Electronic Supplementary Material (ESI) for Analytical Methods.

Electronic Supplementary Information

Surface Charge-Induced Electrospray for High-Throughput Analysis of Complex Samples and Electrochemical Reaction Intermediates Using Mass Spectrometry

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Detailed Experimental Procedures

Chemicals and materials. Serum and artificial urine were obtained from Shaanxi Lebo Biochemical Technology Co., Ltd (Xi'an, China) and Dongguan Xinheng Technology Co., Ltd (Dongguan, China), respectively. The quantitative filter paper was ordered from Hangzhou Special Paper Co. (Fuyang, China). Silica coated paper (SG81 grade, 0.27 mm thickness) was purchased from Whatman International Ltd. (Maidstone, England). The therapeutic drug standards, including amitriptyline, clozapine, amisulpride, quetiapine, risperidone, aripiprazole, D_3-amitriptyline, D_8-clozapine, D_5-amisulpride, D_8-quetiapine, D_4-risperidone, D_8-aripiprazole, were purchased from Sigma-Aldrich (St. Louis, MO) or Toronto Research Chemicals Inc. (Toronto, Canada). Lysozyme, myoglobin and cytochrome c were purchased from Beijing Biodee Biotechnology Co., Ltd. (Beijing, China). The pesticide standards, including alachlor, acetochlor, metolachlor, butachlor, pretilachlor, and benzeneacetamide, were ordered from Shanghai Future Industrial Limited by Share Ltd (Shanghai, China) or Shanghai Beizhuo Biological Technology Co., Ltd (Shanghai, China). Milk and orange juice samples were obtained from a local supermarket. HPLC grade acetonitrile was purchased from Beijing J&K Scientific Ltd. (Beijing, China). Pipette tips were purchased from Xi'an Jingbo Biotechnology Co., Ltd (Xi'an, China). ZnO particles with diameters of approximately 1 μm were purchased from Shanghai Chaowei Material Technology Co., Ltd (Shanghai, China). Amino-modified multi-walled CNTs were ordered from Beijing Deke Daojin Science & Technology Co., Ltd (Beijing, China), and their lengths and diameters were in the range of 10-30 mm and 8-30 nm, respectively. Soluble starch was purchased from Tianjin Kemiou Chemical Reagent Co. (Tianjin, China). The resin pipette tip was fabricated by three-dimensional (3D) printing (Shaanxi Jugao Additive Manufacturing Technology Development Co., Ltd., Weinan, China). The quartz pipette tip was ordered from a local glass processing company.

Preparation of Micropipette Tips Wrapped with Cu Conductive Tape. In a typical example, a piece of commercially available Cu conductive tape (0.05 mm in thick) with 2 mm width and 8 mm length was wrapped around the tip end of a modified micropipette tip (volume: 100 μL) whose front end was cut by a size of 5 mm.

Preparation of Disposable Micropipette Tips Packed with Paper and Adsorbent. For a typical case, a piece of commercial filter paper was first to cut into a triangle with a base of 5 mm and a height of 11 mm. The triangular paper was then inserted into a 100 μL micropipette tip wrapped in conductive Cu tape. Then 20 μL of the acetonitrile solution containing 250 mg mL^{-1} of adsorbent such as ZnO was added from the back of the micropipette tip. After air drying for several hours, a disposable micropipette tip packed with paper and adsorbent would be available.

Preparation of CNTs-Coated Paper Substrates. The procedure for preparing CNTs-coated paper is similar to our recent studies.[1-3] Specifically, 0.1 g of commercially available CNTs were dispersed in 100 mL of deionized water containing 0.2 g of soluble starch as an adhesive agent. To obtain a uniform solution for coating, the mixed solution was sonicated for 40 min, and the resulting suspension solution was directly transferred to a Buchner funnel covered with blank filter paper with 11 cm diameter

for coating. The papers were then hung in a hood to dry for hours and were pressed between glass plates overnight for use.

Preparation of Disposable Micropipette Tips Packed with CNTs-Coated Paper. In a typical case, a piece of CNTs-coated paper was first to cut into a triangle with a base width of 6 mm and a length of 4 cm. The triangular paper was then inserted into a 100 μL micropipette tip wrapped with Cu conductive tape.

Procedure for Measuring the Spray Currents of SCIESI Ionization Sources. The spray current of the SCIESI source was determined by using acetonitrile as the spray solvent. The current was measured using an 8246B TRMS digital multimeter (Xi'an Shengli Instrument Co., Ltd., Xi'an, China). Specifically, the paper tip from the SCIESI was directed at a stainless steel sheet measuring 2 cm (width) x 3 cm (length), which was directly connected to the TRMS digital multimeter. The distance between the paper tip and the stainless steel sheet was 7 mm. After applying 20 μL of spray solvent and 3.5 kV of DC voltage, the spray current was recorded directly.

Procedure for Measuring the Residual Voltages on the Inner Surfaces of Pipette Tip Made of Different Materials. The residual voltages on the inner surfaces of polypropylene, resin and quartz were measured using a Fluke 17B digital multimeter (Shanghai Shilu Instrument Co., Ltd., Shanghai, China). When the DC voltage (3.5 kV) was turned off, the multimeter probe immediately touched the corresponding internal surfaces to measure the residual voltage.

Procedure for Imaging the Spray Plume of SCIESI. The spray plume from the SCIESI source was imaged by using an OMT-5950A horizontal microscope (Suzhou Omnitec Optoelectronics Technology Co., Suzhou, China) coupled to a 195LM00001 HSO LCD monitor (Wuhan Hengfa Technology Co., Wuhan, China).

