prepared quality control samples consisted of Lutein concentrations of 12.533 ng/mL (QCL), 139.258 ng/mL (QCM) and 254.297 ng/mL (QCH). These samples were stored below -50°C until used.

Sample Extraction by Protein Precipitate Method: Calibration standards and QC samples were freshly spiked and processed. Exactly 200 µl of spiked plasma was pipette out into prelabelled polypropylene tubes, added 0.6 ml of mobile phase and vortex for 1 minute, centrifuged at 4000 RPM at 5°C for 10 minutes and 500 µl of supernatant diluted with 500 µl of mobile phase vortexed and these samples were transferred to the auto sampler vial and 10 µl was injected into the chromatographic system.

System Suitability: The system suitability was performed before starting each day's activity according to in-house and it was within acceptance criteria 4 %.

Linearity: Standard curves were constructed at concentrations 2.151, 5.994, 13.018, 29.668, 66.602, 145.313, 260.352 and 302.735 ng/mL of Lutein. The standard calibration curves were shown to be linear in the above mentioned range in wistar rat plasma. Curves were obtained by plotting the peak area against concentrations of these drugs. Linear calibration curves were generated by linear regression analysis and obtained over the respective standard concentrations ranges. The suitability of the calibration models were confirmed by back-calculating the concentrations of the calibration standards. The standard curve, slope, intercept and the correlation coefficient were determined.

Precision and Accuracy: The precision of the assay was measured by the percent coefficient of variation over the concentration range of QCL, QCM and QCH samples respectively during the course of sample analysis. The accuracy of the assay was defined as the absolute value of the ratio of the calculated mean values of the low, middle and high quality control samples to their respective nominal values, expressed in percentage.

Chromatography: Representative chromatograms of blank plasma, STD A, QCL and calibration curve of Lutein are given in Figure. 2 to 4.

Data Processing: The chromatograms were acquired and were processed by peak area ratio method using the Analyst Version 1.5.1 Software.

