SUPPORTING INFORMATION TO ARTICLE:

A tiered procedure for assessing the formation of biotransformation products of pharmaceuticals and biocides during activated sludge treatment

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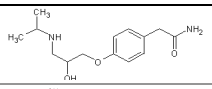
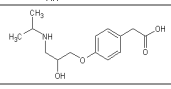
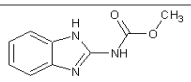
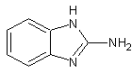
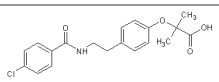
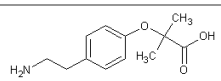
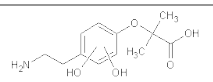
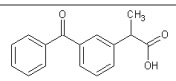
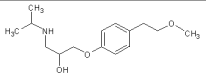
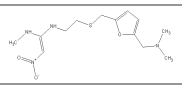
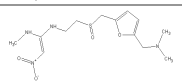
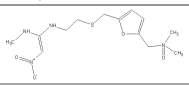
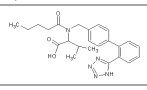
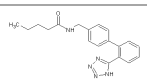
Sampling campaigns and batch experiments

Table S 1 Overview of sampling campaigns and corresponding batch experiments

	Campaign 09 (C09)		Campaign 10 (C10)
WWTP	Kloten/Opfikon in Opfikon	Wüeri in Regensdorf	Wüeri in Regensdorf
Population equivalents	51'000	25'000	
Wastewater per day (dry weather)	17'500 m^3	5'500 m^3	
Hydraulic retention time of biological treatment step (dry weather)	8 h	16-18 h	
Sludge retention time	11 d	16-17 d	
Sampling time	2/3 February 2009		2/3/4 February 2010
Approach	Qualitative		Quantitative
Batch volume	50 mL		70 mL
Starting concentration of parent compound	100 μgL^{-1}		5 μgL^{-1}
Sample volumes withdrawn from batch reactor	2 mL		5 mL
Volume transferred to vial	1 mL		4 mL diluted to 20 mL
SPE	Offline (no SPE for batch samples)		Online
Mass spectrometric detection	LTQ-Orbitrap		Quantum-ultra
Compounds analyzed	Atenolol, bezafibrate, ketoprofen, metoprolol, ranitidine, valsartan, venlafaxine, carbendazim and target list of potential TPs		Atenolol, metoprolol, atenolol acid, ranitidine, ranitidine-S-oxide, valsartan, valsartan acid

Investigated compounds

Table S 2 Investigated compounds with CAS, structure, supplier, HPLC purity, and usage.

Compounds	CAS	structure	Supplier	Purity (HPLC, from supplier)	Usage
Atenolol	29122-68-7		Sigma-Aldrich	≥98%	Beta-blocker
Atenolol acid	56392-14-4		Toronto Research Chemicals Inc	98%	Metabolite/TP
Carbendazim	10605-21-7		Ehrenstorfer	99 %	Fungicide
2-Amino-benzimidazole	934-32-7		Ehrenstorfer	99%	TP
Venlafaxine	93413-69-5		Siegfried	100%	Anti-depressant
N-Desmethyl-venlafaxine	149289-30-5		Toronto Research Chemicals Inc	98%	TP
O-Desmethyl-venlafaxine	93413-62-8		Toronto Research Chemicals Inc	98%	TP
O,N-Didesmethyl-venlafaxine	135308-74-6		Toronto Research Chemicals Inc	98%	TP
Bezafibrate	41859-67-0		Sigma-Aldrich	≥98%	Anti-lipemic
Amino-bezafibrate	55458-78-1		n.a.	n.a.	TP
Dihydroxy-amino-bezafibrate	n.a.		n.a.	n.a.	TP
Ketoprofen	22071-15-4		Sigma-Aldrich	≥98%	Anti-inflammatory
Metoprolol	37350-58-6		Sigma-Aldrich	99%	Beta-blocker
Ranitidine	66357-35-5		Sigma-Aldrich	>99%	Antagonist
Ranitidine-S-oxide	73851-70-4		Toronto Research Chemicals Inc	95%	TP
Ranitidine-N-oxide	73857-20-2		Toronto Research Chemicals Inc	95%	TP
Valsartan	137862-53-4		Toronto Research Chemicals Inc	98%	Anti-hypertensive
Valsartan acid	164265-78-5		Novartis	>99%	TP
Dealkylated valsartan	914465-68-2		n.a.	n.a.	TP
Amino-valsartan	147225-68-1		n.a.	n.a.	TP

Reference standards and materials

The internal reference standards atenolol-d7, atenolol acid-d5, bezafibrate-d4, metoprolol-d7, N-desmethylvenlafaxine-d3, O-desmethylvenlafaxine-d6, ranitidine-d6, valsartan-d3, and venlafaxine-d6 were purchased from Toronto Research Chemicals Inc (Canada). Carbendazim-d4 was purchased from Ehrenstorfer (Germany), valsartan acid-d4 was cordially provided by Novartis International AG (Switzerland). All internal standards had purities ≥ 98%.
Methanol and water (HPLC grade) were obtained from Acros Organics (Belgium). Ethanol p.a., ammonium acetate, NH_3 (25%), and formic acid 98-100% p.a. were purchased from Merck.

Analytical details of the qualitative approach

Table S 3 Analytes for LTQ-Orbitrap measurement.