Mass Spectrometric Analysis of Therapeutic Drugs in Complex Matrices. The TSQ Quantum Access Max mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) was used for detection. Quantitative analysis of therapeutic drugs and pesticides in complex matrices was performed using the selected reaction monitoring (SRM) mode. The corresponding MS detection parameters are listed in Table S1. Mass spectra were recorded in the positive ion mode, and the MS inlet capillary temperature was 270 ^{o}C. Identification of target compound ions was confirmed by tandem mass spectrometry (MS/MS) using collision-induced dissociation (CID), with argon gas of 99.995% purity used as the collision gas. For a typical experiment with SCIESI-MS, a DC voltage of 3.5 kV was applied to the above disposable micropipette tip containing 2 μL of the sample after the addition of 20 μL of spray solvent such as acetonitrile. The distance between the micropipette tip and the MS inlet was approximately 8 mm. A spray time of 30 s was used unless otherwise stated. When optimizing the various experimental parameters, the highest MS signals for each series, in the range of 10^5 to 10^6, were taken as 100% and the others as their relative values.

Probing Electrochemical Reactions using SCIESI-MS. To probe the electrochemical oxidation of N,N-dimethylaniline (DMA) to its dimer N,N,N',N'-tetramethylbenzidine (TMB) in the positive mode, 100 μL of DMA solution at a concentration of 20 μg mL^{-1} (acetonitrile as the solvent) was loaded into the SCIESI emitter. A 4.5 kV DC voltage

was then applied to the ion source to monitor the electrochemical reaction process. For the electrochemical oxidation of 2-naphthalenethiol (2-NT) to 2-napthalenesulfonic acid (2-NSo) in negative mode, the reactant 2-NT was dissolved in 1:1 ethanol/water containing 10 mM ammonium acetate and 0.1% ammonia, and its concentration was 50 μg mL^{-1}. In the electrochemical reaction, a voltage of -3.5 kV was applied. For the electrochemical reduction of 6-nitroquinoline (6-NQ) to 6-aminoquinoline (6-AQ) in the positive mode, 30 μg mL^{-1} of 6-NQ in methanol containing 0.5% acetic acid was used, and the applied spray voltage was 4.5 kV. For the electrochemical reduction of 4-nitrobenzoic acid (4-NA) to 4-aminobenzoic acid (4-AA) in negative mode, 20 μg mL^{-1} of 6-NQ in methanol containing 0.5% acetic acid was used, and the applied spray voltage was -4.5 kV.

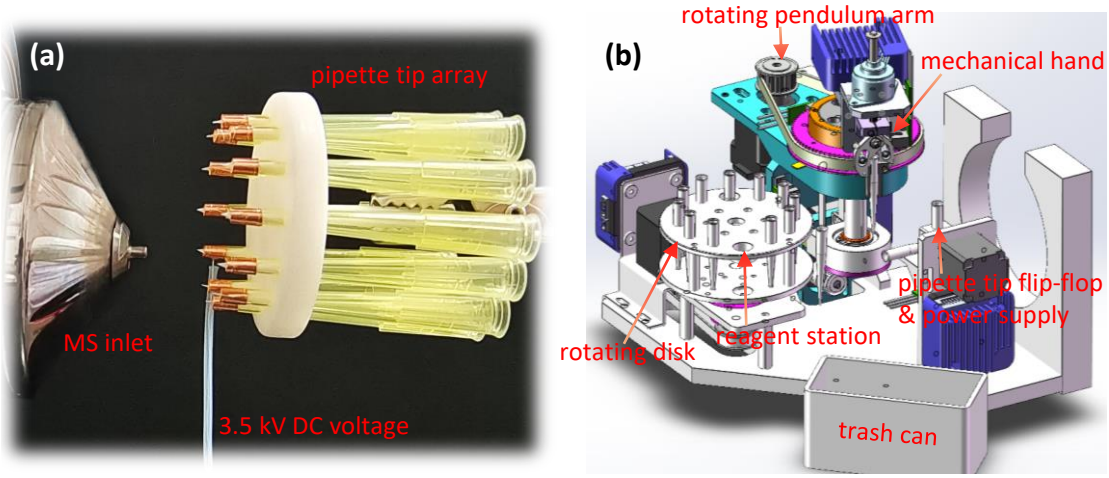

Figure S1. a) Photograph of the high-throughput SCIESI array coupled to a mass spectrometer; b) Proposed schematic diagram of the high-throughput SCIESI array for automated operation.

