Supporting Information

Fast Online Separation and Identification of Electrochemically Generated Isomeric Oxidation Products by Trapped Ion Mobility-Mass Spectrometry

Jens Fangmeyer, Simon G. Scheeren, Robin Schmid, Uwe Karst*

Westfälische Wilhelms-Universität Münster, Institut für Anorganische und Analytische Chemie, Corrensstraße 30, 48149 Münster, Germany

Corresponding author

*Uwe Karst, e-mail: uk@uni-muenster.de

telephone: +49 251 83-36658, fax: +49 251 83-36013

SI-1: Settings and operating parameters of the mass spectrometer

Electrochemical oxidation and detection of Metoprolol and its metabolites by means of EC/MS.

All mass spectra were recorded using a timsTOF Pro (Bruker Daltonik, Bremen, Germany) with the following parameters: ESI(+); nebulizer (N_2), 0.3 bar; dry gas (N_2), 3.0 L/min; dry heater, 200 °C; capillary, 3.5 kV; endplate offset, -500 V; funnel 1 RF, 115.0 Vpp; funnel 2 RF, 300.0 Vpp; isCID energy, 0.0 eV; multipole RF, 200.0 Vpp; deflection delta, 220.0 V; quadrupole ion energy, 5.0 eV; low mass, mass-to-charge-ratio (*m*/*z*) 50.0; collision energy, 10.0 eV; collision RF, 500.0 Vpp; transfer time, 70.0 µs; pre pulse storage, 5.0 µs; *m*/*z* 100-1000. Each measurement was performed three times in order to guarantee reproducibility.

Online separation of Metoprolol and its oxidative electrochemical generated metabolites via trapped ion mobility mass spectrometry.

TIMS separation experiments were performed using above mentioned timsTOF Pro. Nitrogen was used as drift gas at a temperature of 300 K. The gas flow velocity was adjusted by the pressure difference between the entrance and the exit funnel set to 2.5 and 0.8 mbar, respectively. Additional to the general mass spectrometric conditions, six different delta potentials were set for TIMS separation: deflector (transfer electrode) / capillary exit (∆1), 0 V; deflector (transfer electrode) / deflector (discharge electrode) (∆2), -120 V; funnel 1 entrance / deflector (transfer electrode) (∆3), 20 V; accumulation tunnel entrance / funnel 1 entrance (∆4), 50 V; accumulation tunnel exit / accumulation tunnel entrance (transfer electrode) (∆5), 0 V; TIMS tunnel entrance (ramp start electrode) / accumulation tunnel exit (∆6), 50 V. The TIMS ramp was applied within 340.0 ms between 0.81 and 0.94 Vs/cm^2 while the ramp end accumulation was set to 2.00 Vs/cm^2. Each measurement was performed three times in order to guarantee reproducibility.

Parallel accumulation, serial fragmentation (PASEF) mode for fragmentation experiments of the TIMS separated Metoprolol metabolites.

The PASEF mode was used to perform fragmentation experiments after TIMS separation. Therefore, the PASEF collision energy base was set to 30 eV. Target masses were picked using exclusion windows for the reduced mobility and the mass set to 0.020 Vs/cm^2 and 0.015 Da, respectively. Target masses were fragmented automatically when reaching a scheduled target intensity of at least 40000 counts. Each measurement was performed three times in order to guarantee reproducibility.

