Analysis of Metabolites by UHPLC-MALDI-MS/MS on a Triple Quadrupole Linear Ion Trap

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Overview

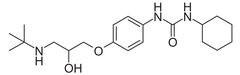
LC-MALDI-MS/MS analysis of talinolol phase I metabolites

1. Application sub 2 µm particles for separation of isobaric metabolites
2. On-line LC-MS with fraction collection onto MALDI plates
3. MALDI on a triple quadrupole linear ion trap
4. Investigations of various MALDI matrices for low molecular weight compounds (CHCA, DBH, SA)

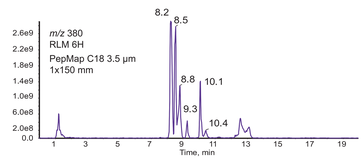
Introduction

Conventional liquid chromatography often suffers from limited separation efficiency. This is particularly critical in phase I drug metabolism studies were many isobaric metabolites can be generated. Using smaller particle sizes (sub 2 µm) significant gains in chromatographic efficiency can be obtained even at higher flow rates. The consequences are that the columns need to be operated at higher pressure (up to 1000 bars). Also the chromatographic peak width is decreasing from typically 10-20 s. to a few seconds which conflicts with the duty cycle in MS/MS of most instruments. A solution is to fraction collect the samples to eliminate the time constraints.

In the present work were evaluated the use of UHPLC-MALDI-MS/MS for the analysis of metabolites of talinol generated in-vitro. An analytical system was put in place which allows direct LC-MS analyses of the in-vitro samples, fraction collection onto a MALDI plate and subsequent re-analysis of the fraction of interest on a triple quadrupole linear ion trap equipped with a MALDI source using a high repetition frequency laser. The stragegy is similar to that presented previously by our group with the difference that the analytes are collected onto a MALDI plate instead of into a 96-wells plate [1].
Talinolol (Cordanum®) a racemic, highly selective, a1-adrenoceptor antagonist has been used in once daily dosages as a therapy for arterial hypertension and coronary heart disease, in Germany and Eastern Europe, for many years. In-vitro, mainly isobaric hydroxylate metabolites are generated which are challenging to separate.



Structure of Talinolol - MW = 364 u

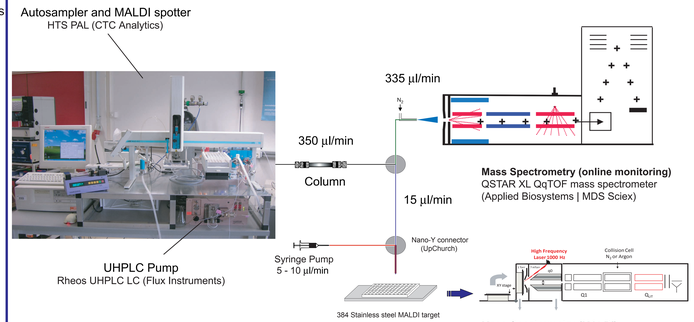


Typical LC-MS chromatogram for hydroxylated talinolol metabolites

[1] R.F. Staack *et al., Rapid Commun. Mass Spectrom.* **2005**, 19: 618-626

Methods

Instrumental Setup



Microsomes incubations

The incubations of talinolol have been made in two types of microsomes *i.e.* in human liver microsomes (HLM) and in rat liver microsomes (RLM). The experiments were designed to obtain metabolites phase I. All concentrations that are given hereafter are final concentration in the incubation mixtures. A pre-incubation on ice has been performed for the first 3 minutes at 37°C by adding appropriate volume of phosphate buffer (250 µL – volume of other ingredients), 1mg/mL of microsomes (HLM or RLM) and 100 µM of talinolol. The incubations were then initiated by adding 1.2 mM NADPH. Incubations have been quenched after 6, 24 or 48 h by adding 750 µL of acetonitrile. A subsequent centrifugation for 10 min at 4°C and 10000 rpm has been then performed and the supernatant taken out and evaporated to dryness. The pellets have been then re-suspended in 100 µL or 50 µL of MeOH/H_2O (10:90, v/v).

Liquid Chromatography

Column A: Hypersil GOLD (Thermo Scientific)
50 x 2.1 mm, 1.9 µm, flow rate 350 µl/min
Gradient from 5% B to 100 % B in 3.75 min
A: 95/5 H_2O/CH_3CN (v/v) 0.1 % HCOOH
B: 5/95 H_2O/CH_3CN (v/v) 0.1 % HCOOH
2.5 µl injected

Column B: Hypersil GOLD (Thermo Scientific)
200 x 2.1 mm, 1.9 µm, flow rate 350 µl/min
Gradient from 5% B to 100 % B in 15 min
A: 95/5 H_2O/CH_3CN (v/v) 0.1 % HCOOH
B: 5/95 H_2O/CH_3CN (v/v) 0.1 % HCOOH
10 µl injected

LC-MS and MALDI spotting

LC-MS analysis was performed on a QSTAR XL using the Turbo Ionspray interface in the positive ionization mode. TOF data were acquired with an accumulation time of 200 ms over a mass range of *m/z* 50 to *m/z* 1000. The matrix α-cyano- 4-hydroxy cinnamic acid (CHCA), 2,5-dihydrobenzoic acid (DHB) or sinapinic acid (SA) was added post-column at flow rate of 5 to 10 µl/min. The concentration of the matrix was of 5 mg/ml in 25/75 H_2O/CH_3CN (v/v) 0.1 % HCOOH (v/v). The LC effluent was spotted onto the MALDI target at a frequency of 0.3-1.0 Hz.