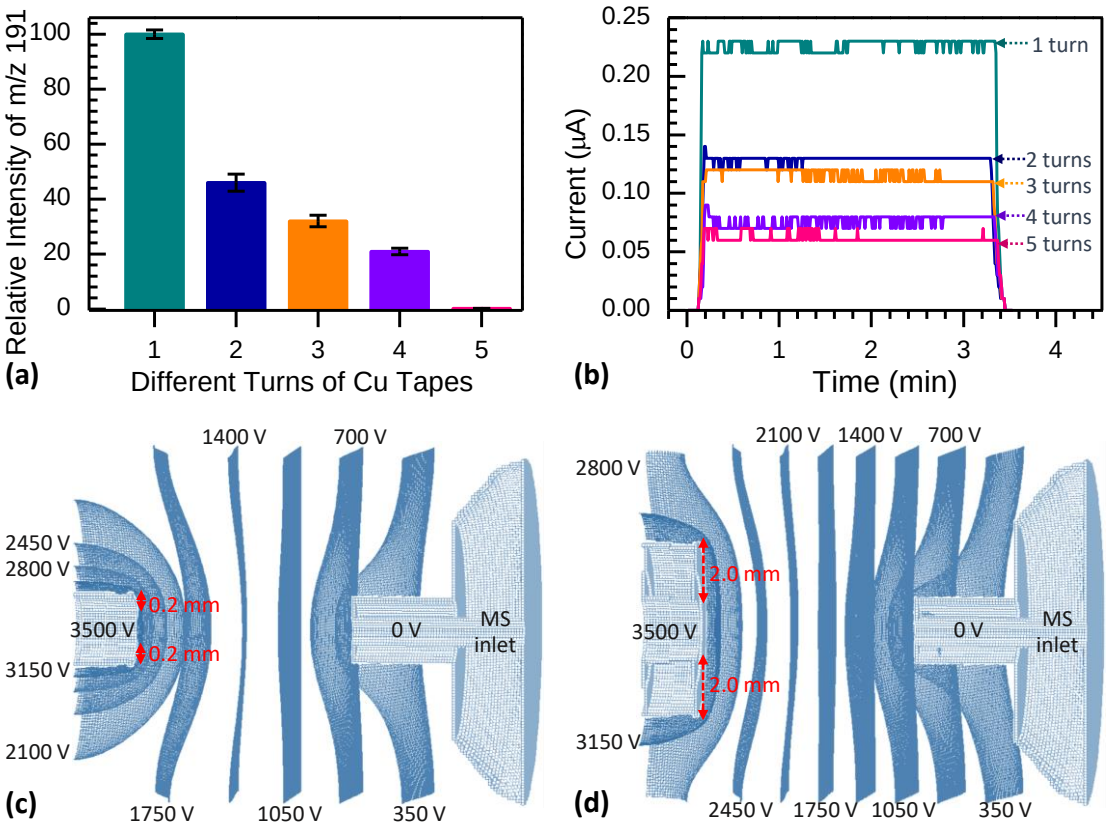

Figure S2. Influence of the different turns of Cu conductive tapes wrapped around the pipette tip a) on SCIESI-MS for the analysis of risperidone and b) on the current from the resulting spray plume; electric field profiles between SCIESI and an MS inlet when the thickness of the Cu conductive tape was c) 0.2 mm and d) 2.0 mm.

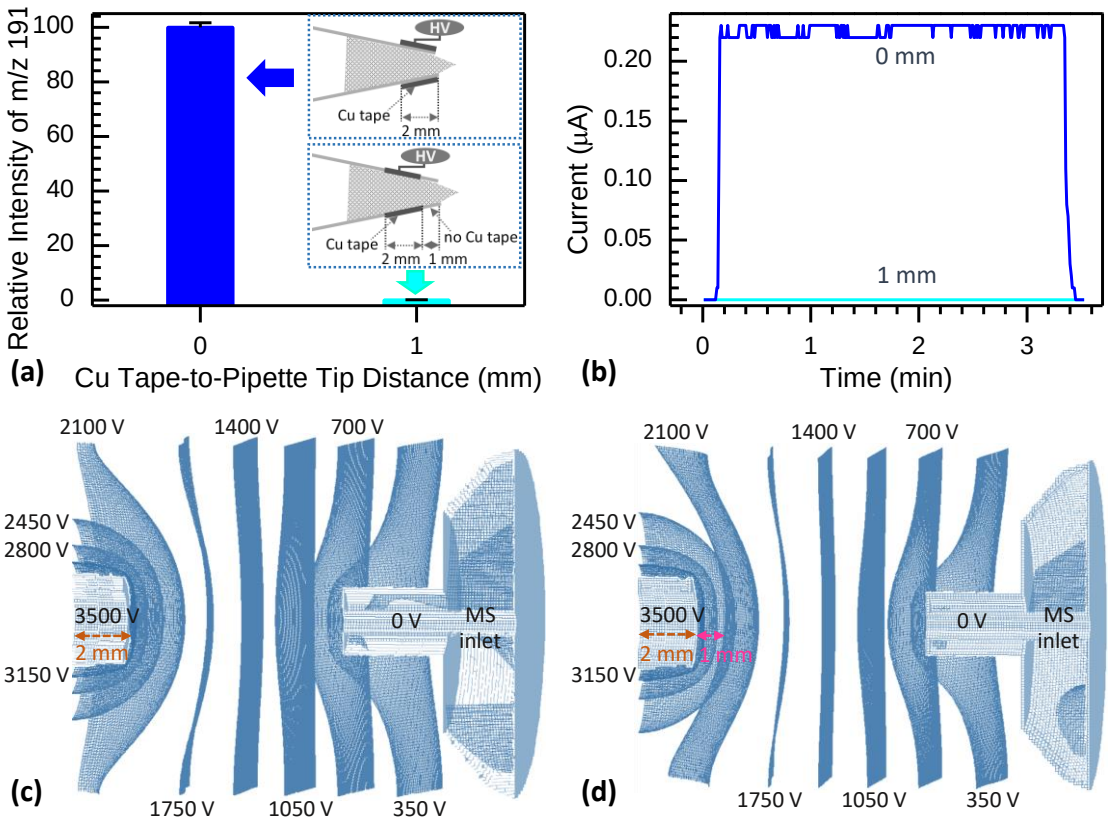

Figure S3. Influence of the distance of the Cu conductive tape to the front end of the pipette tip a) on the SCIESI-MS for the analysis of risperidone and b) on the current from the resulting spray plume; electric field profiles between SCIESI and an MS inlet when the distance of the Cu conductive tape to pipette tip was c) 0 mm and d) 1 mm.