Analyte	Retention time (X-Bridge C18)	Exact mass $[M+H]^+$	Fragments for comparison to reference standard	HCD (-)
Atenolol	2.4 min	267.1703	190.0863 ($C_{11}H_{12}O_2N_1$), 225.1234 ($C_{11}H_{17}O_3N_2$), 145.0644 ($C_{10}H_9O_1$)	45
Atenolol acid	3.9 min	268.1543	191.0707 ($C_{11}H_{11}O_3$), 145.0647 ($C_{10}H_9O_1$), 226.1077 ($C_{11}H_{16}O_4N_1$)	45
Carbendazim	3.7 min	192.0768	160.0506 ($C_8H_6O_1N_3$), 132.0557 ($C_7H_6N_3$)	60
2-Aminobenzimidazole	2.7 min	134.0713	92.0491 ($C_6H_6N_1$), 107.0600 ($C_6H_7N_2$)	80
Venlafaxine	6.4 min	278.2115	260.2009 ($C_{17}H_{26}O_1N_1$), 58.0644 ($C_{12}H_8N_1$), 215.1429 ($C_{15}H_{19}O_1$)	40
N-Desmethyl-venlafaxine	6.5 min	264.1958	215.1430 ($C_{15}H_{19}O_1$), 121.0644 ($C_8H_9O_1$), 147.0801 ($C_{10}H_{11}O_1$)	45
O-Desmethyl-venlafaxine	5.1 min	264.1958	246.1852 ($C_{16}H_{24}O_1N_1$), 58.0644 ($C_3H_8N_1$), 107.0486 ($C_7H_7O_1$)	45
O,N-Didesmethyl-venlafaxine	4.7 min	250.1802	201.1273 ($C_{14}H_{17}O_1$), 133.0644 ($C_9H_9O_1$), 232.1696 ($C_{15}H_{22}O_1N_1$)	45
Bezafibrate	10.6 min	362.1154	138.9941 ($C_7H_4O_1Cl_1$), 316.1102 ($C_{18}H_{19}O_2N_1Cl_1$), 276.0787 ($C_{15}H_{15}O_2N_1Cl_1$)	35
Amino-bezafibrate	4.4 min	224.1276	no reference standard	50
Dihydroxy-amino-bezafibrate	6.7 min	256.1174	no reference standard	15
Ketoprofen	9.7 min	255.1016	209.0963 ($C_{15}H_{13}O_1$), 105.0331 ($C_7H_5O_1$), 177.0547 ($H_{19}H_9O3$)	15
Metoprolol	5.2 min	268.1907	191.1068 ($C_{12}H_{15}O_2$), 159.0803 ($C_{11}H_{11}O_1$), 116.1067 ($C_6H_{14}O_1N_1$)	45
Ranitidine	2.5 min	315.1485	176.0489 ($C_5H_{10}O_2N_3S_1$), 130.0557 ($C_5H_{10}N_2S_1$), 224.0979 ($C_{11}H_{16}O_1N_2S_1$)	35
Ranitidine-S-oxide	1.0 min	331.1435	138.0911 ($C_8H_{12}O_1N_1$), 110.0960 ($C_7H_{12}N_1$), 94.0409 ($C_6H_6O_1$)	35
Ranitidine-N-oxide	2.6 min	331.1435	176.0487 ($C_5H_{10}O_2N_3S_1$), 130.0556 ($C_5H_{10}N_2S_1$), 224.0977 ($C_{11}H_{16}O_1N_2S_1$)	35
Valsartan	11.1 min	436.2343	207.0913 ($C_{14}H_{11}N_2$), 235.0975 ($C_{14}H_{11}N_4$), 291.1490 ($C_{19}H_{19}O_1N_2$)	30
Valsartan acid	6.8 min	267.0877	206.0603 ($C_{14}H_8O_1N_1$), 221.0716 ($C_{14}H_9O_1N_2$), 178.0653 ($C_{13}H_8N_1$)	45
Dealkylated valsartan	8.3 min	336.1819	no reference standard	35
Amino-valsartan	4.6 min	252.1244	no reference standard	45

Analytical details of the quantitative approach

For sample injection and buffer addition, a tri-directional autosampler (HTC PAL, CTC Analytics, Switzerland) with a 100-µL side-port syringe (80-mm needle, Hamilton, Switzerland) was connected with a large volume dispenser module (10-mL dispenser syringe with 10-mL loop, CTC Analytics) and four sample trays with 128 positions for 20 mL vials (GB Analytik, Switzerland). Sample enrichment was achieved with a 20-mL sample loop (custom product, BGB Analytik, Switzerland) on a manually filled SPE cartridge 20 mm x 2.1 mm I.D., 25 µm particle size (analogue to cartridges for offline SPE with 9.1 mg Oasis and 9.8 mg of Strata XCW, Strata XAW and Isolute ENV+ in a mixing ratio of 1:1:1.5). The HPLC pump system consisted of a binary pump for sample loading, a quaternary low pressure mixing gradient pump, an isocratic pump (all Rheos 2000, Flux Instruments, Switzerland) and a column oven (Jones, Omnilab, Switzerland).

