Identification of 2-alkenals, 4-hydroxy-2-alkenals and 4-hydroxy-2,6-alkadienals in Exhaled Breath Condensate by UHPLC-HRMS and Breath Analysis in Real Time

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Introduction

- Breath analysis has become a non-invasive alternative for the diagnosis of several diseases
- Exhaled aldehydes, produced by lipid peroxidation, have been proposed as a way for assessing oxidative stress
- UHPLC-HRMS/MS shows the capabilities needed to properly identify these non-volatile compounds in Exhaled Breath Condensate

Results

Identified aldehydes

- Six 2-alkenals
- Eleven 4-hydroxy-2-alkenals
- Nine 4-hydroxy-2,6-alkadienals

- For aldehydes from 6 to 16 carbons, chromatographic signals were found for [M+NH₄]⁺ and [M+H]⁺ adducts in positive ion mode ESI and for [M+HCOO]⁻ adducts in negative ion mode ESI

UHPLC separation of EBC

- The main limitation of UHPLC-HRMS is that compounds with less than 6 carbon atoms are not detected, probably because of their high volatilities
- On the other hand, the method is capable of detecting aldehydes with up to 16 carbons that are rarely analyzed by GC or SIFT-MS because of their high molecular weights (>300u) and their low volatilities

Secondary-ESI Breath Analysis in Real Time

- The aldehydes identified in EBC can also be detected in breath in real time, a technique more suitable for diagnosis purposes
- The inlet of a LTQ Orbitrap mass spectrometer was modified to set a new low flow secondary electrospray ionization source (LF-SESI) that allowed the admission of breath samples through a heated tube

UHPLC-HRMSⁿ comparison with Standards

- One of the main advantages of UHPLC-HRMS is the possibility of acquiring tandem mass spectra that greatly increase the structural information obtained and, therefore, allow much better compound identification
- To further improve the fidelity of the identification of the groups of aldehydes studied, a set of standards were analyzed. MS analyses of these standards showed excellent matches, regarding retention times, MS² spectra and MS³ spectra

Conclusions

- The use of UHPLC-HRMS has allowed the identification of a set of aldehydes, metabolites of lipid peroxidation, in EBC
- This identification has been possible thanks to the capabilities of UHPLC-HRMS for identifying unknowns by means of retention times, tandem mass spectra that allowed the generation of fragmentation trees, and comparison with standards
- It was also demonstrated that these compounds can be analyzed, and confirmed by tandem MS, in breath in real time
- This technique could be of great interest for diagnosis of diseases related with oxidative stress, such as COPD or cancer

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