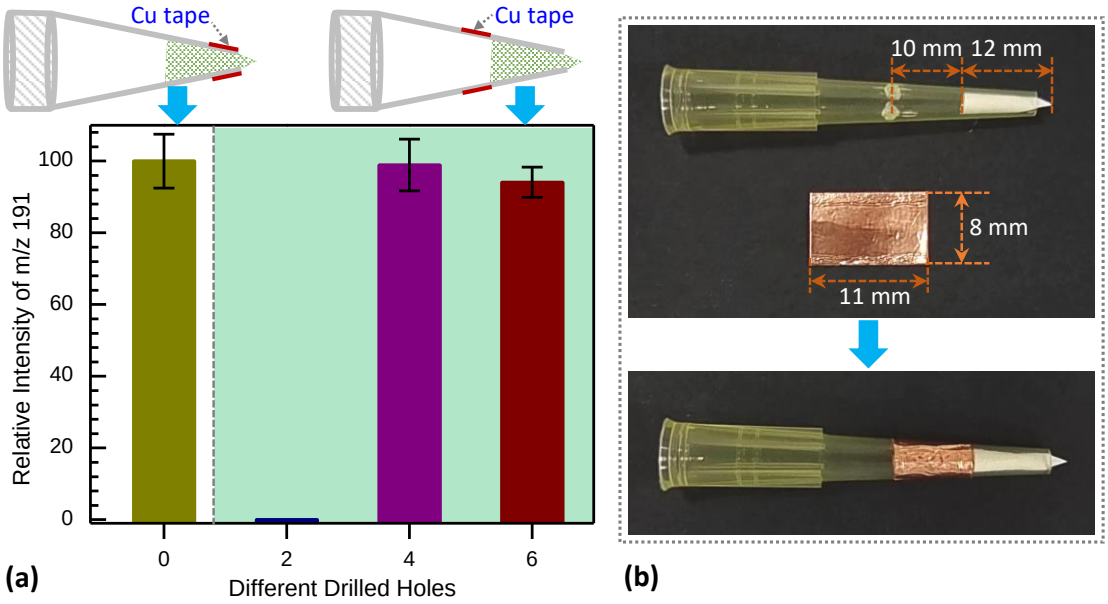

Figure S4. a) Comparison of the performance of the geometries of the pipette tip wrapped with Cu conductive tape at its front end and the pipette tip drilled with holes at a distance of 10 mm from the rear end of the paper substrate and subsequently wrapped with Cu conductive tape for the analysis of risperidone; b) Photographs of the preparation procedure of the pipette tip drilled with holes and subsequently wrapped with Cu conductive tape.

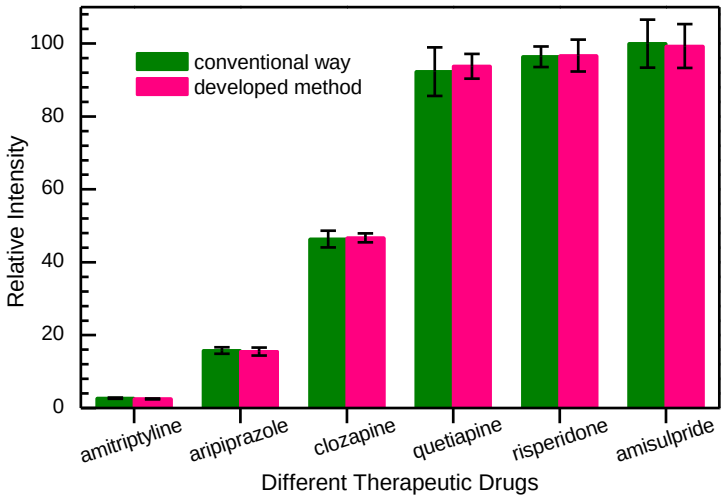

Figure S5. Comparison of the performance of the SCIESI source (developed way) and portable paper-based ESI by contacting a metal wire with a paper substrate (conventional method) for the analysis of various therapeutic drugs.

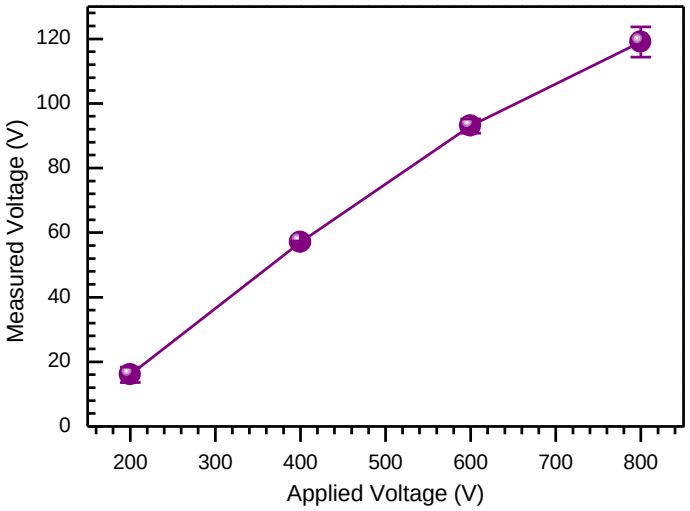

Figure S6. Correlation between the voltage applied to the Cu conductive tape and the voltage measured on the paper substrate (Note: To measure the voltage on the paper substrate, a metal wire was inserted into the pipette tip used and touched to the paper substrate. The corresponding voltage was then measured using a multimeter. Due to the limitations of our experimental conditions, a maximum voltage of 800 V was applied to the Cu conductive tape).

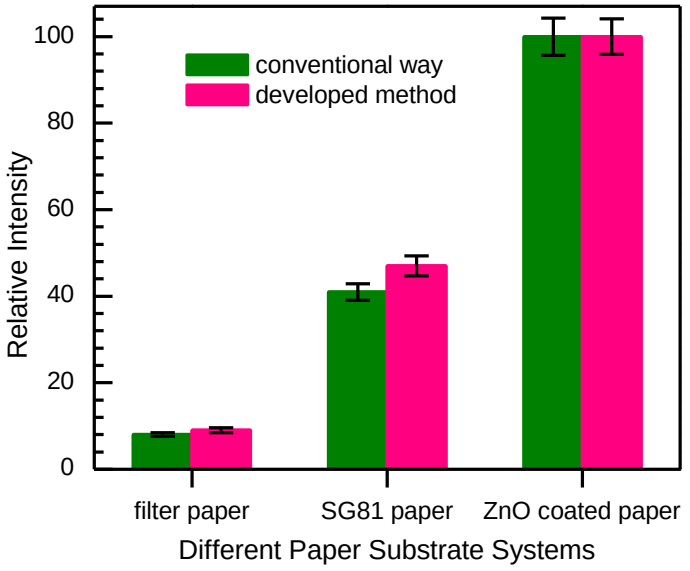

Figure S7. Comparison of the performance of the SCIESI source (developed way) and portable paper-based ESI by contacting a metal wire with a paper substrate (conventional method) for the analysis of 1 μg mL^{-1} risperidone in serum using different paper substrates (sample volume: 2 μL; spray solvent: 20 μL acetonitrile; applied voltage: 3.5 kV).