For the first method, the gradient was run from 90% water (5mM NH_4Ac, pH 5) and 10% methanol (0.5% 25%-NH_3) to 95% methanol and 5% water within 23 minutes. The gradient of the second method was run from 90% water (+0.1% formic acid) and 10% methanol to 95% methanol and 5% water within 30 minutes. Retention times for both methods are given in Table S4, as well as the assigned internal standards, transition quantifiers, identifiers and collision energy for the quantitatively measured analytes.

For the batch experiment, metoprolol, atenolol, atenolol acid, valsartan and valsartan acid were analyzed with the first method (basic conditions) due to better selectivity and sensitivity for the two transformation products, whereas ranitidine and ranitidine-S-oxide were analyzed with the second method (neutral/acidic conditions). The LOQ for all analytes in the batch was between 5-50 ng/L (except for atenolol acid LOQ 100 ng/L).

For influent, secondary effluent, final effluent, and surface water, all analytes were analyzed with both methods, but the second method (neutral/acidic conditions) was taken for comparison to model predictions. The LOQ was <10 ng/L for all matrices and analytes except for atenolol acid (LOQ 30-60 ng/L) and valsartan acid (LOQ 30-40 ng/L). The spike recoveries of influent, secondary effluent, final effluent, and surface water were all between 90-110% except for ranitidine-S-oxide which had no isotopically labelled internal standard (spike recovery 70-80% for all four different matrices).

Table S 4 LC-MS/MS data of analytes for the two methods: Retention time (first method with neutral elution and acidic chromatography: Waters T3 Atlantis column, second method with basic elution and chromatography: Nucleodur C18 Gravity column), identifier and quantifier ions, and collision energy on triple quadrupole instrument.

Analyte	Internal standard	Retention time (Atlantis)	Retention time (Nucleodur)	Transition quantifier	Transition identifier	Collision energy
Atenolol	Atenolol-d7	14.4	7.8	267.2→190.0	267.2→225.1	18/16
Atenolol acid	Atenolol acid-d5	14.4	6.9	268.2→145.0	268.2→191.1	24/17
Metoprolol	Metoprolol-d7	19.4	18.0	268.2→159.0	268.2→133.0	17/21
Ranitidine	Ranitidine-d6	14.6	9.5	315.2→176.0	315.2→130.0	17/26
Ranitidine-S-oxide	Atenolol-d7	12.3	9.0	331.1→138.1	331.1→110.1	16/26
Valsartan	Valsartan-d3	26.1	14.2	436.2→207.0	436.2→291.1	27/18
Valsartan acid	Valsartan acid-d4	21.9	9.3	267.0→206.0	267.0→151.0	18/40

Ionization source parameters for ESI are given in Table S 5:

Table S 5 ESI source parameters

Parameters	Unit	ESI+	ESI-
Spray voltage	V	3800	3000
Sheath gas	arbitrary	50	50
Auxiliary gas	arbitrary	10	10
Capillary temperature	°C	350	350

Data were analyzed with Xcalibur (Thermo Scientific, USA) in the QuanBrowser.

Results of control experiments

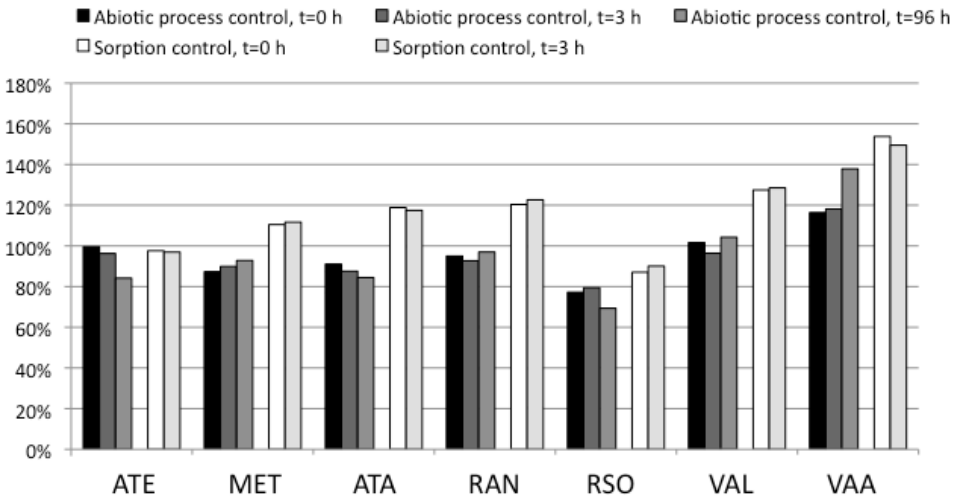


Figure S 1 Results of effluent and autoclaved control experiments for each of the seven quantitatively analyzed compounds. Sorption equilibrium is assumed to be reached within the first three hours. ATE = Atenolol, MET = Metoprolol, ATA = Atenolol acid, RAN = Ranitidine, RSO = Ranitidine-S-oxide, VAL = Valsartan, VAA = Valsartan acid.

