Supplementary Information

Transition metal cations catalyze $^{16}O/^{18}O$ exchange of catechol motifs with $H_2{}^{18}O$

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Materials and methods

Materials

Catechol (≥99 wt%), 4-chlorocatechol (≥97 wt%), 4-methylcatechol (≥95 wt%), 4-nitrocatechol (≥97 wt%), dopamine hydrochloride (≥98 wt%). Copper(II) chloride (≥97 wt%), aluminum(III) chloride hexahydrate (≥99 wt%), hydrochloric acid (HCl, 37 wt%), sodium hydroxide (NaOH , ≥98 wt%) and ethanol (EtOH) were obtained from Merck (St. Louis, MO, USA). 3,4-Dihydroxybenzoic acid (≥98 wt%) was purchased from TCI Europe NV (Zwijndrecht, Belgium). 4-Methoxycatechol (≥95 wt%) was purchased from Toronto Research Chemicals (North York, Canada). Iron(III) chloride (≥97 wt%), zinc(II) chloride (≥98 wt%), and 3,4-dihydroxybenzaldehyde (≥95 wt%) were purchased from Fisher Scientific (San Jose, CA, USA). $H_2{}^{18}O$ (GMP grade, $^{18}O \geq 97$ %) was purchased from ABX advanced biochemical compounds-Biomedizinische Forschungsreagenzien GmbH (Radeberg, Germany). ULC-MS grade acetonitrile (ACN) and water, both containing 0.1 vol.% formic acid (FA) were purchased from Biosolve (Valkenswaard, the Netherlands). Water for other purposes than UHPLC was prepared using a Milli-Q water purification system (Merck Millipore, Billerica, MA, USA).

General experimental procedure

High resolution RP-UHPLC-PDA-ESI-MS of catechol and derivatives

Stock solutions of catechol and derivatives in acetone were added to an Eppendorf and the acetone was evaporated under nitrogen flow. Water ($H_2{}^{18}O$) and metal chloride solution (10 mM in $H_2{}^{18}O$) were added to obtain a final concentration of 1.0 mM of metal (*i.e.*, $FeCl_3$, $CuCl_2$, $AlCl_3$, and $ZnCl_2$) and ligand for the initial screening with catechol and 3,4-DHBA and 0.1 mM of $FeCl_3$ and catechol and derivatives (*i.e.*, 4-chlorocatechol, 4-methylcatechol, 3,4-DHBA, dopamine, 4-nitrocatechol, 3,4-DHBZ, and 4-methoxycatechol) for the continuation experiments. The pH was adjusted to 3 or 7 for the initial screening and to 3 for the continuation experiments using 0.5 M NaOH or HCl prepared in $H_2{}^{18}O$. Samples were incubated at 37 °C at 1000 rpm using an Eppendorf Thermomixer® F1.5 (Eppendorf, Hamburg, Germany). For UHPLC-MS analysis, 80 µL aliquots of the reaction mixture were taken after 2 and 24 h for the initial screening and 0.5, 1, 4, and 24 h for the continuation experiments, and centrifuged (5 min, 15,000 × *g*) prior to RP-UHPLC-PDA-ESI-MS analysis.

RP-UHPLC-PDA-ESI-MS^n analysis was performed using a Thermo Vanquish UHPLC system (Thermo Scientific, San Jose, CA, USA), equipped with a pump, degasser, and autosampler, coupled to a PDA detector and a Thermo Q Exactive Focus hybrid quadrupole-orbitrap mass spectrometer. Samples (1 µL) were injected onto an Acquity UPLC BEH C18 column (150 × 2.1 mm, particle size 1.7 µm) with a VanGuard guard column (5 × 2.1 mm) of the same material (Waters, Milford, MA, USA). The flow rate was 400 µL min^{-1} and the column temperature was 45 ºC. Water (A) and ACN (B), both acidified with 0.1% FA, were used as eluents. The following solvent gradient was used: 0-1.09 min at 1% B (isocratic), 1.09-6.18 min from 1 to 15% B (linear gradient), 6.18-7.27 min from 15 to 100% B (linear