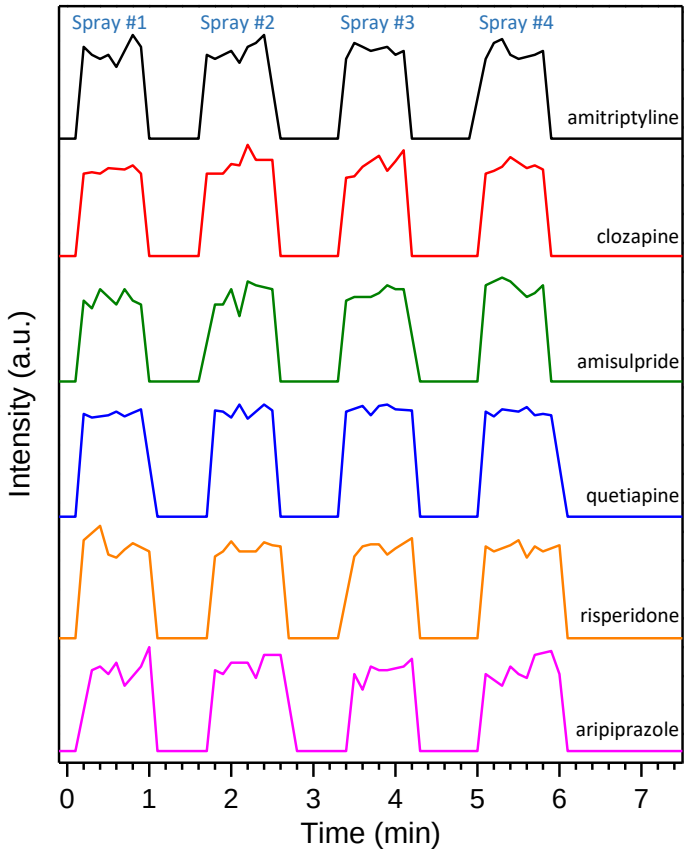

Figure S8. Ion chronograms for four spray events of amitriptyline, clozapine, amisulpride, quetiapine, risperidone, and aripiprazole in serum (100 ng mL^{-1} for each drug; sample volume: 2 µL; spray solvent: 20 µL acetonitrile; applied voltage: 3.5 kV).

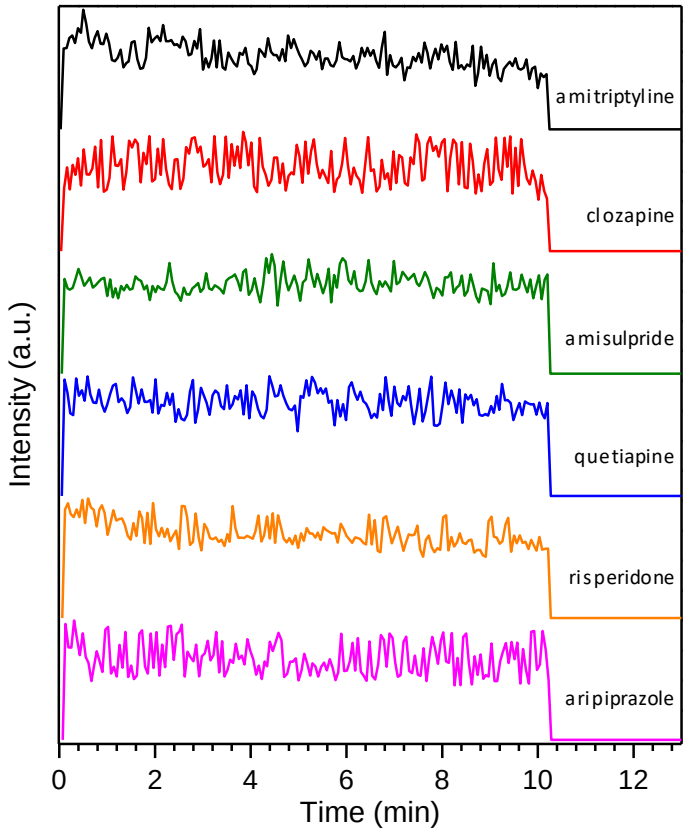

Figure S9. Ion chronograms for a single spray event of amitriptyline, clozapine, amisulpride, quetiapine, risperidone, and aripiprazole in serum (100 ng mL^{-1} for each drug; sample volume: 2 µL; spray solvent: 20 µL acetonitrile; applied voltage: 3.5 kV).