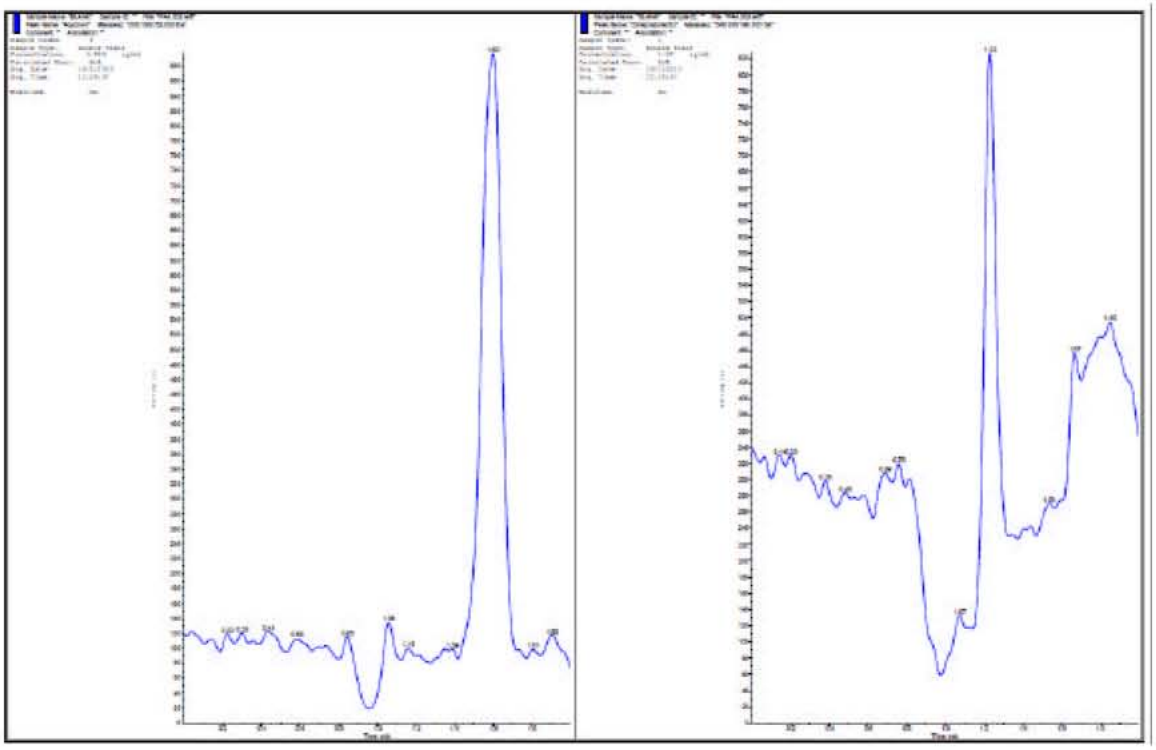

Fig. 2: A Representative Chromatogram of a Blank Plasma

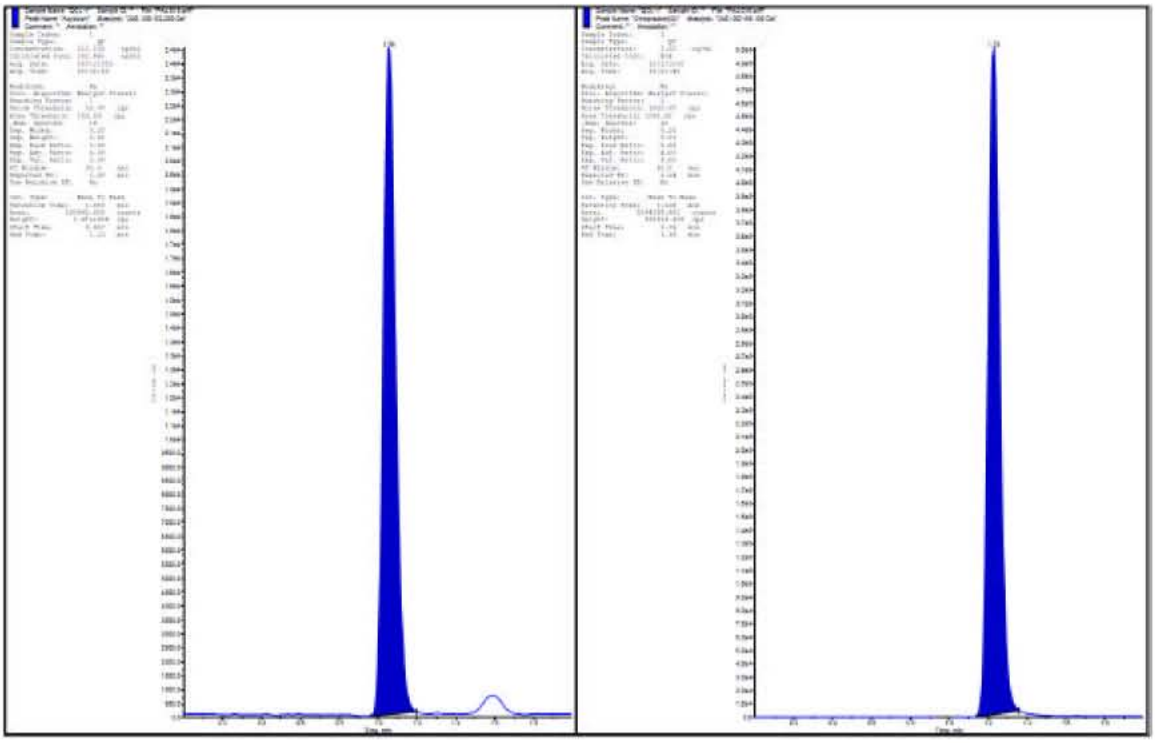

Fig. 3: A Representative Chromatogram of a QCL

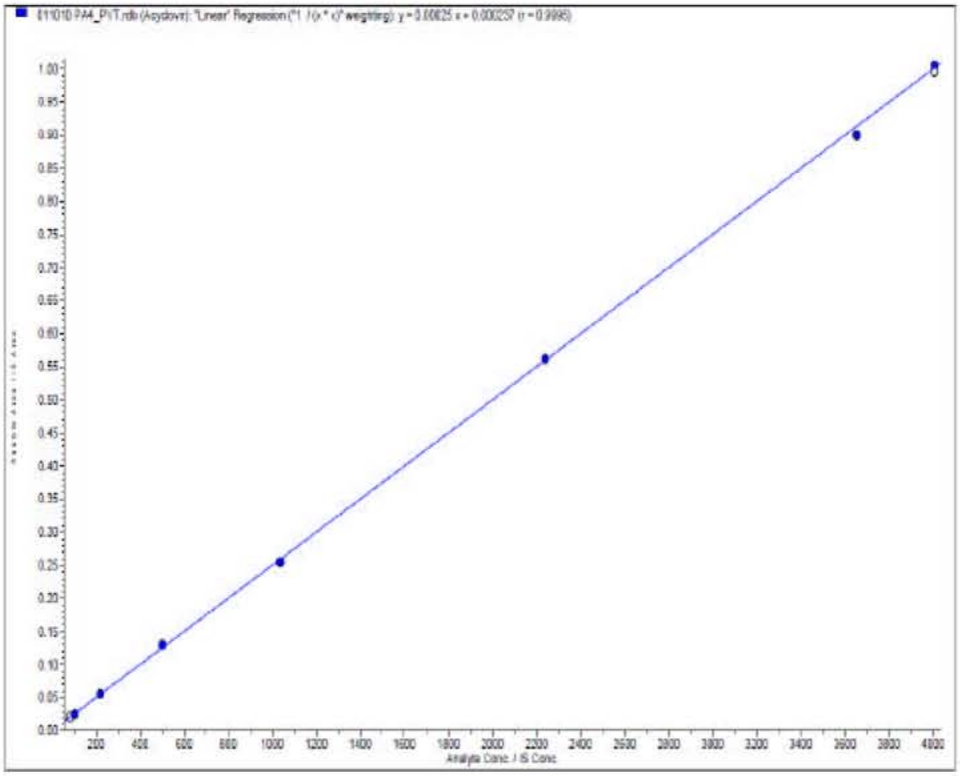

Fig. 4: A Representative Calibration Curve for Acyclovir

The concentration of the unknown was calculated from the following equation using regression analysis of spiked standard with the reciprocal of the ratio of the (drug concentration)^2 to internal standard concentration as a weighing factor ($1/x^2$):

$y = mx + c$

Where,

Y = Peak area ratio of Lutein to internal standard
M = Slope of calibration curve
X = Concentration of Lutein
C = Y-axis intercept of the calibration curve

CONCLUSION

An Isocratic LCMS method was successfully developed for the estimation of Lutein in rat plasma. The method was selective, precise, accurate, linear and robust. The run time (2.2 min) enables for rapid determination of drug. The developed method was successfully applied for bioavailability and preclinical studies

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