SI-2: Mobilo voltammogram of *m/z* 238.14370

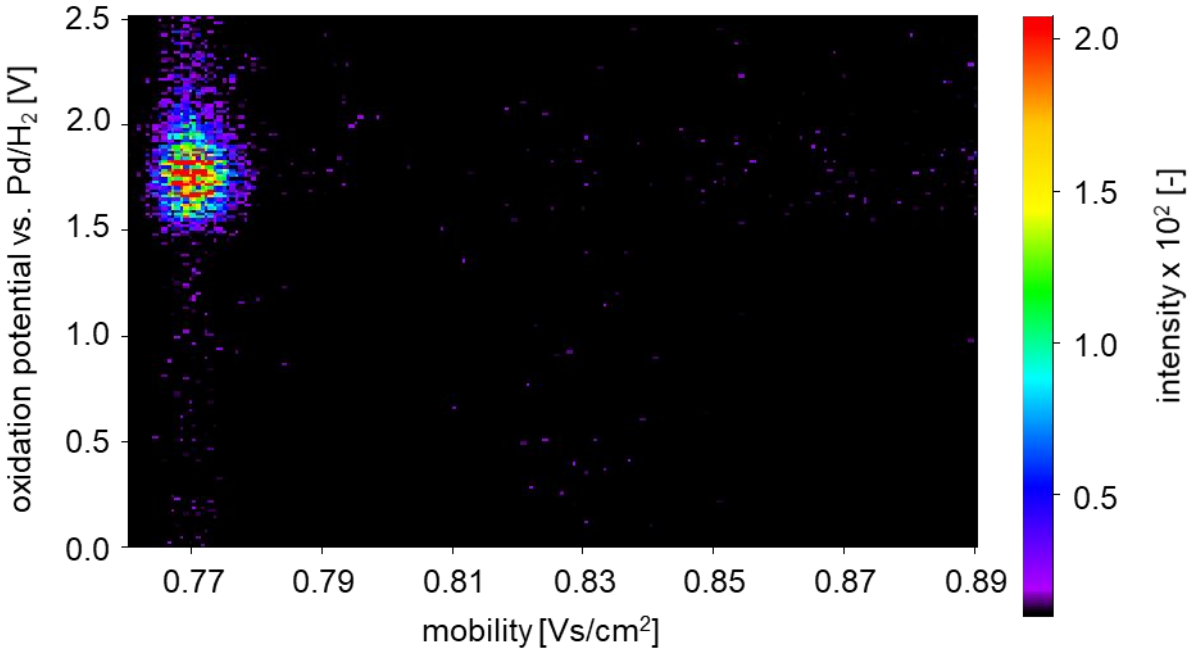

Figure SI-2: Mobilo voltammogram, generated by online EC/ESI-TIMS-ToF-MS in ESI(+) mode for Metoprolol-C_2H_6 ($[M+H]^+$ *m/z* 238.14370).

SI-3: Mobility alignment of fragment ions and the respective parent compounds in a 2D-IMS-MS/MS plot

Figure SI-3 shows a 2D-IMS-MS/MS plot, where the alignment of the mobility of the fragment ions and their respective parent compounds are visualized. The green boxes highlight the region of interest for the mobility values of 0.846 and 0.858 Vs/cm^2, which corresponds to the two hydroxylated metabolites, respectively. For both compounds, the loss of one water molecule can be observed in the fragment spectra (yellow highlight box). The second water loss is characteristic for the benzylic hydroxylated product and therefore only detectable for the mobility of 0.858 Vs/cm^2 (right blue box). The blue box next to that signal highlights the signal for a fragment ion with *m*/*z* 224.12795, which represents the *N*-demethylated parent after the first water loss. The second water loss again is only detected for the mobility of 0.858 Vs/cm^2 and assigned by the signal *m*/*z* 189.09119 (blue box) after the loss of ammonia. For the mobility of 0.846 Vs/cm^2, two major characteristic signals can be detected (highlighted in white boxes). One corresponds to the loss of the methoxy group from the parent (*m*/*z* 252.16063), while the other one describes the loss of C_3H_6 afterwards (*m*/*z* 210.11259) and can only be seen rarely for the other isomer.

