Analyst

Therapeutic drug monitoring in dried blood spots using liquid microjunction surface sampling and high resolution mass spectrometry

Tanja Gaissmaier, Markus Siebenhaar, Vanya Todorova, Volker Hüllen, Carsten Hopf

Electronic supplementary information (SI)

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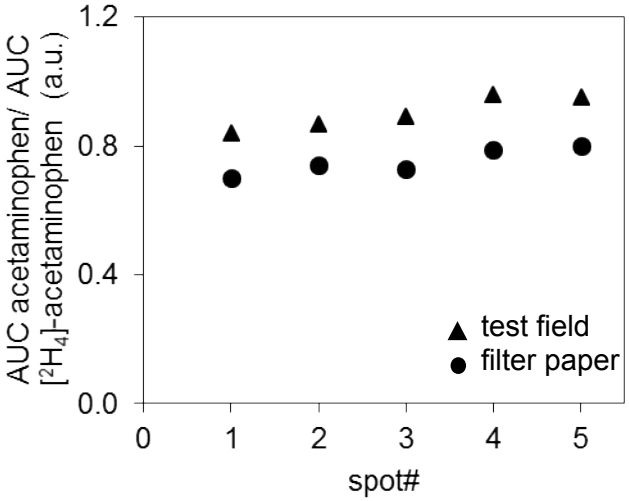


Figure S1: Low variance and good reproducibility for Flowprobe-MS analyses of acetaminophen and internal standard [$^2H_4$]-acetaminophen in DBS on filter paper and glucose test fields. DBS from one donor containing acetaminophen and [$^2H_4$]-acetaminophen as internal standard (IS) (N=5) were prepared on filter paper (1 µg/mL each). CV% was 9.98 and the corresponding average IS-normalized AUC for acetaminophen was 0.78. The normalized acetaminophen AUC for five DBS on glucose measurement test fields containing acetaminophen and IS (0.75 µg/mL each) were reproducible for a single donor. The CV% was 5.73, and the mean normalized acetaminophen AUC was 0.90.

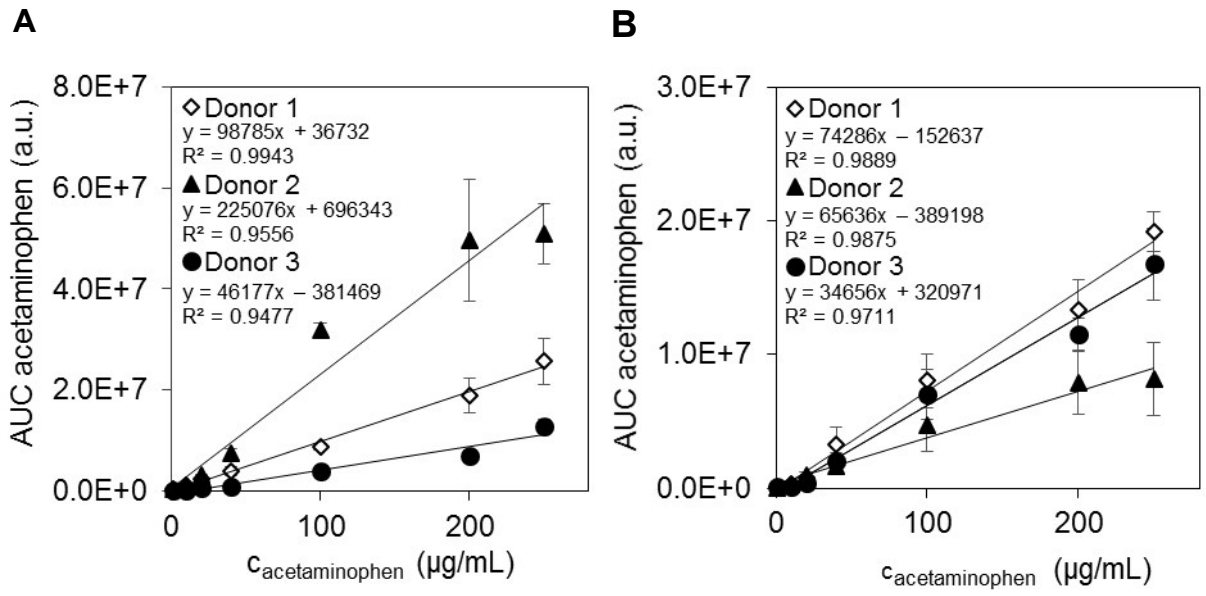
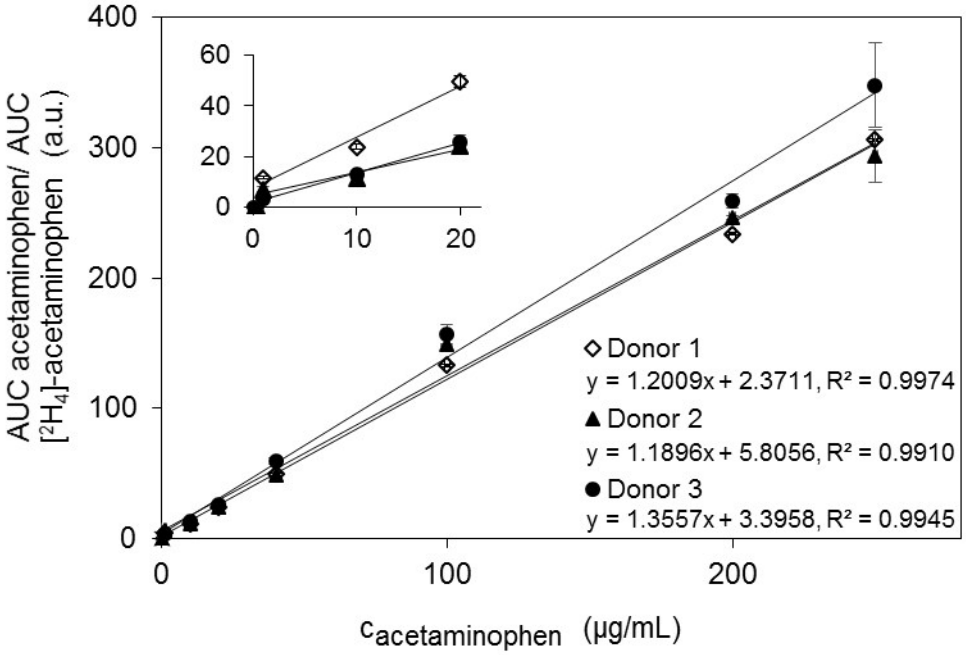