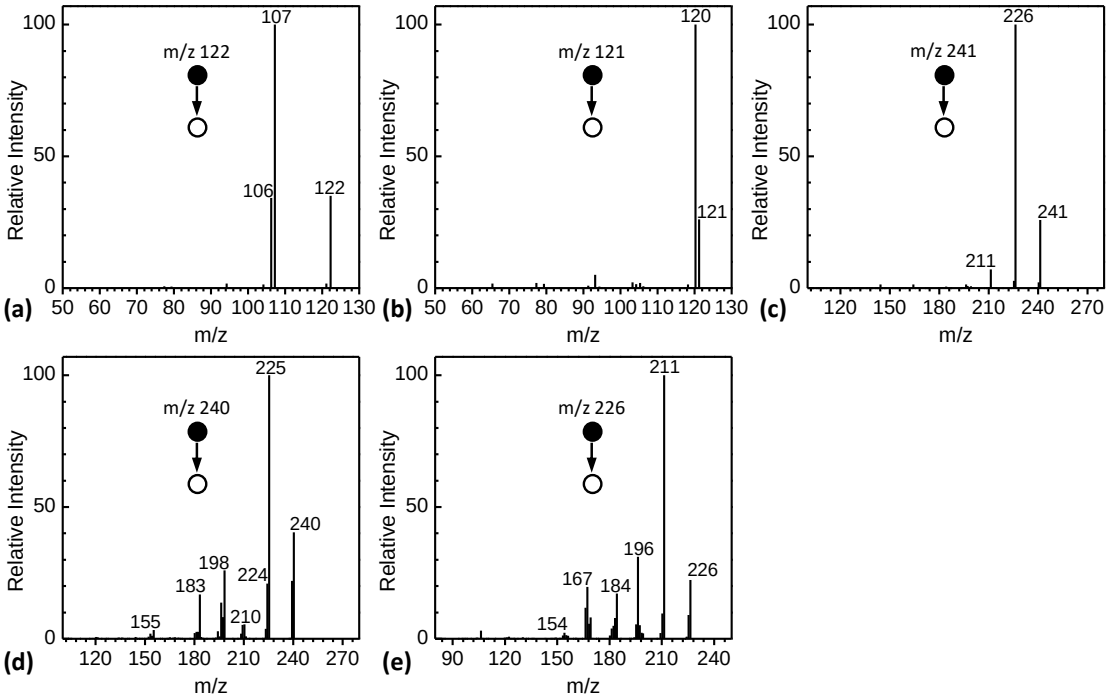

Figure S10. MS/MS spectra of the a) reactant (m/z 122), b) intermediate (m/z 121), and c-e) products (m/z 241, m/z 240, and m/z 226) involved in the oxidation reaction of N,N-dimethylaniline to its dimer N,N,N',N'-tetramethylbenzidine.

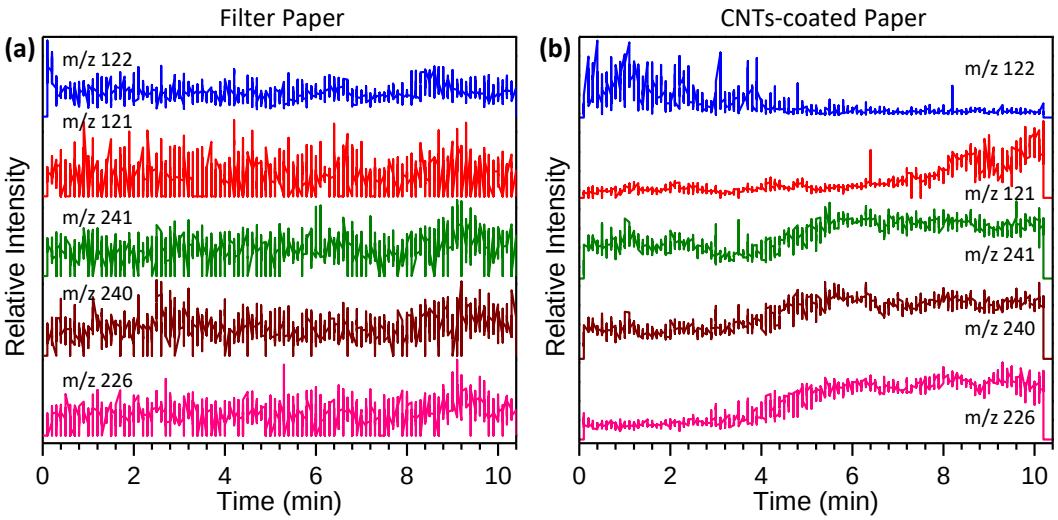

Figure S11. Extracted ion chronograms (EIC) of the reactant (m/z 122), intermediate (m/z 121), and products (m/z 241, m/z 240, and m/z 226) involved in the oxidation reaction of N,N-dimethylaniline to its dimer N,N,N',N'-tetramethylbenzidine using a) filter paper and b) CNTs-coated paper as electrodes.

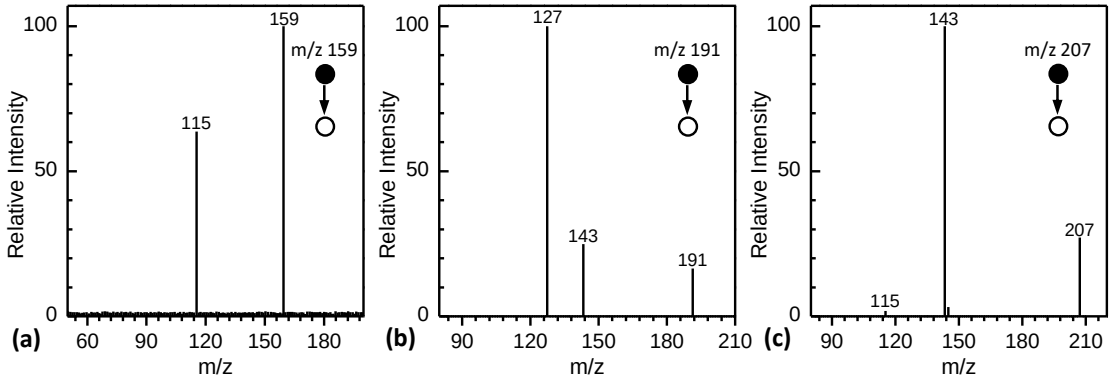

Figure S12. MS/MS spectra of the a) reactant (m/z 159), b) intermediate (m/z 191), and c)-e) product (m/z 207) involved in the oxidation reaction of 2-naphthalenethiol (2-NT) to 2- naphthalenesulfonic acid (2-NSo).