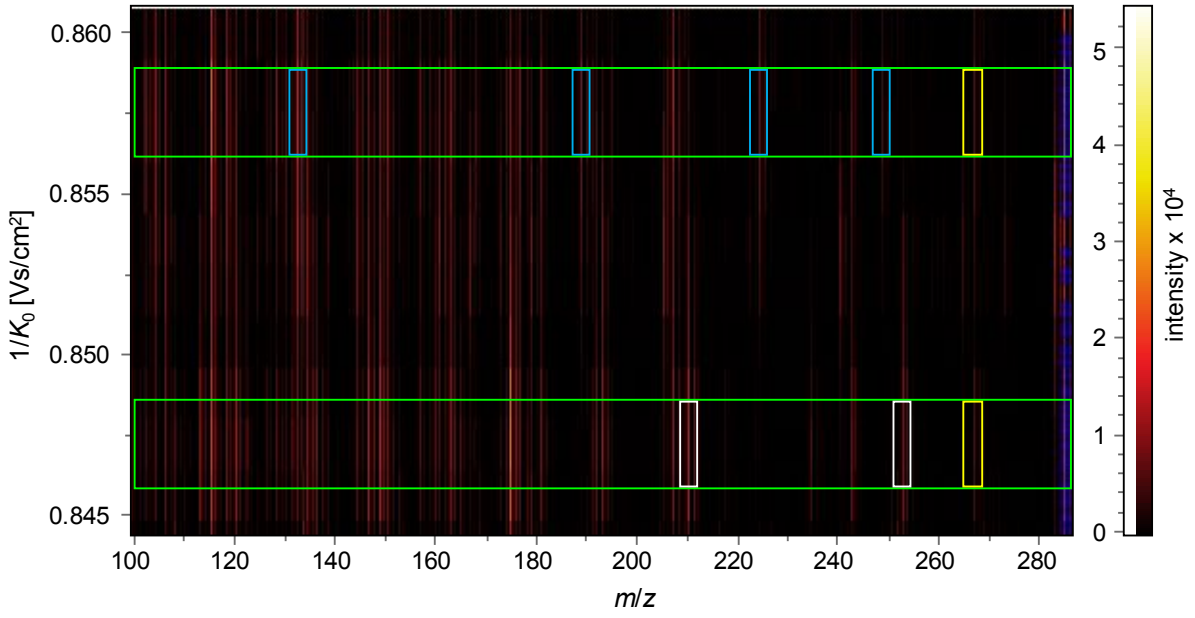

Figure SI-3: 2D-IMS-MS/MS plot for mobility alignment visualization of fragment ions and their respective parent compounds of different mobility values. Green boxes show the two mobility regions of the two isomeric compounds of *m*/*z* 284.18566. Blue boxes show unique fragmentation features only occurring for

M2, white boxes show the same for M1. The two yellow boxes highlight the signal assigned to the first water loss during fragmentation.

SI-4: $1/K_0$ versus *m/z* plots for selected oxidation potentials

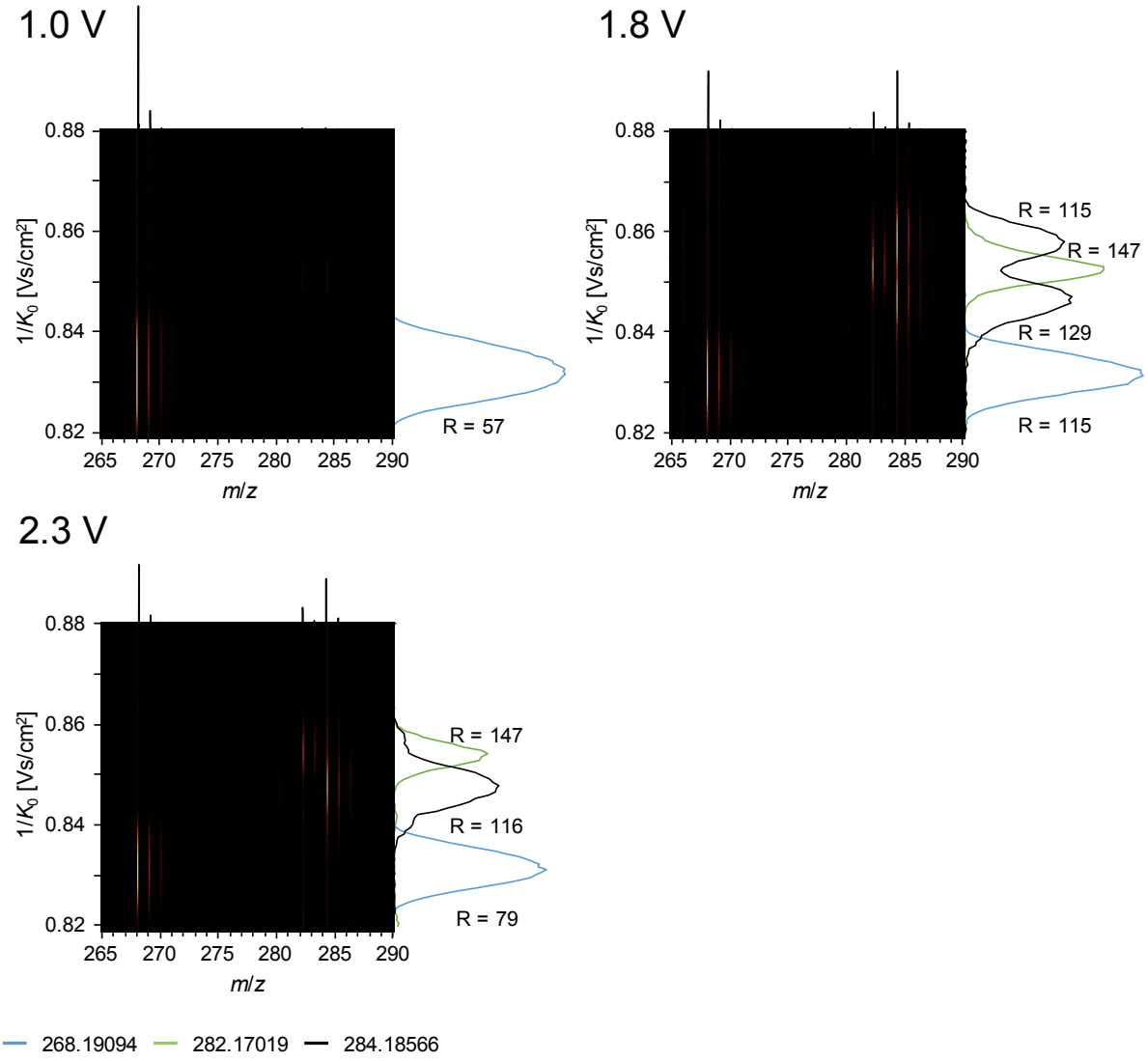

Figure SI-4: $1/K_0$ vs. *m/z* plots of three different oxidation potentials (1.0 V, 1.8 V and 2.3 V) as illustrated. The mobility is shown from 0.82 to 0.88 Vs/cm². The *m/z* range is shown from *m/z* 265 to 290.