Kinetic analysis and CSTR model setup for valsartan and valsartan acid

Eq. 2b given in the main paper is not suited to estimate the rate constants from the valsartan batch because valsartan acid is a third generation TP and hence formation of two intermediate TPs has to be accounted for in the rate equations. Eq. S1 gives the analytical solution for the concentration of valsartan acid as a function of time, assuming that the three TPs of valsartan form a sequence, i.e., valsartan → dealkylated valsartan (TP1) → amino-valsartan (TP2) → valsartan acid (TP3).

$$c^{TP3} = -(\exp(-a*t - b*t - c*t - d*t)*(-a^3*b^2*c*\exp(a*t + b*t + c*t)*FF1*FF2*FF3 + a^2*b^3*c*\exp(a*t + b*t + c*t)*FF1*FF2*FF3 + a^3*b*c^2*\exp(a*t + b*t + c*t)*FF1*FF2*FF3 - a*b^3*c^2*\exp(a*t + b*t + c*t)*FF1*FF2*FF3 - a^2*b*c^3*\exp(a*t + b*t + c*t)*FF1*FF2*FF3 + a*b^2*c^3*\exp(a*t + b*t + c*t)*FF1*FF2*FF3 + a^3*b^2*c*\exp(a*t + b*t + d*t)*FF1*FF2*FF3 - a^2*b^3*c*\exp(a*t + b*t + d*t)*FF1*FF2*FF3 - a^3*b*c*d*\exp(a*t + b*t + d*t)*FF1*FF2*FF3 + a*b^3*c*d*\exp(a*t + b*t + d*t)*FF1*FF2*FF3 + a^2*b*c*d^2*\exp(a*t + b*t + d*t)*FF1*FF2*FF3 - a*b^2*c*d^2*\exp(a*t + b*t + d*t)*FF1*FF2*FF3 - a^3*b*c^2*\exp(a*t + c*t + d*t)*FF1*FF2*FF3 + a^2*b*c^3*\exp(a*t + c*t + d*t)*FF1*FF2*FF3 + a^3*b*c*d*\exp(a*t + c*t + d*t)*FF1*FF2*FF3 - a*b*c^3*d*\exp(a*t + c*t + d*t)*FF1*FF2*FF3 - a^2*b*c*d^2*\exp(a*t + c*t + d*t)*FF1*FF2*FF3 + a*b*c^2*d^2*\exp(a*t + c*t + d*t)*FF1*FF2*FF3 + a*b^3*c^2*\exp(b*t + c*t + d*t)*FF1*FF2*FF3 - a*b^2*c^3*\exp(b*t + c*t + d*t)*FF1*FF2*FF3 - a*b^3*c*d*\exp(b*t + c*t + d*t)*FF1*FF2*FF3 + a*b*c^3*d*\exp(b*t + c*t + d*t)*FF1*FF2*FF3 + a*b^2*c*d^2*\exp(b*t + c*t + d*t)*FF1*FF2*FF3 - a*b*c^2*d^2*\exp(b*t + c*t + d*t)*FF1*FF2*FF3)*P0)/((-a + b)*(b - c)*(-a + c)*(a - d)*(b - d)*(c - d))$$

eq. S1

with a = k_{deg}^{PC}, b = k_{deg}^{TP1}, c = k_{deg}^{TP2}, d = k_{deg}^{TP3}, FF1 = fraction of TP1 forming out of PC, FF2 = fraction of TP2 forming out of TP1, FF3 = fraction of TP3 forming out of TP2. To fit eq. S1 to the data, FF1 and FF2 were assumed to be 1, and only FF3 was fit to the data. This assumption was considered justified since no other relevant TPs had been observed to be formed in parallel, and based on the mechanisms of transformation, which suggest quantitative conversion, at least for the amide hydrolysis from TP1 to TP2 [1]. Also, k_{deg}^{TP1} and k_{deg}^{TP2} were assumed to differ from k_{deg}^{PC} by fixed factors, which were derived from fitting the qualitative data given in Figure 2 (main paper) for WWTP Wüeri. This is acceptable since the shape of the TP curves is determined by the relative rate constants of precursor and TP and is independent of the absolute heights of the curves.

To calculate the expected effluent concentrations of valsartan acid with the mass balance model for WWTP Wüeri, the model was supplemented with two more sets of differential equations for TP2 and TP3, where the precursor, PC, in eqs. 4a/b (main paper) was replaced with TP1 and TP2, respectively, and the product, TP, was replaced with TP2 and TP3 respectively.

Table S 6 WWTP-specific parameters for mass balance model

Parameters	Symbol	Unit	Value
Discharge	Q	m^3/d	2230±870
Internal recirculation	IR	m^3/d	1985±345
Return sludge	RS	m^3/d	1680±190
Suspended solids concentration	X_{ss}	kg/m^3	3.0±0.1
Volume reactors 1+2	$V[1…2]$	m^3 each	312
Volume reactor 3	$V[3]$	m^3	260
Volume reactors 4–6	$V[4…6]$	m^3 each	400