gradient), 7.27-12.72 min at 100% B (isocratic), 12.72-13.81 min from 100 to 1% B (linear gradient) and 13.81-19.27 min at 1% B (isocratic). The PDA detector was set to measure the wavelength range of 190–680 nm. Mass spectrometric data were collected over the *m/z* range of 100–1,000 in negative and positive ionization mode by using source voltages of 2.5 and 3.5 kV, respectively. Nitrogen was used as a sheath gas (50 arbitrary units) and auxiliary gas (13 arbitrary units). For both modes, the S-lens RF level was set at 50 %, the ion transfer tube temperature was 263 °C and the source heater temperature 425 °C. Data acquisition and processing were performed using Xcalibur (version 4.1, Thermo Scientific). Recoveries of catechol and derivatives were calculated based on integrals of the chromatographic UV_{280} peaks and comparison to an external calibration curve of the corresponding authentic standard (0.0125-0.1 mM, in duplicate, R^2 >0.99). Labeling yields were determined as the average distribution of the accurate masses corresponding to unlabeled, singly labeled and doubly labeled compound under the chromatographic peak. The labeling yield was corrected for labeling percentage of the used $H_2{}^{18}O$ (97%), by dividing the uncorrected percentage of doubly ^{18}O labeled catechols by 0.941 (the theoretical percentage expected after complete ^{18}O labeling of the two catechol hydroxyl groups; 0.97^2).

Time-resolved ESI-MS of catechol and derivatives in $H_2{}^{18}O$ in presence of $FeCl_3$

Solutions (400 µL) of catechol and derivatives at 0.2 mM were prepared in $H_2{}^{18}O$ and placed in a glass vial, the pH of the solution was adjusted to 3 by 0.5 M HCl in $H_2{}^{18}O$. The solution was stirred at 37 °C for 5 minutes after which it was directly infused into the ESI-IT-MS source via a capillary by applying a slight overpressure of N_2 (approximately 0.25 bar). When the MS signal was stable, the measurement was started and after 0.5 min measurement of the ligand, a solution (400 µL) of 0.2 mM $FeCl_3$ in $H_2{}^{18}O$ (pH 3) was added to the glass vial to obtain final concentrations of 0.1 mM catecholate and iron. The ESI-IT-MS measurements were acquired using an LTQ Velos Pro linear ion trap mass spectrometer (Thermo Scientific) equipped with a heated electrospray ionization (ESI) probe. Data were collected for 60 min over the *m/z* range of 100-600 in negative or positive ionization mode by using source voltages of 4.0 and 3.5 kV in positive ionization (PI) and negative ionization (NI) mode, respectively. The S-lens RF level was set at 67.9% in PI and 69.5% in NI, the ion transfer tube and the source heater temperatures were 275 and 45 °C, respectively. Nitrogen was used as a sheath gas (50 arbitrary units) and auxiliary gas (13 arbitrary units). Data were processed using Xcalibur 4.1 (Thermo Scientific).

HCD fragmentation spectra of unlabeled and doubly ^{18}O labeled catechol derivatives