Figure S2: Donor-to-donor variance in non-normalized data is >20% and therefore donor-independent calibration curves cannot be defined. Plotting acetaminophen peak areas versus the nominal acetaminophen concentrations of DBS showed linearity on filter paper **(A)** and glucose measurement test fields **(B)**. The coefficients of determination R^2 on filter paper were calculated to be 0.9943 (donor1), 0.9556 (donor2) and 0.9477 (donor3). For glucose test fields the R^2 were 0.9889 (donor1), 0.9875 (donor2) and 0.9711 (donor 3). However, %CV for one acetaminophen concentration in blood samples from different donors were >20%. Error bars indicate mean +/- SD.

A



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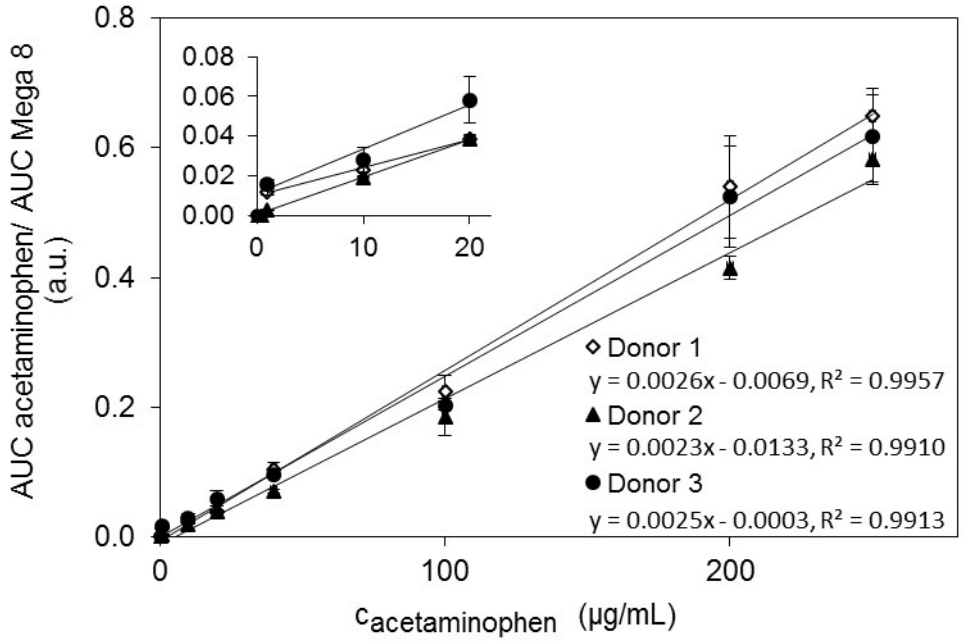