MS/MS spectra for bezafibrate and its TPs

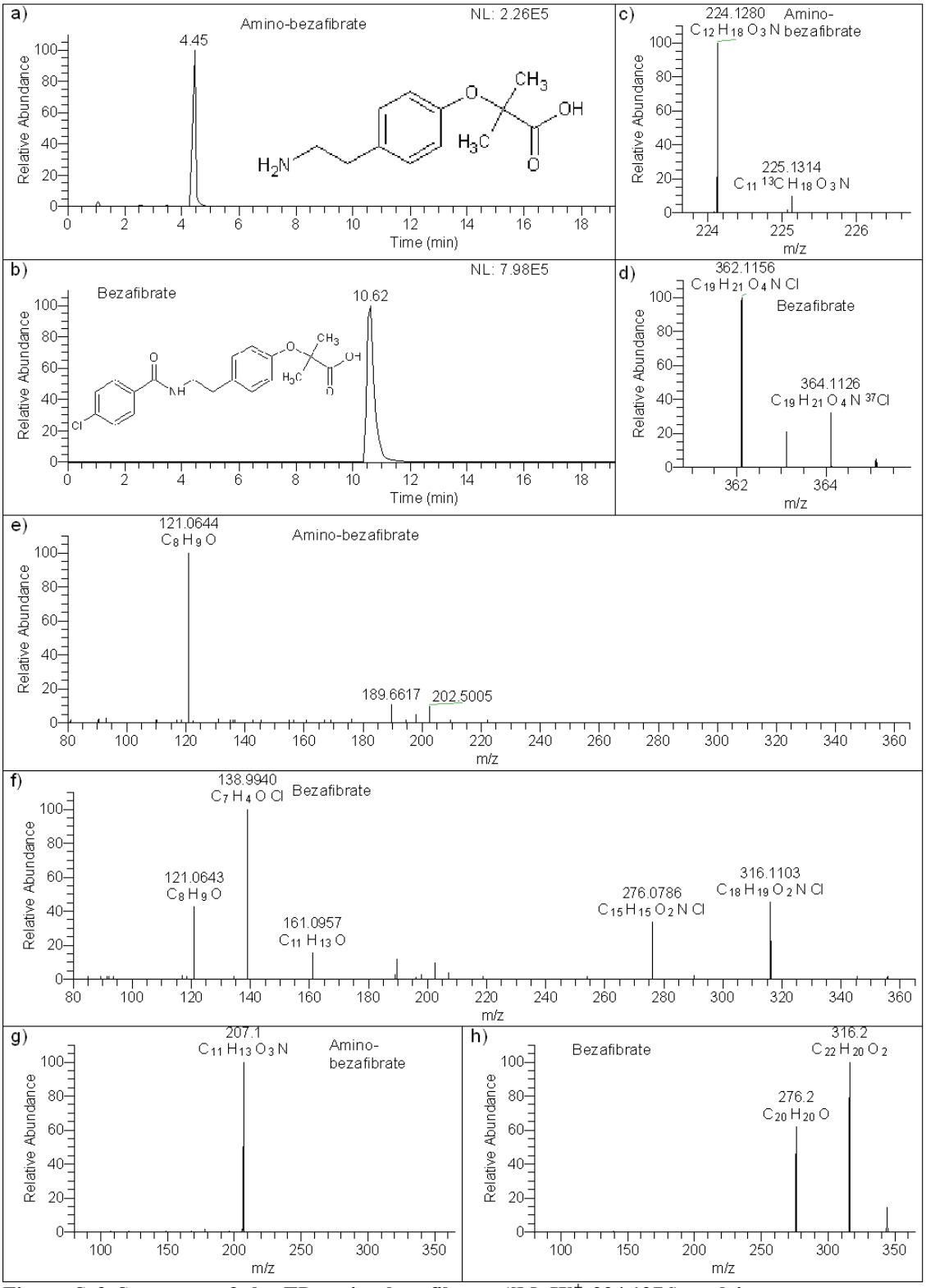


Figure S 2 Structure of the TP amino-bezafibrate ($[M+H]^+$ 224.1276) and its parent compound bezafibrate ($[M+H]^+$ 362.1154) and corresponding chromatograms (a and b), HR-MS spectra of the molecular ions, including isotopic patterns (c and d), the HR-MS/MS spectra from HCD (e and f), and MS/MS spectra from CID (g and h). All spectra are shown for the Wüeri 09 batch after 3 hours.

