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Determination of Cholesterol and its Oxides

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Introduction

Several cholesterol oxidation products might play important roles in the development of atherosclerosis [1]. This concept is fostered by mounting interest in the role of oxidatively modified lipoproteins and cholesterol in atherogenesis. Cholesterol oxides are widely encountered in foods and biological tissues. Here the analysis of plasma cholesterol oxidation products by high-performance liquid chromatography-mass spectrometry (HPLC-MS) [2,3] is described

Protocol

1. Bllod is drawn into 10-mL Vacutainer tubes containing EDTA (15% w/v, 0.10 mL).
2. Lipids are extracted from plasma (1.0-mL) in 12-mL borosilicate screw cap tubes by addition of chloroform-methanol (2:1, v/v 6.0 mL) containing BHT (0.01% w/v).
3. Internal standard stock solution (5α-cholestane in toluene, 1.0 mg mL^{-1}, 100 µL) is added to each sample and the tubes are sealed under argon and mixed in a rotary drum for 30 min at room temperature.
4. After centrifugation at 3,000 rpm for 15 min, the organic phase is isolated and the aqueous phase is re-extracted with chloroform-methanol (3.0 mL).
5. The pooled organic phases are evaporated to dryness under nitrogen and the residue, dissolved in ethanol or hexane-isopropanol (1:1, v/v), is analysed by HPLC-MS. (HPLC-MS analysis was performed by means of a Hewlett-Packard HPLC 1090A chromatograph, a particle beam interface, and a Hewlett-Packard 5988A mass spectrometer. Compounds were separated on an Altex-Ultrasphere-Si 250 column with 96:4 (v/v) hexane-isopropanol as mobile phase at a flow rate of 0.4 mL min^{-1}).

Results

The principal ions monitored for analysis of cholesterol oxides are listed in Table 1.

Conclusion

Together with HPLC-MS, capillary gas chromatography-mass spectrometry (GC-MS) can also be used for the analysis of cholesterol oxides after derivatization, usually to the trimethylsilyl ethers.

References

1. Peng SK, Hu B, Morin RJ (1992) Effects of cholesterol oxides on atherogenesis. In Biological Effects of Cholesterol Oxides. CRC Press London 167-189.
2. Sevanian A, McLeod LL (1987) Cholesterol autoxidation in phospholipid membrane bilayers. Lipids 22: 627-636.
3. Sevanian A, Seraglia R, Traldi P, Rossato P, Ursini F, et al. (1994) Analysis of plasma cholesterol oxidation products using gas- and high-performance liquid chromatography/mass spectrometry. Free Radic Biol Med 17: 397-409.

Compound name	Major ions	Minorions	Detection limit (ng)
Cholesterol	386, 275	301, 368, 353	0.70
Cholestanetriol	402, 384	369, 303,331	1.50
Cholesterol-5β,6β-epoxide	402	384, 369, 275	1.20
Cholesterol-7-hydroperoxide	400	367, 382, 287	0.75
7-Ketocholesterol	400	367, 382, 287	0.75
5α-Cholestane	372	-	-
7a-Hydroxycholesterol	384	402, 369	1.0
Cholesta-3, 5-diene-7-one	174	382, 367, 398	1.0
Cholesterol-5α,6α-epoxide	402	384, 369, 275	1.20
25-Hydroxycholesterol	402	400	1.0

Table 1: The principal ions monitored for analysis of cholesterol oxides by HPLC-MS with the particle-beam interface.

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