Figure S3: Donor-to-donor variance in normalized data is negligible. A, Normalized acetaminophen AUC were linear for DBS on filter paper (1 - 250 µg/mL) for three different blood donors. [2H4]-acetaminophen IS concentration was kept constant (1 µg/mL). The coefficients of determination were 0.9974, 0.9910 and 0.9945. **B,** On glucose measurement test fields, the acetaminophen AUC normalized against Mega 8 were linear (1 - 250 µg/mL). The coefficient of determination were 0.9957, 0.9910 and 0.9913. The CV% of one concentration between the three different donors were <20% for both investigated surfaces.

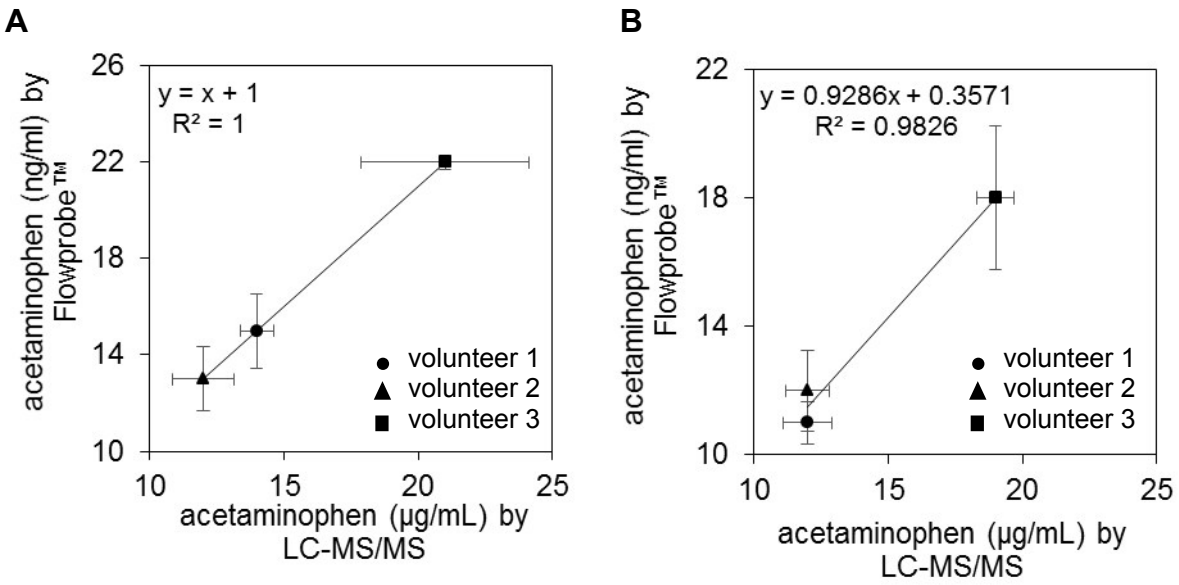

Figure S4: Flowprobe micro extraction and LC-MS/MS correlated very well with R^2>0.99 for the analysis of dried blood on filter paper and test fields in an endpoint pharmacokinetic study. Quantification of acetaminophen in DBS on filter paper using the three-layer set-up and test fields from three volunteers after oral uptake of 1000 mg acetaminophen by Flowprobe micro extraction and LC-MS/MS was performed. **A,** A coefficient of determination of R^2=1 was obtained for analysis performed on filter paper using LC-MS/MS and Flowprobe micro extraction. **B,** On test field the coefficient of determination was R^2=0.9826. Error bars indicate mean +/- SD.