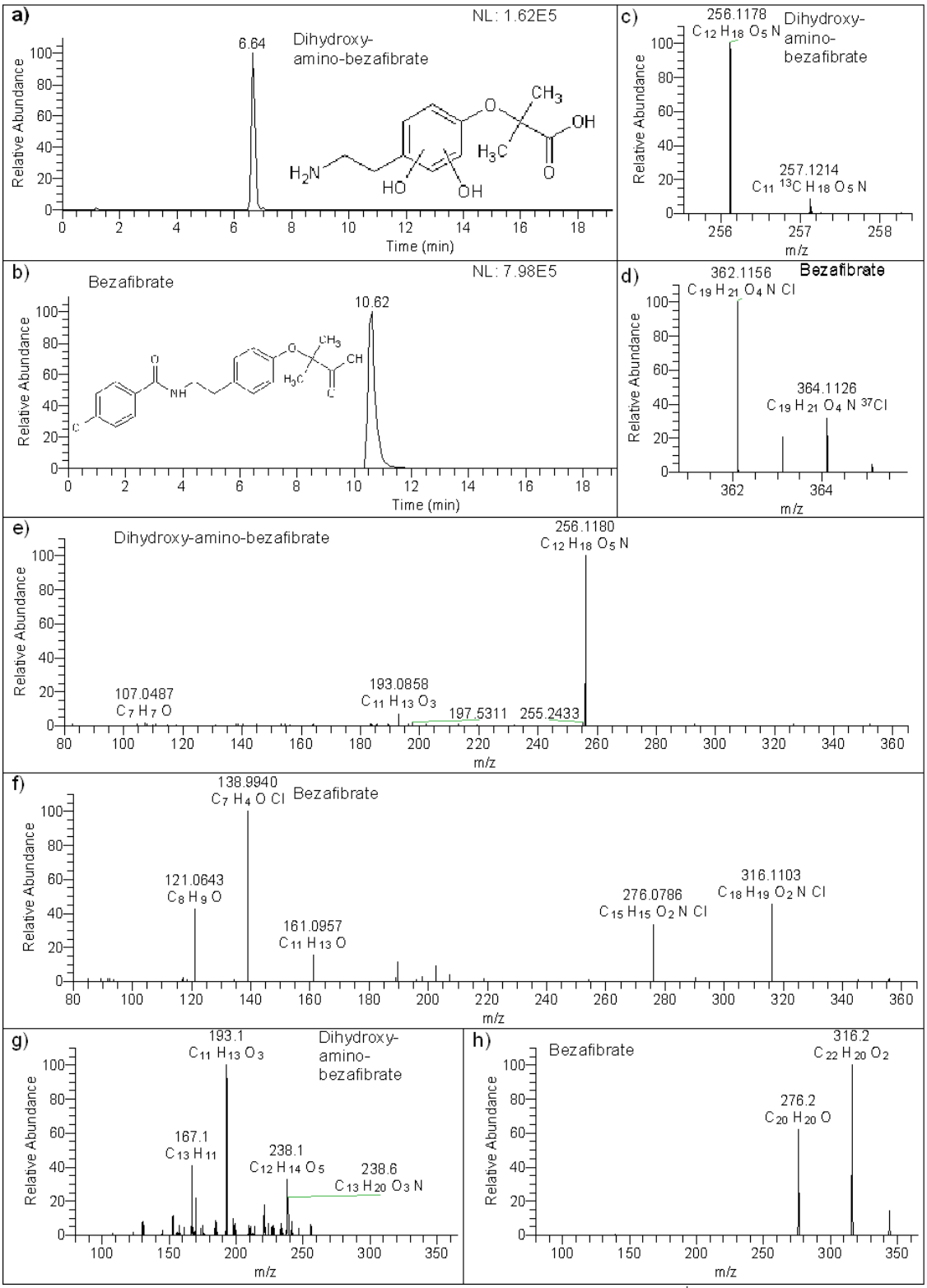


Figure S 3 Structure of the TP dihydroxy-amino-bezafibrate ($[M+H]^+$ 256.1174) and its parent compound bezafibrate ($[M+H]^+$ 362.1154) and corresponding chromatograms (a and b), HR-MS spectra of the molecular ions, including isotopic patterns (c and d), the HR-MS/MS spectra from HCD (e and f), and MS/MS spectra from CID (g and h). All spectra are shown for the Wüeri 09 batch after 48 hours.

