Optimization of HPLC method for determination of cholesterol

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Introduction

The aim of this work was to evaluate the optimal conditions for determination of cholesterol by HPLC chromatography when optimal composition of mobile phase, its influence on the retention time, absorption wavelength, and limit of quantification have been studied. The main parameters, which influence the effectivity of liquid chromatography are the composition of mobile phase, type of stationary phase, or the size particle of column. The cholesterol content can be determined by several analytical methods but the most used are gas or liquid chromatography. In the HPLC method, most of the mobile phases are composed of acetonitrile, isopropanol, or methanol[1].

Material and methods

HPLC analysis was performed using an Agilent Technologies 1260 infinity system equipped with a UV-DAD detector. Isocratic elution was performed at a flow rate of 0.5 mL.min^{-1} using the various mobile phase composed of water, methanol, and acetonitrile. The injection volume was 10 µL and the temperature was set at 30 °C. As a stationary phase, a Zorbax Eclipse Plus SB-C_{18} column (2.1x100 mm, 3.5 µm particle size) was used with the guard column Zorbax SB-C_{18} (4.6x12.5 mm, 5 µm particle size).

Conclusion

It can be concluded that the optimal mobile phase for cholesterol determination by HPLC is mixture of methanol and acetonitrile (40:60, v/v), with the retention time of cholesterol in 5,701 min at the flow rate of 0.5 mL.min^{-1} and the absorption wavelength at 205 nm. At these conditions, the LOQ was 24.21 µg.ml^{-1}.

Results and discussion

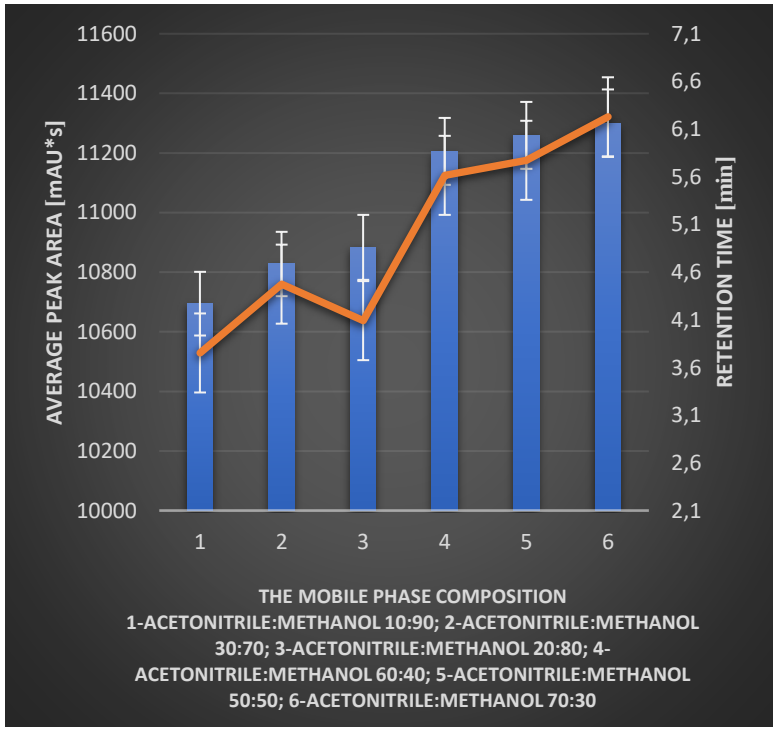

Fig. 1 The influence of the mobile phase composition on the peak area and retention time of cholesterol standard

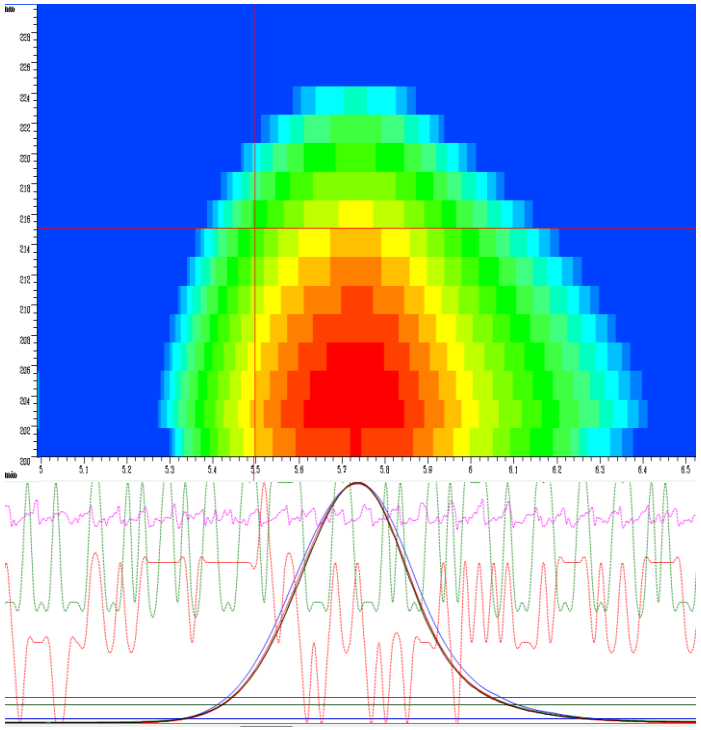

Fig. 2 The isothermal record of cholesterol standard. The red area refers to absorption maximum at 205 nm

REFERENCES

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ACKNOWLEDGMENT

This work is the result of the project implementation of grant APVV-061-2018 and ITMS 26230120009 financially supported by the Research & Development Operational Programme funded by the ERDF.