

ABSTRACT

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Title of the diploma thesis: Development and validation of UHPLC-MS/MS method for determination of urea in exhaled breath condensate and its application in clinical research

Key words: urea, exhaled breath condensate, UHPLC-MS/MS

Exhaled breath condensate analysis is a simple non-invasive method of measuring biomarkers not only from the respiratory tract. For the proper clinical interpretation of the measured data it is necessary to find the dilution biomarker due to dilution of target analytes with condensed water vapour. Urea seems to be the molecule of choice.

The aim of this diploma thesis was to develop a fast, simple and sensitive analytical method for determining urea concentration levels in exhaled breath condensate for clinical research.

Ultra-high performance liquid chromatography with mass spectrometry detection was chosen for urea analysis. Kinetex HILIC 100 A column, 1.7 µm particle size, 100 × 2.10 mm (Phenomenex, USA) was used for the separation. The mobile phase was composed of acetonitrile, methanol and water in a ratio of 81 : 9 : 10, the organic part was enriched with 0.1% formic acid. An isotopically labeled urea molecule (¹⁵N₂-urea) was used as an internal standard.

After optimizing the extraction process, separation and detection conditions, the newly developed method was applied to exhaled breath condensate samples from 54 patients. Urea concentrations ranged from 0.22 µmol/l–11.95 µmol/l.