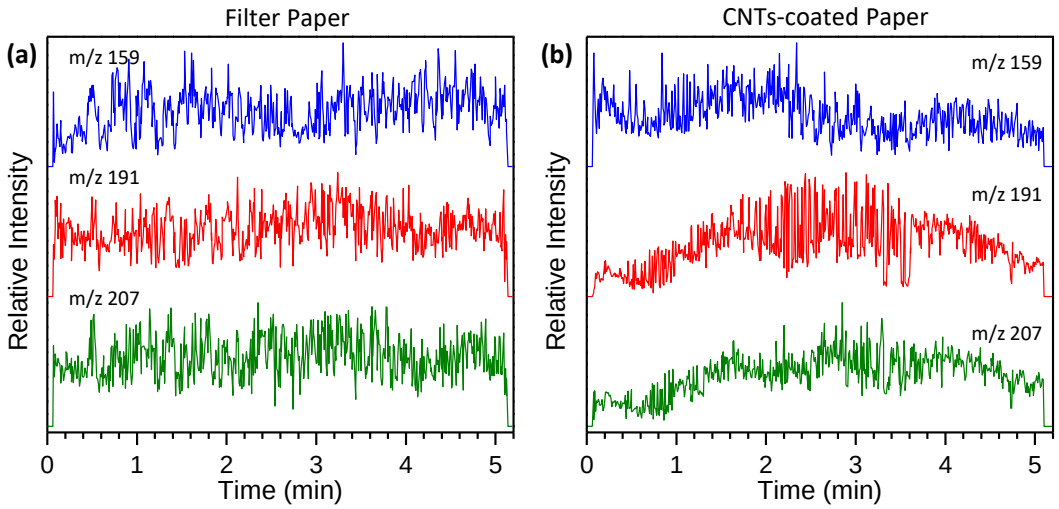

Figure S13. Extracted ion chronograms (EIC) of the reactant (m/z 159), intermediate (m/z 191), and product (m/z 207) involved in the oxidation reaction of 2-naphthalenethiol (2-NT) to 2- naphthalenesulfonic acid (2-NSo) using a) filter paper and b) CNTs-coated paper as electrodes.

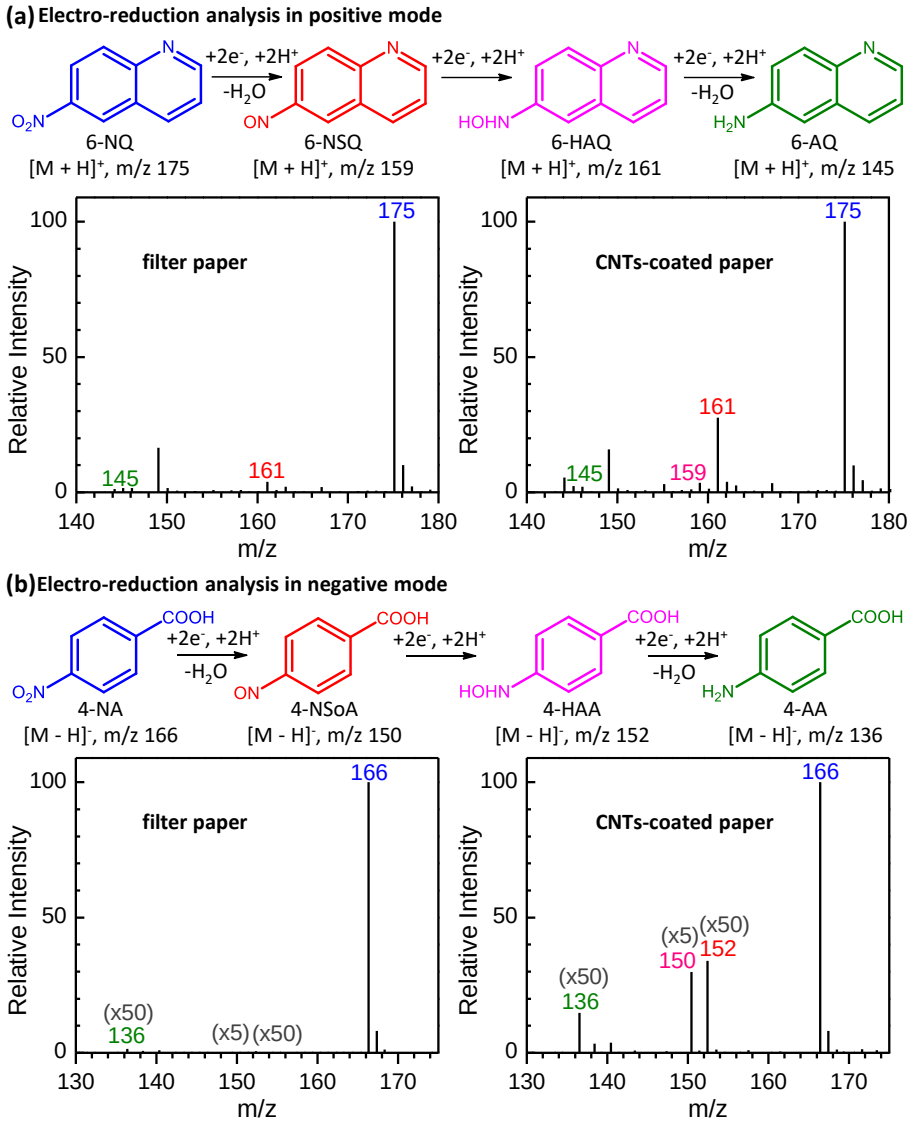

Figure S14. Electrochemical reduction of a) 6-nitroquinoline (6-NQ) to 6-aminoquinoline (6-AQ) in the positive mode and b) 4-nitrobenzoic acid (4-NA) to 4-aminobenzoic acid (4-AA) in the negative mode.