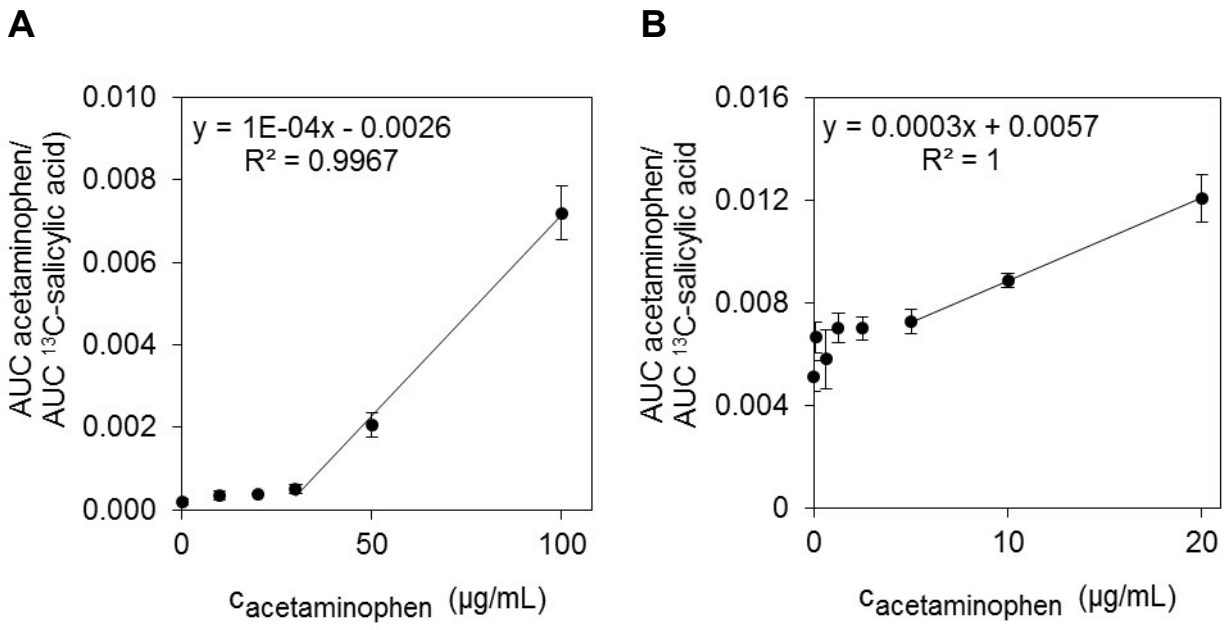

Figure S5: 6-fold increased sensitivity for acetaminophen analysis in DBS using the Flowprobe micro extraction system compared to DESI.

Acetaminophen concentrations in DBS were analyzed by DESI MS (10 - 100 µg/mL) and Flowprobe micro extraction MS (0.08 - 20 µg/mL). **A**, For DESI analysis a linear correlation is obtained in an acetaminophen concentration range in DBS from 30 – 100 µg/ml (R^2=0.9967) with a LOD of 5 µg/ml. **B**, For Flowprobe micro extraction a linear correlation is obtained in an acetaminophen concentration range on DBS from 5 – 20 µg/mL (R^2=1) with a 5-fold increased LOD of 5 µg/mL. Error bars indicate mean +/- SD. For both experiments, a solvent composition of MeOH/H_2O (1:1) was used.

DESI-MS parameter were: nitrogen gas pressure 150 psi, spray voltage -3.5 kV, solvent flow rate 5 µL/min, mass resolution 50,000, max. ion inlet time 500 ms, inlet capillary temperature 250 °C. DBS were analyzed in single line scans with 1.47 min total acquisition time.

Flowprobe-MS parameter were: nitrogen gas pressure 29 psi, spray voltage -3.5 kV, solvent flow rate 15 µL/min, mass resolution 50,000, max. ion inlet time 250 ms, inlet capillary temperature 250 °C. DBS were analyzed in spot sampling mode with 10 seconds sampling time and a total acquisition time of 1.07 min.

