Overview

LC-MALDI-MS/MS analysis of talinolol phase I metabolites

Application sub 2 µm particle for separation of isotopic metabolites

Online LC/MS with fraction collection on MALDI plates

MALDI on a triple quadrupole linear ion trap

Investigations of various MALDI matrices for low molecular weight compounds (CHCA, DHB, SA)

Introductions

Conventional liquid chromatography suffers from limited separation efficiency. This is particularly obvious in case of drug metabolite analysis, in which samples need to be operated at higher pressure (up to 1500 bars). Furthermore, the chromatographic peak width is determined from typically 5 to 10 min by the analysis time which conflicts with the daily use of MS/MS in most laboratories. A solution to this problem is to collect the samples in multiple fractions to allow re-analysis of the chromatographic peak. The present work was aimed to evaluate the use of LC-MALDI-MS/MS for the analysis of metabolites of talinolol generated in-vitro. In this analytical system, the peak width is reduced to approx. 2 to 3 min. Analysis time of the MALDI fraction collection is therefore decreased from typically 10 min to less than 1 min which conflicts with the daily use of MS/MS in most laboratories. A solution to this problem is to collect the samples in multiple fractions to allow re-analysis of the chromatographic peak. The present work was aimed to evaluate the use of LC-MALDI-MS/MS for the analysis of metabolites of talinolol generated in-vitro. In this analytical system, the peak width is reduced to approx. 2 to 3 min. Analysis time of the MALDI fraction collection is therefore decreased from typically 10 min to less than 1 min.

Results

1 LC separation with performance with sub 2 µm particle

3 MALDI-MS/MS and MS/MS spectra of talinolol metabolites

Microsomes incubations

The incubations of talinolol have been made in two types of microsomes, i.e. human liver microsomes (HLM) and rat liver microsomes (RLM). The experiments were performed to obtain metabolites of talinolol. The incubations of the samples were performed at 37 °C by shaking at 100 rpm. The samples were incubated for 1 h by adding 750 µL of acetonitrile. A subsequent centrifugation for 10 min at 4 °C and 10000 rpm has been then performed after a given time (2, 6, 24 or 48 h) and the supernatant was taken out and evaporated to dryness. The pellets have been then re-suspended in 100 µL water or 100 µL water and 100 µL of acetonitrile. 2.5 µl injected

Gradient from 5% B to 100% B in 3.75 min

Flow rate 0.5 ml/min

100% B: 5/95 H2O (UpChurch)

Column: Hypersil GOLD (Thermo Scientific)

Mass Spectrometry (MALDI)

MALDI on a triple quadrupole linear ion trap

PepMap C18 3.5

Log 0.5

N

1000. The matrix was ejected with an accumulation time of 200 ms over a mass range of m/z 100-1000.

LC-MS and MALDI spotting

LC-MS analysis was performed on a QTRAP 5500 using the Turbo ion source in the positive ionization mode. MS/MS data were acquired with an accumulation time of 600 ms over a mass range from 100 to 1000. The mass spectrometer used was a QTRAP 5500 by Applied Biosystem (MH). In this system, samples were injected post-column at a flow rate of 5 to 10 µl/min. The concentration of the matrix was 0.5-4% (v/v) of CHCA, DHB, SA or sinapinic acid. The MALDI laser was operated with a frequency of 0.1-0.3 Hz.

MALDI - O TRAP analysis

The MALDI-TOF TRAP was operated with a high repetition frequency operated at 300 µm and 2.5 µJ. The laser frequency was set between 1000 and 10000 Hz. Data were acquired in the linear mode (0.5-100 Hz) or in the asynchronous mode. For enhanced peak identification, nitrogen was used as a collimating gas.