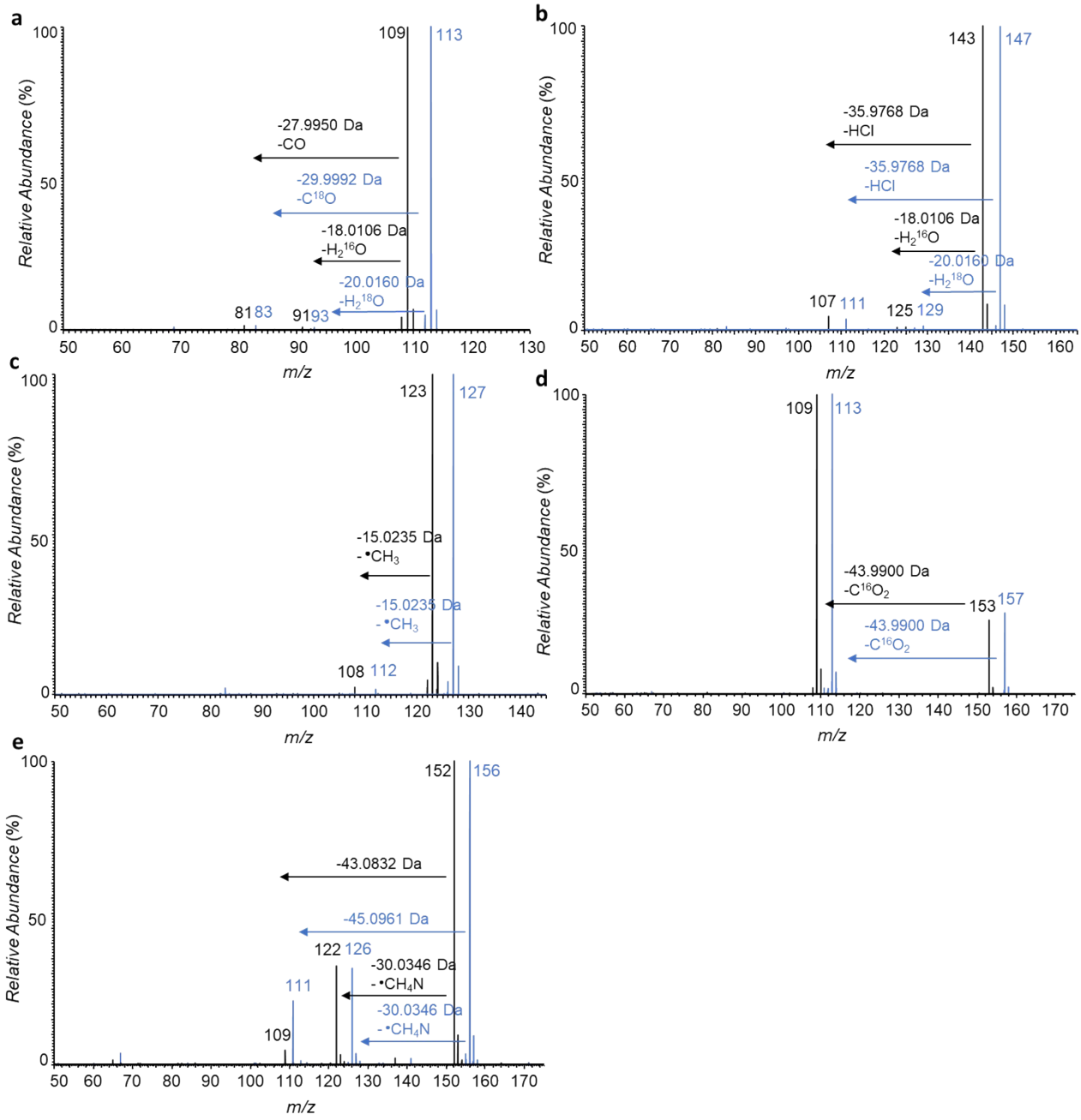

Fig. S1 HCD fragmentation spectra of unlabeled (black) and doubly ^{18}O-labeled catechol (a), 4-chlorocatechol (b), 4-methylcatechol (c), 3,4-dihydroxybenzoic acid (d), and dopamine (e).

