

Determination of 4-Hydroxy-2-Nonenal in Plasma

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Introduction

4-Hydroxy-2-nonenal (HNE) is a peroxidation product of n-6 poly-unsaturated fatty acids, which probably requires traces of catalytic iron for its formation.

Spectrophotometric, thin-layer chromatographic (TLC), and high-performance liquid chromatographic (HPLC) techniques for determination of HNE in fluids and cellular preparations are covered in detail elsewhere [1,2]. Here we describe a GC-MS method for use with plasma. HNE reacts with proteins to form stable complexes. This technique measures only 'free' HNE in plasma [3].

Protocol

1. Place freshly taken plasma (1.0 mL), BHT (2.0 mM, 0.010 mL), and nonanoic acid (internal standard; 0.006 mL) in new clean glass tubes.
2. Mix and divide into equal parts 'A' and 'B'.
3. Add chloroform-methanol (2: 1 v/v, 4.0 mL) and NaCl (0.15 M, 0.4 mL) to each tube.

4. Purge with oxygen-free nitrogen for 2 min, cap, and vortex mix for 30 s.
5. Centrifuge at 5,000 rpm for 6 min at 4°C.
6. Remove lower (chloroform) layer, and store at 4°C \bar{A} , under N_2 .
7. Extract the remaining (upper) residue with chloroform-methanol (2:1 v/v 2 mL) by vortex mixing for 30 s.
8. Centrifuge at 5,000 rpm for 6 min at 4°C.
9. Collect the lower phase and combine with previous extract.
10. Evaporate combined extracts to dryness under N_2 at 25°C.
11. Store at -20°C for less than 1 week.
12. Derivatize the samples:
 - a. Add dry acetone (0.2 mL) and bis(trimethylsilyl) trifluoroacetamide (BSTFA) containing 1% (w/v) trimethylchlorosilane (0.2 mL) to the dried extracts.
 - b. Cap tubes and leave for 60 min at 25°C.
 - c. Centrifuge at 6,000 rpm for 5 min to deposit residues.
13. Analyse by GC-MS.

Calculation

Selected ions characteristic of HNE-TMS are m/z 129, m/z 157, and m/z 199; for nonanoic acid-TMS the ions are m/z 117, m/z 129, and m/z 215. Quantitation is achieved by preparing a standard curve relating to m/z 117 for nonanoic acid-TMS and m/z 157 for HNE-TMS.

Results

A typical mass spectrum is shown in Figure 1.

References

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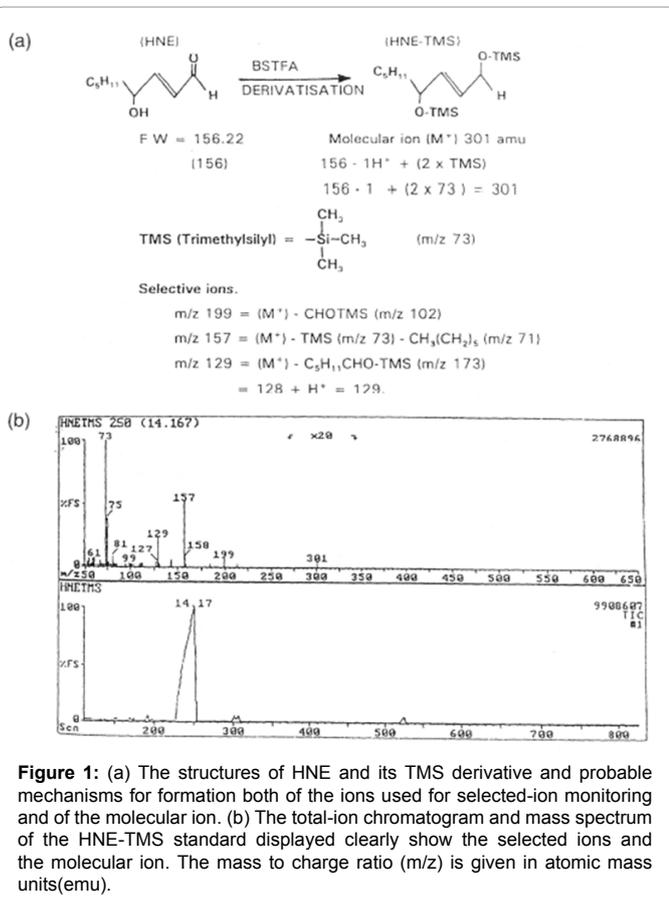


Figure 1: (a) The structures of HNE and its TMS derivative and probable mechanisms for formation both of the ions used for selected-ion monitoring and of the molecular ion. (b) The total-ion chromatogram and mass spectrum of the HNE-TMS standard displayed clearly show the selected ions and the molecular ion. The mass to charge ratio (m/z) is given in atomic mass units(emu).

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