

# GC/MS 法测定人血清中角鲨烯与 6 种非胆固醇类固醇含量

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## Determination of Squalene and 6 Non-cholesterol Sterols in Human Serum by GC/MS

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**Abstract:** Besides cholesterol, a few of squalene and non-cholesterol sterols exist in human serum, which include cholesterol synthesis precursors (e.g. desmosterol and lathosterol) and plant sterols (e.g. campesterol, stigmasterol, sitosterol and cholestanol). In present studies, squalene and cholesterol precursors could be considered as cholesterol synthesis markers, which reflect synthesis efficiency of cholesterol, while plant sterols are considered as cholesterol absorption markers which reflect cholesterol absorption efficiency. Because of the similar molecule structure among cholesterol and non-cholesterol sterols, and the less content of non-cholesterol sterols, the present method, such as radioactive isotope, high-performance liquid chromatography (HPLC), and gas chromatography (GC), have some difficulties in the separation and quantification of non-cholesterol sterols.

In our studies, the method of sample preparation was improved, and the chemical derivation process was also optimized, and GC/MS were used in the quantitative determination of cholesterol and non-cholesterol sterols. Compared to GC method, our method has a better resolution and repeatability, and the accuracy is also good, so it is feasible to use the method in analysis for clinical individual difference of cholesterol metabolism. Serum of 30 patients of hyperlipemia were determined by our method, and the mean values of squalene and sterols are squalene  $0.18 \text{ mg} \cdot \text{dL}^{-1}$ , cholestanol  $0.69 \text{ mg} \cdot \text{dL}^{-1}$ , desmosterol  $0.03 \text{ mg} \cdot \text{dL}^{-1}$ , lathosterol  $0.31 \text{ mg} \cdot \text{dL}^{-1}$ , campesterol  $0.27 \text{ mg} \cdot \text{dL}^{-1}$ , stigmasterol  $0.05 \text{ mg} \cdot \text{dL}^{-1}$  and sitosterol  $0.39 \text{ mg} \cdot \text{dL}^{-1}$ . The total content of squalene and non-cholesterol sterols is  $1.53 \text{ mg} \cdot \text{dL}^{-1}$ , which is 0.89% of cholesterol.

Linear regression analysis is made between all kinds of sterols. Cholesterol has positive correlation with all 6 non-cholesterol sterols. Whereas, the positive correlations with cholestanol and stigmasterol are not significant probably due to the serum quantity is not enough. More serum data will need to obtain more representative results.

**Key words:** non-cholesterol sterol; squalene; cholesterol; derivation; gas chromatography mass spectrometry

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人体血清中除含有胆固醇 (cholesterol) 外, 还含有微量的角鲨烯 (squalene) 和非胆固醇类固醇。非胆固醇类固醇包括胆固醇合成的前体物质, 如 2,4-脱氢胆固醇 (desmosterol), 7-烯胆固醇 (lathosterol); 源自膳食的植物固醇, 如菜油固醇 (campesterol), 豆固醇 (stigmasterol),  $\beta$ -谷固醇

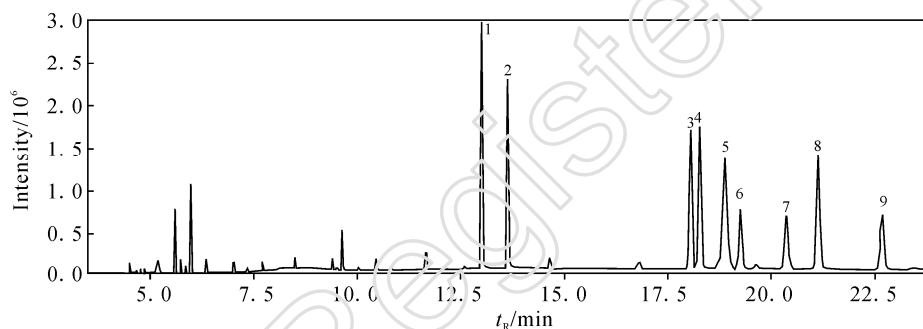
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醇 (sitosterol) 和二氢胆固醇 (cholestanol)。最近研究发现, 血清中的角鲨烯和胆固醇前体固醇可作为胆固醇合成标志物 (cholesterol synthesis markers) 反映机体的内源性胆固醇合成效率, 植物固醇可作为胆固醇吸收标志物 (cholesterol absorption markers) 反映机体的外源性胆固醇吸收效率<sup>[1]</sup>。由于以上非胆固醇类物质与胆固醇的结构相似、化学性质相近, 但含量比胆固醇要低很多, 已有的放射性同位素法、高效液相色谱法、气相色谱法在测定这些固醇类物质常会受到不同程度的干扰, 影响了其在血清样品中的准确定量<sup>[2-4]</sup>。

本工作改进了样品的前处理方法, 优化了化学衍生法的最佳条件, 利用气相色谱-质谱联用法 (GC/MS) 同时测定了血清中角鲨烯与 6 种非胆固醇类固醇。本方法与气相色谱法相比, 分离度好、重现性好、精密性高, 适于大规模研究和检测试验研究过程中胆固醇吸收与合成的变化, 可为个体化治疗中临床诊断提供依据。针对目标化合物, 对 30 例高血脂患者的血清进行含量测定, 平均值分别为角鲨烯  $0.18 \text{ mg}\cdot\text{dL}^{-1}$ 、二氢胆固醇  $0.69 \text{ mg}\cdot\text{dL}^{-1}$ 、2, 4-脱氢胆甾烷醇  $0.03 \text{ mg}\cdot\text{dL}^{-1}$ 、7-烯胆甾烷醇  $0.31 \text{ mg}\cdot\text{dL}^{-1}$ 、菜油固醇  $0.27 \text{ mg}\cdot\text{dL}^{-1}$ 、豆固醇  $0.05 \text{ mg}\cdot\text{dL}^{-1}$  和谷固醇  $0.39 \text{ mg}\cdot\text{dL}^{-1}$ 。血清中 6 种固醇及角鲨烯的总和平均值为  $1.53 \text{ mg}\cdot\text{dL}^{-1}$ , 约为胆固醇总量的 0.89%。

另外, 对血清中非胆固醇类固醇及胆固醇之间进行了线性回归分析, 所有 6 种非胆固醇类固醇均与胆固醇成正相关, 但二氢胆固醇、豆固醇与胆固醇的正相关不显著, 进一步的规律还需大量样品的数据, 进行统计分析, 得出更具代表性的结果。



注: 1. 角鲨烯; 2. 内标; 3. 胆固醇; 4. 二氢胆固醇; 5. 2,4-脱氢胆甾烷醇; 6. 7-烯胆甾烷醇; 7. 菜油固醇; 8. 豆固醇; 9.  $\beta$ -谷固醇

图 1 角鲨烯和 6 种固醇类物质标准品衍生后的固醇总离子流图

Fig. 1 The total ion chromatogram of squalene and derivatized standard sterols

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