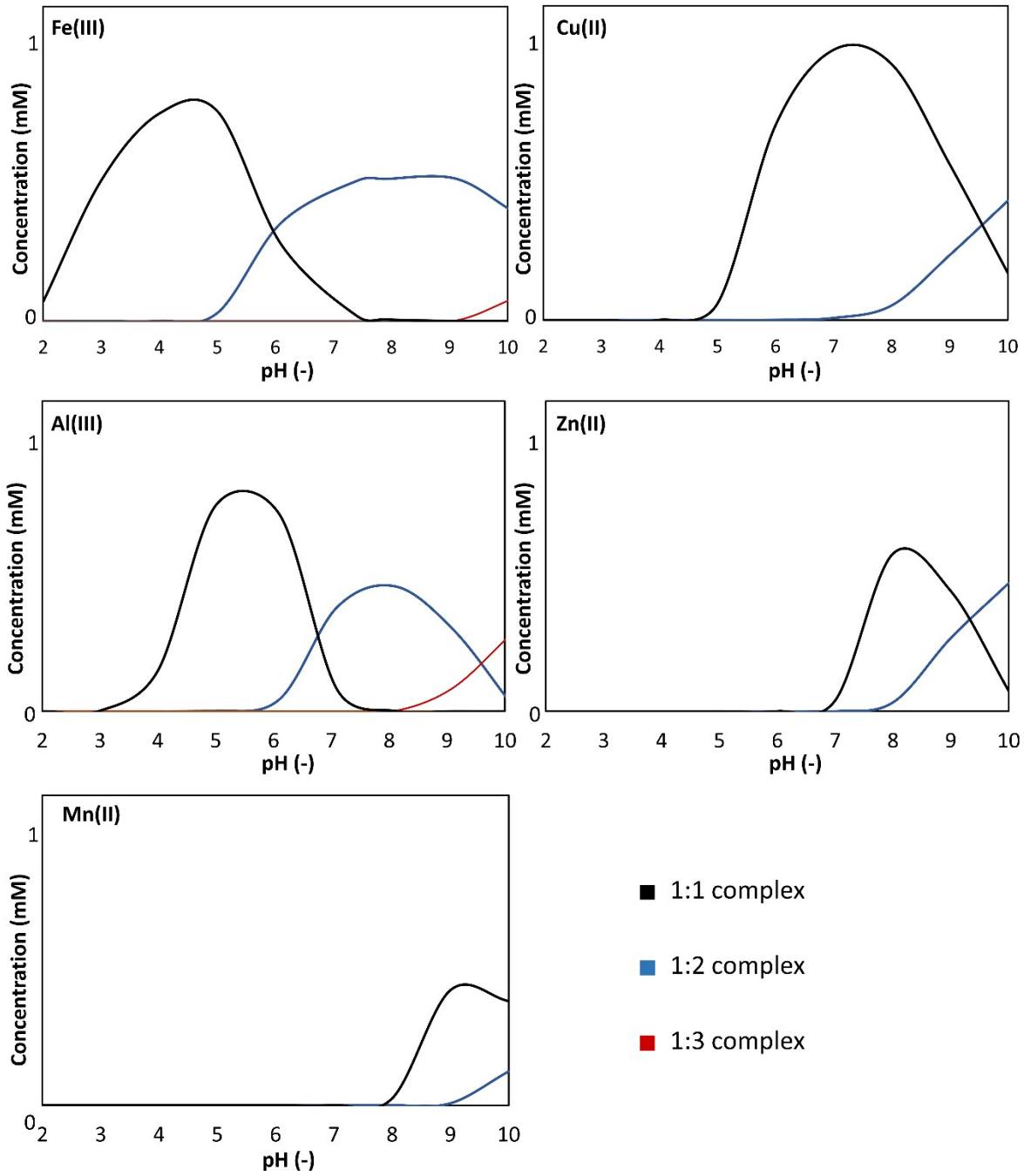

Fig. S2 Predicted concentration of 1:1, 1:2, and 1:3 complexes of catechol with Fe(III), Cu(II), Al(III), Zn(II) and Mn(II) at different pH. Visual MINTEQ (version 3.1) was used for the simulation. The metal-catechol and metal hydrolysis species and stability constants (log K) were obtained from the standard database in Visual MINTEQ 3.1 and not fixed to a constant ionic strength.