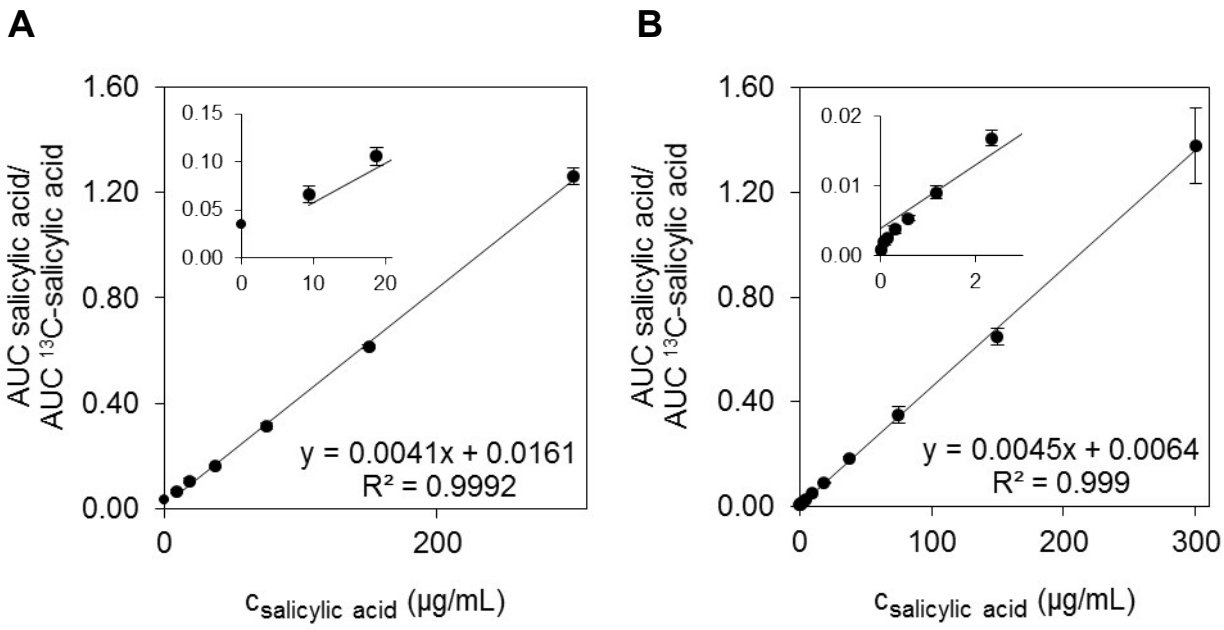

Figure S6: Significant lower LOD for salicylic acid analysis by using Flowprobe MS. Comparison of the calibration curves using desorption electrospray ionization (DESI; **A**) and Flowprobe (**B**) obtained significant differences in sensitivity. Both calibration curves were generated by a serial dilution from a stock solution of 300 µg/ml salicylic acid in whole blood. Analysis by DESI MS obtained 100-fold decreased LOD. Using the Flowprobe source instead lead to an improved LOD of 0.07 µg/mL. The slopes of both calibration curves are comparable with 0.0041 using DESI and 0.0045 using Flowprobe with CVs of ≤14% and ≤12%, respectively.