MS/MS spectra for valsartan and its TPs

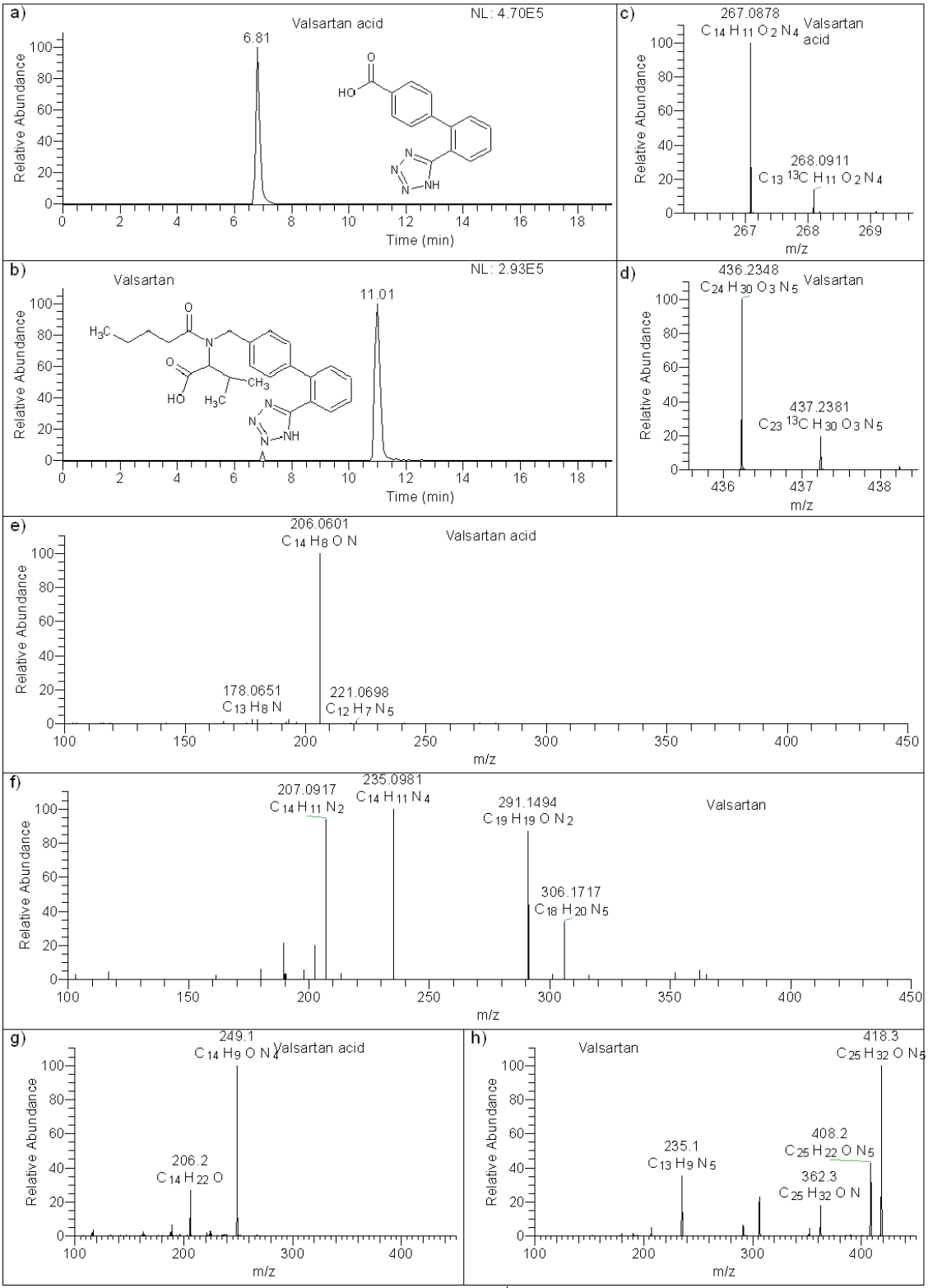


Figure S 4 Structure of the TP valsartan acid ($[M+H]^+$ 267.0877) and its parent compound valsartan ($[M+H]^+$ 436.2343) and corresponding chromatograms (a and b), HR-MS spectra of the molecular ions, including isotopic patterns (c and d), the HR-MS/MS spectra from HCD (e and f), and MS/MS spectra from CID (g and h). All spectra are shown for the Wüeri 09 batch after 10 hours.