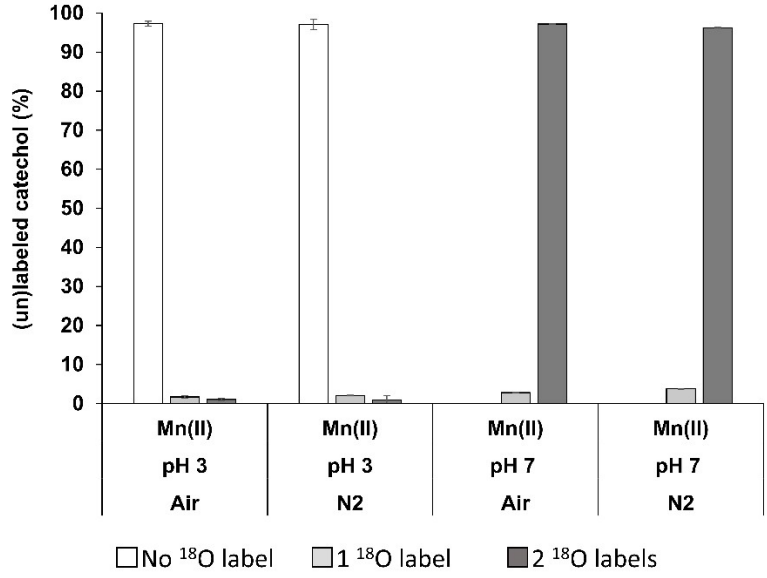

Fig. S3 Percentages of unlabeled, singly labeled, and doubly labeled catechol after equimolar incubations (1 mM) with $MnCl_2$ at 37 °C for 2 h, at pH 3 or 7 in presence of air or after purging with N_2. Purging with N_2 was performed for 2 min through the $H_2{}^{18}O$ solvent and headspace of the Eppendorf tube. Data are presented as average and standard deviation of two separate incubations. The large difference in $^{16}O/^{18}O$ exchange between pH 3 and 7 matches with the low tendency of Mn(II) to form catecholato complexes at low pH.

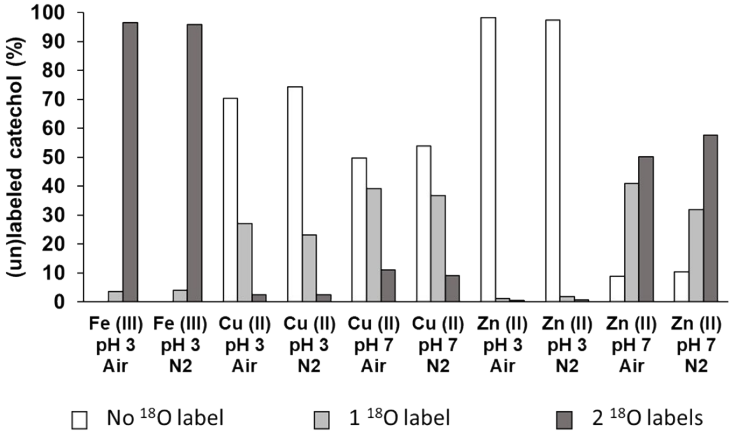

Fig. S4 Relative abundance of unlabeled, singly labeled and doubly labeled catechol in equimolar (1 mM) incubations of catechol with $FeCl_3$, $CuCl_2$, or $ZnCl_2$ at pH 3 or 7 in presence of air or after purging with N_2. Purging with N_2 was performed for 2 min through the $H_2{}^{18}O$ solvent and headspace of the Eppendorf tube. Incubation times were 30 min for the samples containing $FeCl_3$ and 24 h for the samples containing $CuCl_2$ or $ZnCl_2$.