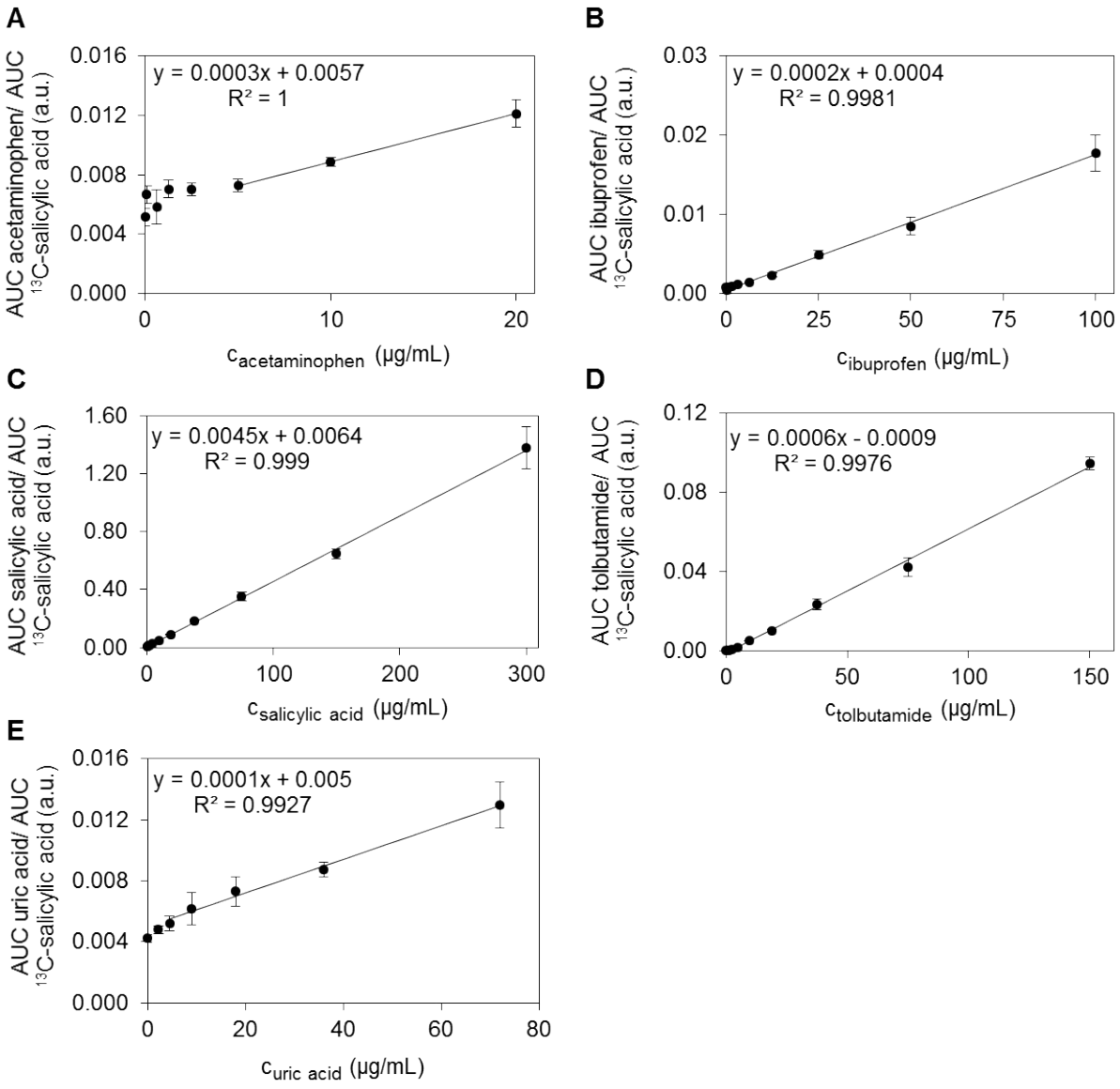

Figure S7: Linear calibration curves were obtained for (A) acetaminophen, (B) ibuprofen, (C) salicylic acid, (D) tolbutamide, and (E) uric acid using Flowprobe micro extraction. Each substance was analyzed in dried blood spots. All calibration curves showed R^2 values of 0.99 or higher. Variances for each recorded data point were CV ≤14%. The obtained linear equations were used for the multi component analysis evaluation.

Each data point consists of N=3 dried blood spots on one 3-layer slide. The internal standard of the second layer was 6-fold ^{13}C labelled salicylic acid.

Flowprobe-MS parameter were: nitrogen gas pressure 29 psi, spray voltage -3.5 kV, solvent flow rate 15 µL/min, mass resolution 50,000, max. ion inlet time 250 ms, inlet capillary temperature 250 °C. DBS were analyzed in spot sampling mode with 10 seconds sampling time and a total acquisition time of 1.07 min. Solvent composition was 1:1 Methanol to water.