MALDI - Q TRAP analysis

The MALDI-Q TRAP was equipped with a high frequency laser operating at 355 nm (Nd:YAG x 3). The laser frequency was set between 200-1000 Hz. Data were acquired in the rastering mode (0.5-1 mm/s) or in the discrete mode. For enhanced product ion spectra, nitrogen was used as a collision gas.

Results

1 LC separation performance with sub 2 µm particle

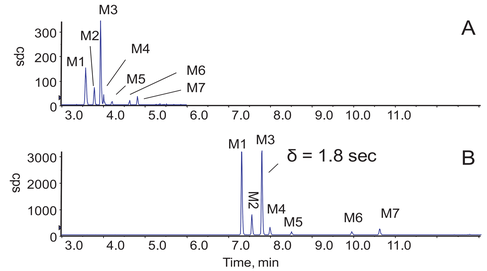


Figure 1: LC-MS trace at *m/z* 380 corresponding to the hydroxylated metabolites of talinolol (6H incubation of talinolol in human liver microsomes).
A) short column 50 x 2.1 mm, B) long column 200 x 2.1 mm the pressure was about 700-800 bars

2 LC-MALDI-MS/MS analysis

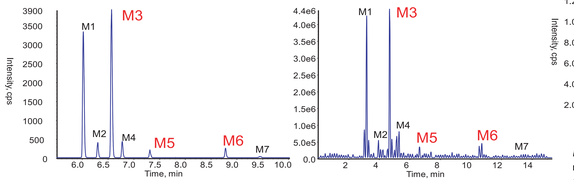


Figure 2: LC-MS trace at *m/z* 380 corresponding to the hydroxylated metabolites of talinolol (24H incubation of talinolol in human liver microsomes).
LC column 200 x 2.1 mm

Figure 3: LC-MALDI trace at *m/z* 209 of the enhanced product ion specrum of the precursor at m/z 380 (24H incubation of talinolol in human liver microsomes).
LC column 200 x 2.1 mm, DHB was used as matrix

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

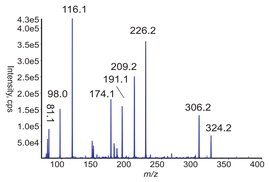


Figure 4: MALDI Enhanced product ion spectrum of metabolites M3 (precursor ion *m/z* 380) collision energy 40 eV.

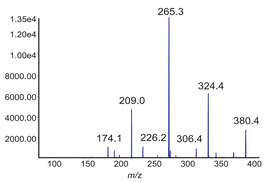


Figure 5: MALDI Trap product ion spectrum of metabolites M3 (precursor ion *m/z* 380) collision energy 10 eV, AF 60.

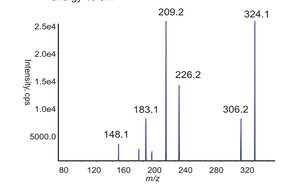
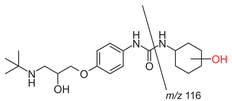


Figure 6: MALDI MS^3 spectrum of metabolite M3 precursor *m/z* 380 → *m/z* 324 collision energy 40 eV, AF 60.



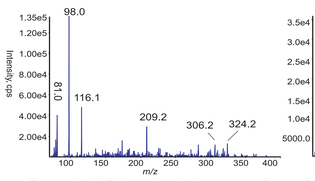
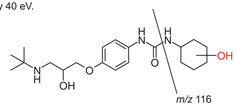


Figure 7: MALDI Enhanced product ion spectrum of metabolites M5 (precursor ion *m/z* 380) collision energy 40 eV.



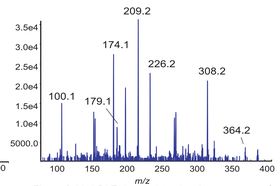
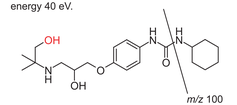


Figure 8: MALDI Enhanced product ion spectrum of metabolites M6 (precursor ion *m/z* 380) collision energy 40 eV.



Conclusions

- The seven hydroxylated metabolites of talinolol were baseline separated with the UHPLC 200 x 2.1 I.D. column
- Good quality MALDI MS/MS and MS^3 spectra could be obtained for the metabolites with DHB as a matrix
- It was not possible to use CHCA as a matrix due to strong matrix interferences. SA give also good results
- Idealy the system should be run with micro UHPLC columns
- With the MALDI fraction collection, the LC separation can be archived and the samples can be re-interrogated at any time