RP-UHPLC-PDA-ESI-MS chromatograms and spectra

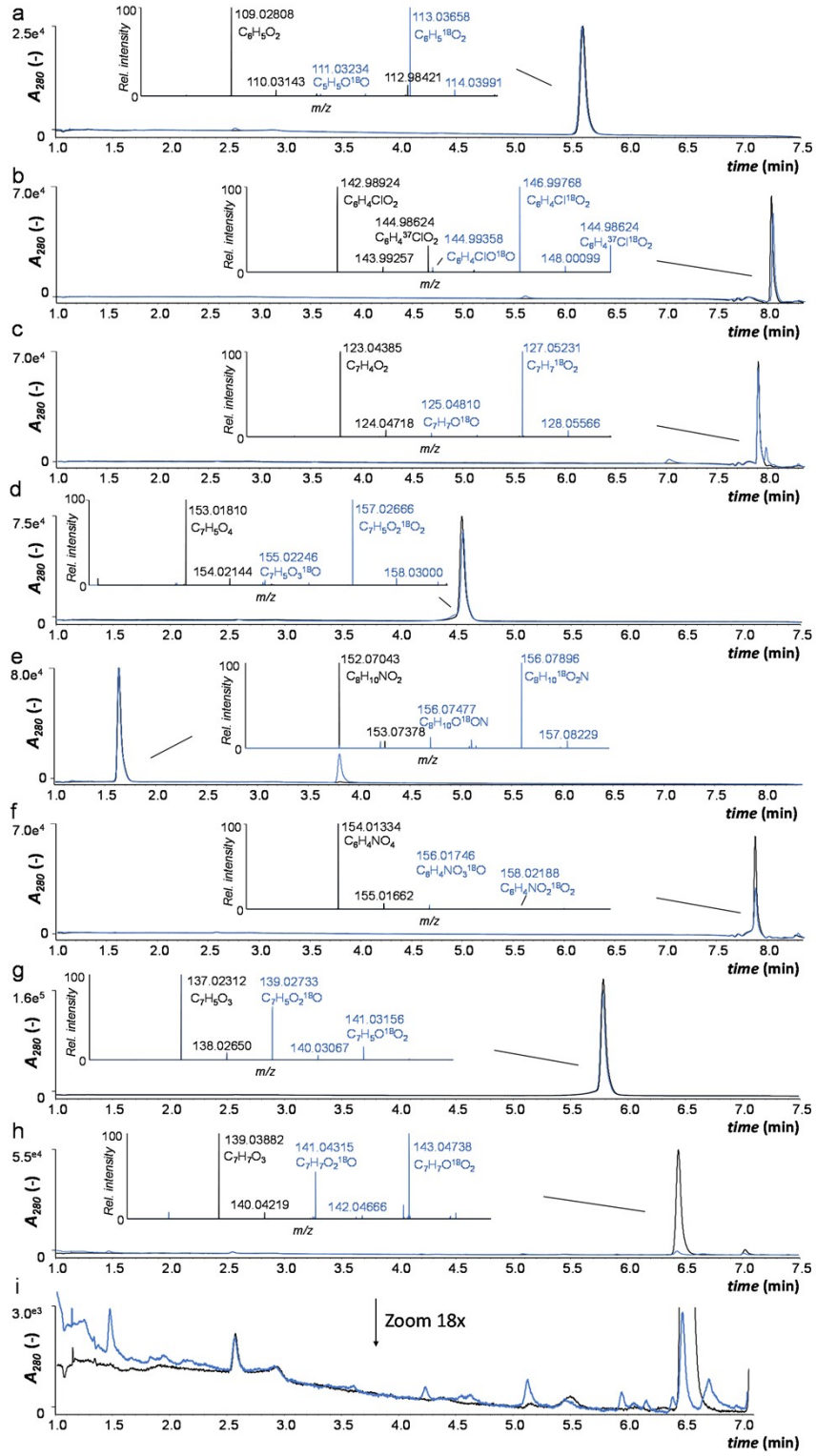

Fig. S5 Overlayed RP-UHPLC-UV_{280} chromatograms and HRMS spectra of catechol (a), 4-chlorocatechol (b), 4-methylcatechol (c), 3,4-dihydroxybenzoic acid (d), dopamine (e), 4-nitrocatechol (f), 3,4-dihydroxybenzaldehyde (g), and 4-methoxycatechol (h) after incubation at 37 °C in absence (black) and presence (blue) of $FeCl_3$. Incubation times were 1 h for a-c and e, 4 h for d and 24 h for f-i. Chromatogram i is an 18-fold zoomed version of h, displaying several (unidentified) degradation products of 4-methoxycatechol. Equimolar concentrations (0.1 mM) were used in all incubations. The inserts depict the overlayed high resolution Full MS spectra under the corresponding peaks. Masses without molecular formula label correspond to the natural ^{13}C isotope peak of the preceding peak.