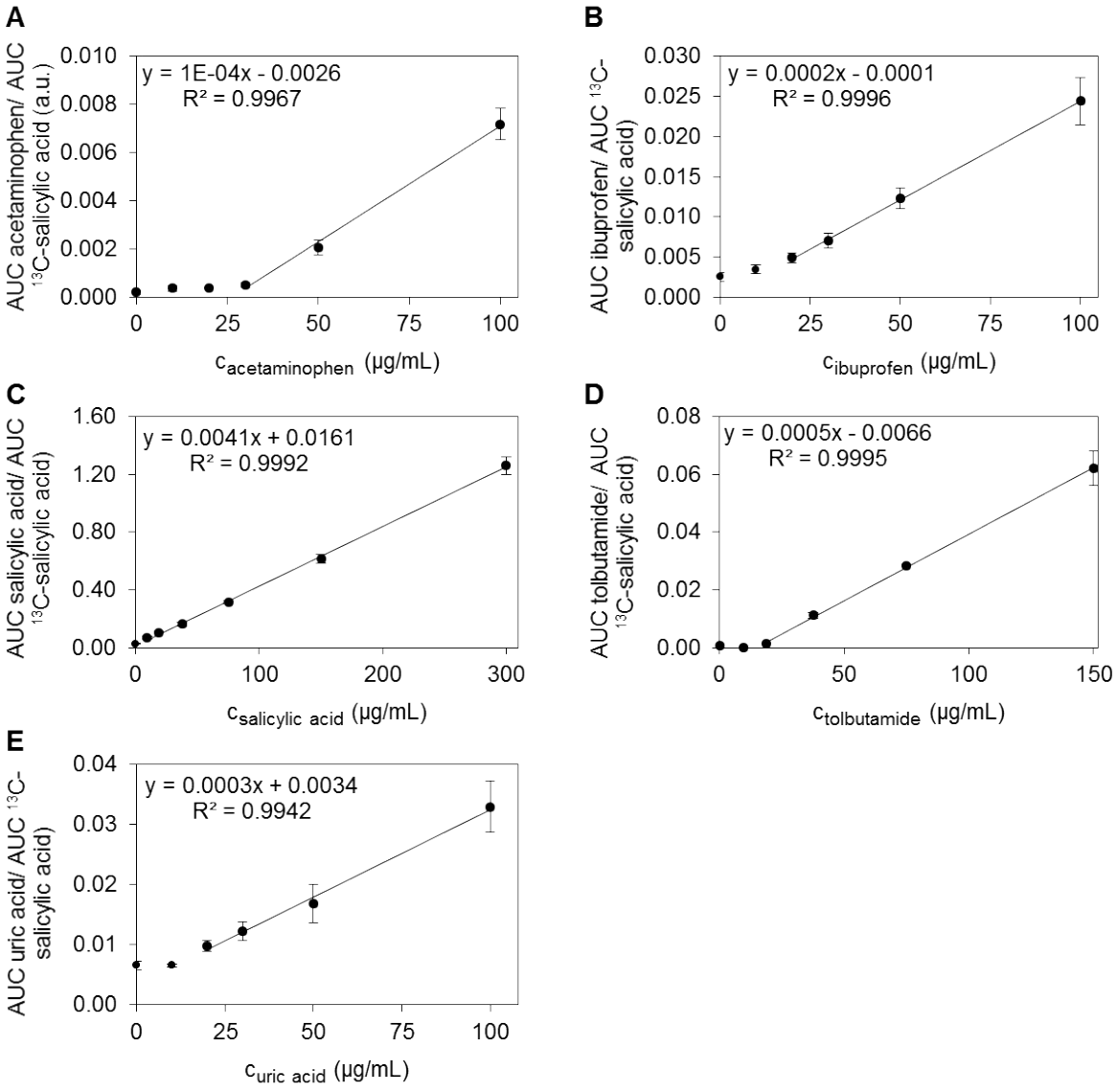

Figure S8: Linear calibration curves were obtained for (A) acetaminophen, (B) ibuprofen, (C) salicylic acid, (D) tolbutamide, and (E) uric acid using DESI-MS. Each substance was analyzed in dried blood spots. All calibration curves showed R^2 values of 0.99 or higher. Variances for each recorded data point were CV ≤14% except for uric acid (CV ≤19%) and acetaminophen (CV ≤ 20%).

Each data point consists of N=3 dried blood spots on one 3-layer slide. The internal standard of the second layer was 6-fold ^{13}C labelled salicylic acid.

DESI-MS parameter were: nitrogen gas pressure 150 psi, spray voltage -3.5 kV, solvent flow rate 5 µL/min, mass resolution 50,000, max. ion inlet time 500 ms, inlet capillary temperature 250 °C. DBS were analyzed in single line scans with 1.47 min total acquisition time. Solvent composition was 1:1 Methanol to water.