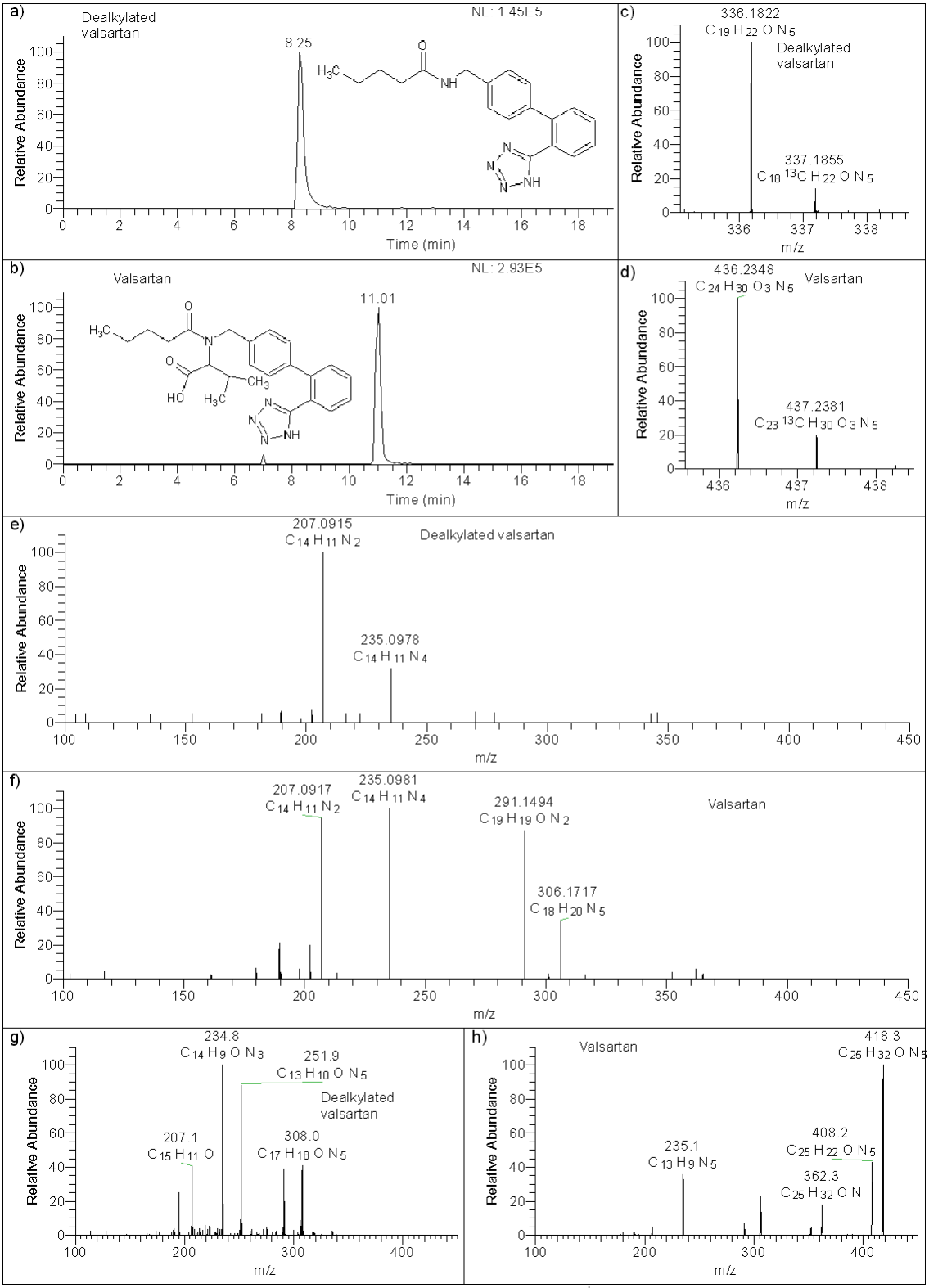


Figure S 5 Structure of the TP dealkylated valsartan ($[M+H]^+$ 336.1819) and its parent compound valsartan ($[M+H]^+$ 436.2343) and corresponding chromatograms (a and b), HR-MS spectra of the molecular ions, including isotopic patterns (c and d), the HR-MS/MS spectra from HCD (e and f), and MS/MS spectra from CID (g and h). All spectra are shown for the Wüeri 09 batch after 10 hours.

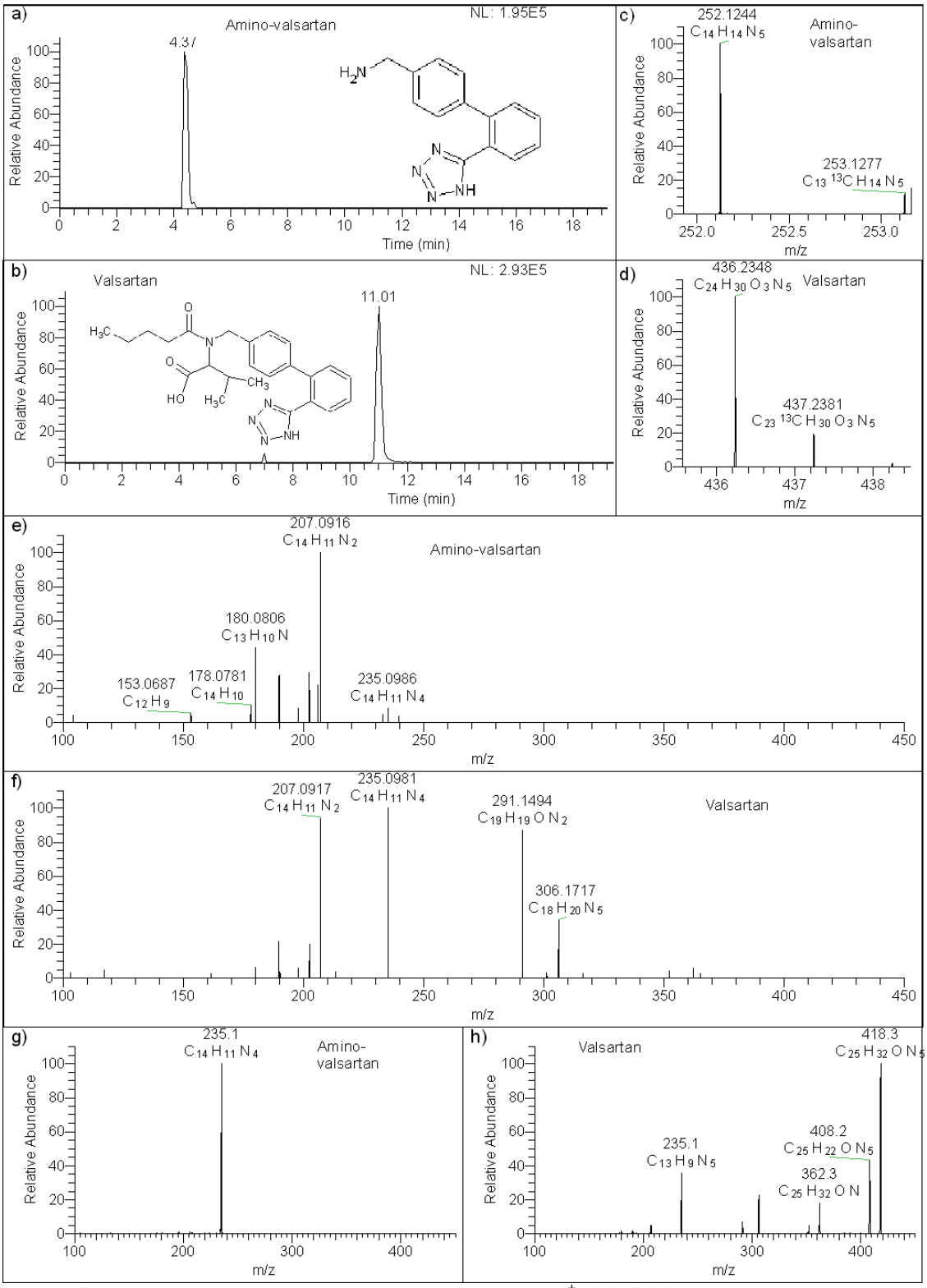


Figure S 6 Structure of the TP amino-valsartan ([M+H]$^+$ 252.1244) and its parent compound valsartan ([M+H]$^+$ 436.2343) and corresponding chromatograms (a and b), HR-MS spectra of the molecular ions, including isotopic patterns (c and d), the HR-MS/MS spectra from HCD (e and f), and MS/MS spectra from CID (g and h). All spectra are shown for the Wüeri 09 batch after 10 hours.

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