SI-5: Liquid chromatography mass spectrometry analysis of metoprolol and its electrochemically generated oxidative metabolites

In addition to TIMS separation, LC/MS analysis was conducted. For that, the reaction mixture was collected from the effluent of the cell in a vial at a constant potential of 1.8 V. The Ultimate 3000 LC system (Thermo Fisher Scientific, Bremen, Germany) was equipped with a WPS-

3000RS autosampler, a SRD 3400 degasser, two HPG-3200RS pumps and a TCC-2000RS column oven. For separation a ProntoSIL C18 ace-eps 100 x 2 mm with 3 µm particle size from Bischoff Chromatography, Leonberg, Germany. Gradient elution was performed using 0.1% formic acid and methanol as eluents. The analysis run started at a methanol ratio of 5% and was kept for five minutes. The ratio was increased to 90% after 13 minutes and kept for additional four minutes. After 18 minutes the ratio was reduced again to 5% until the end of the run at 20 minutes. A flow rate of 400 µL/min was used. The temperature of the column oven was set to 40 °C. For each analysis 1 µL were injected. The respective chromatograms are illustrated in Figure SI-5.

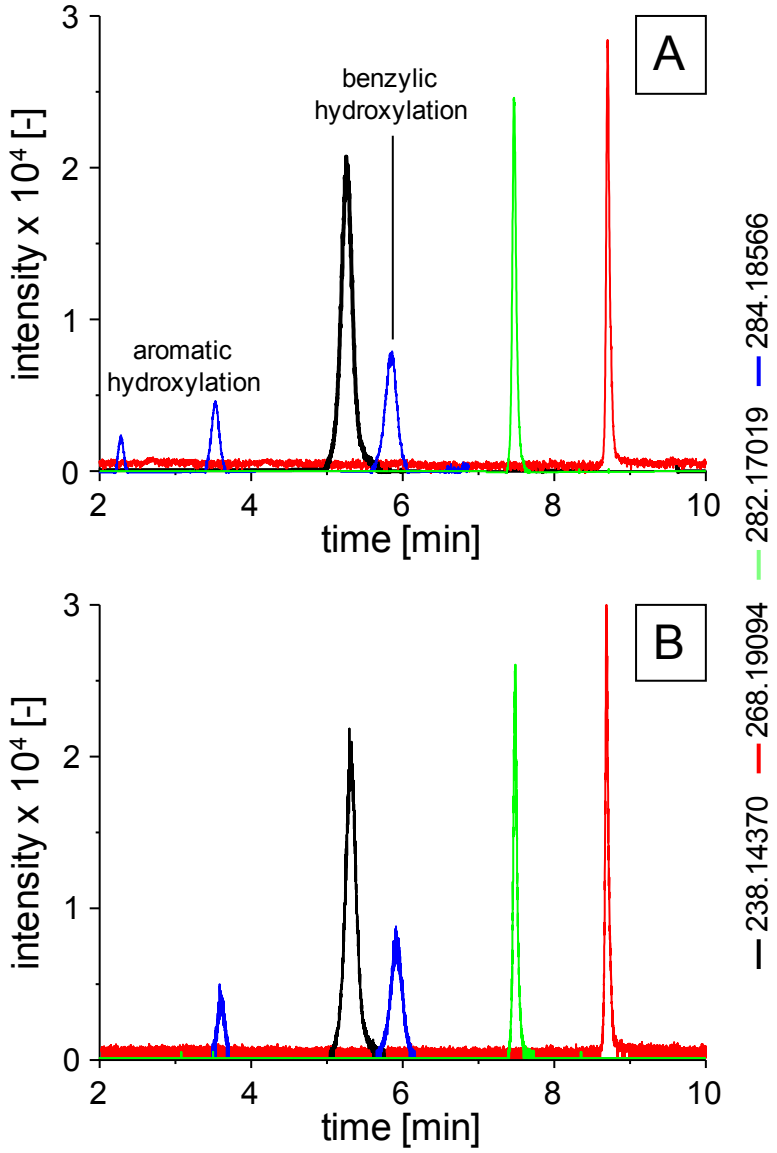

Figure SI-5: Chromatograms of the LC-separation using the conditions mentioned in section SI-4. Chromatogram A shows an analysis directly performed after electrochemical metabolite generation. B represents the same sample with a delay of measurement of 20 minutes.