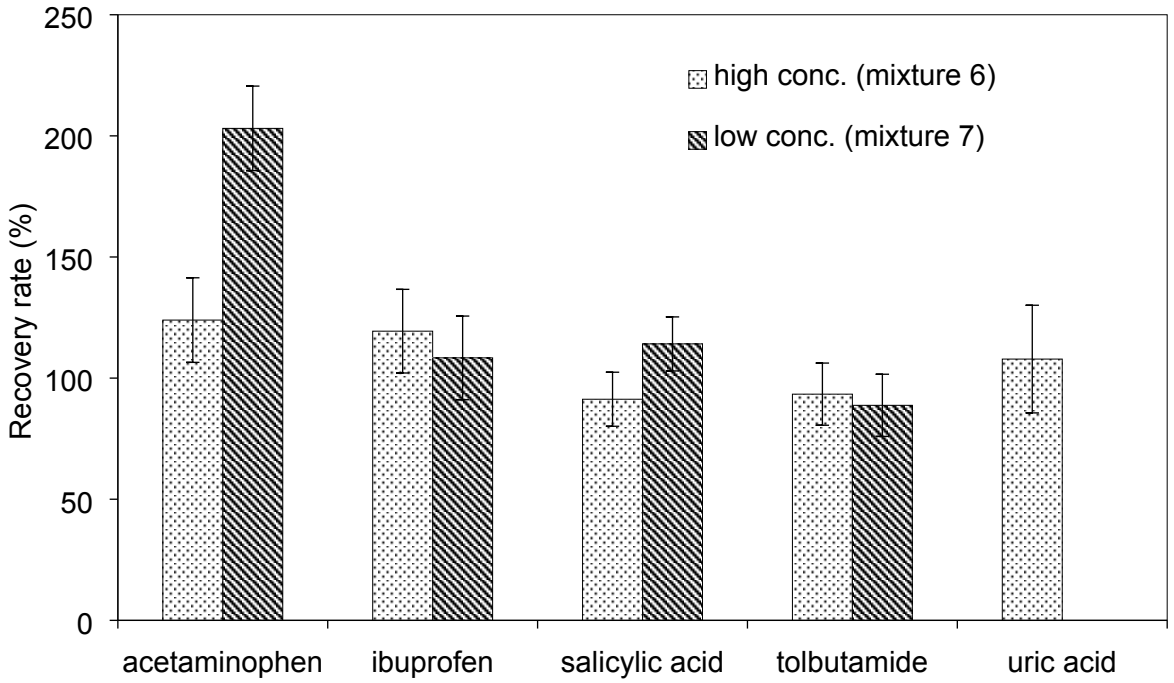

Figure S9: Recovery rates for high concentrations of acetaminophen, ibuprofen, salicylic acid, tolbutamide, and uric acid, as assessed by multi-component analysis using Flowprobe micro extraction MS. Parallel acquisition of five substances in DBS. Blood mixture 6 contained all substances in high concentrations (see Table S2) representing the upper therapeutic concentrations. Recovery rates were in the range from 124% (acetaminophen) to 91% (salicylic acid).
Blood mixture 7 contained all substances in low concentrations, representing a concentration just above or equal to the respective LOD. Recovery rates were 203% for acetaminophen, 108% for ibuprofen, 114% for salicylic acid and 89% for tolbutamide. Uric acid showed complete ion suppression in the presence of all other substances.
Each data point consists of N=3 dried blood spots on one 3-layer slide. The internal standard of the second layer was 6-fold ^{13}C labelled salicylic acid.
Flowprobe-MS parameter were: nitrogen gas pressure 29 psi, spray voltage -3.5 kV, solvent flow rate 15 µL/min, mass resolution 50,000, max. ion inlet time 250 ms, inlet capillary temperature 250 °C. DBS were analyzed in spot sampling mode with 10 seconds sampling time and a total acquisition time of 1.07 min. Solvent composition was 1:1 methanol to water.

		concentration (µg/mL)													
Substance	**Solvent**	Stock	d1	d2	d3	d4	d5	d6	d7	d8	d9	d10	d11	d12	bl
Acetaminophen	EtOH	20	10	5	2.5	1.25	0.625	0.313	0.156	0.078					0
Ibuprofen	MeOH	100	50	25	12.5	6.25	3.125	1.563	0.781	0.391	0.195				0
Salicylic Acid	Saline	300	150	75	37.5	18.75	9.375	4.688	2.344	1.172	0.586	0.293	0.146	0.073	0
Tolbutamide	EtOH	150	75	37.5	18.75	9.38	4.688	2.344	1.172	0.586	0.293	0.146			0
Uric Acid	Saline	72	36	18	9	4.5	2.25	1.125	0.563	0.281	0.141				0

Table S1: Final compound concentrations in whole blood dilution series for calibration curves and multi-component analysis. For blood mixtures in multi-component analysis, only concentrations highlighted in green were used. Blank samples (bl) contained only the respective solvent. (d=dilution).

	analyte concentration (µg/mL)				
mixture #	acetaminophen	ibuprofen	salicylic acid	tolbutamide	uric acid
1	10	100	300	150	72
2	20	1.56	300	150	72
3	20	100	2.3	150	72
4	20	100	300	9.38	72
5	20	100	300	150	9
6	10	1.56	2.3	9.38	9
7	20	100	300	150	72

Table S2: Compound concentrations in blood mixtures for multi-component analysis.