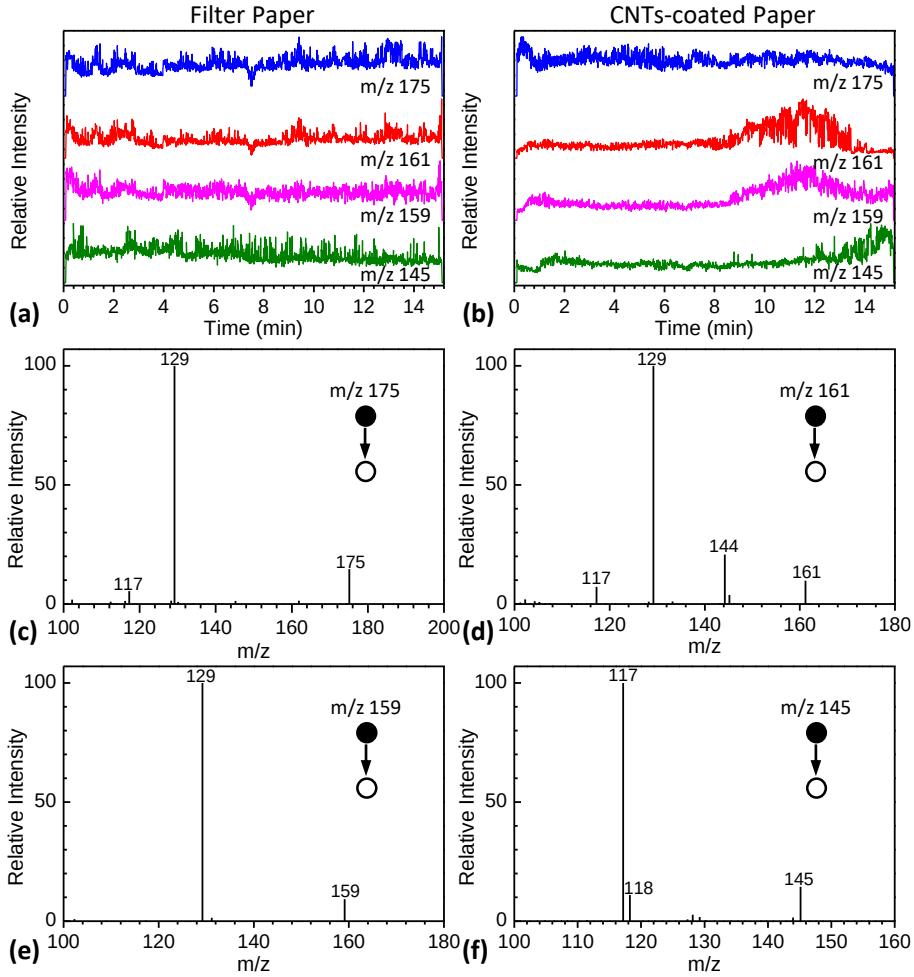

Figure S15. Extracted ion chronograms (EIC) of the reactant (m/z 175), intermediates (m/z 161 and m/z 159) and product (m/z 145) involved in the reduction reaction of 6-nitroquinoline (6-NQ) to 6-aminoquinoline (6-AQ) in positive mode using a) filter paper and b) CNTs-coated paper as electrodes, and c-f) their corresponding MS/MS spectra.

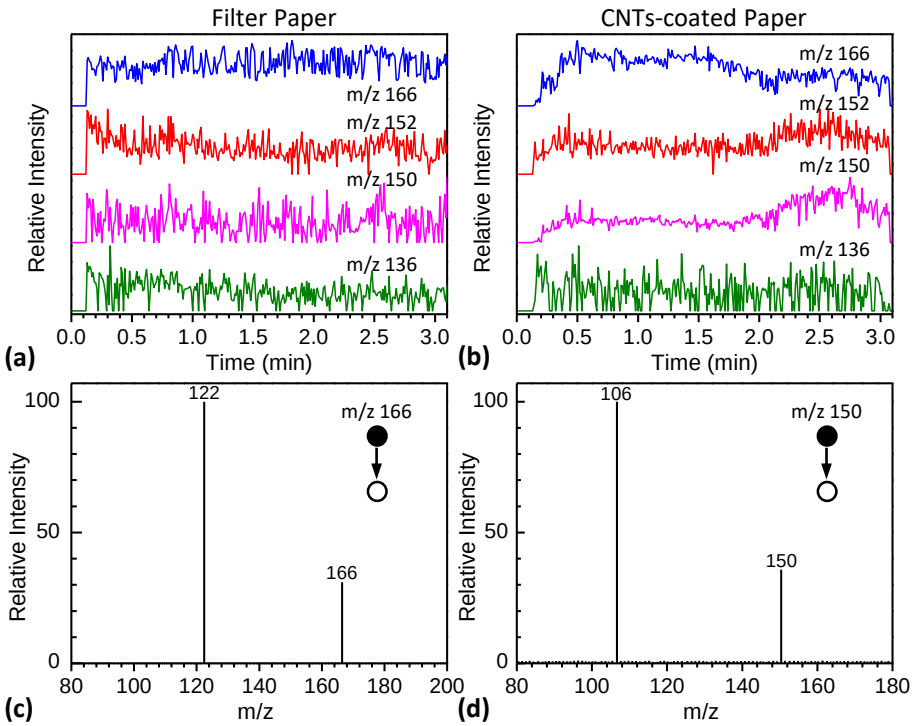

Figure S16. Extracted ion chronograms (EIC) of the reactant (m/z 166), intermediates (m/z 152 and m/z 150), and product (m/z 136) involved in the reduction reaction of 4-nitrobenzoic acid (4-NA) to 4-aminobenzoic acid (4-AA) in negative mode using a) filter paper and b) CNTs-coated paper as electrodes, and c-d) their corresponding MS/MS spectra (note: MS/MS spectra of m/z 152 and m/z 136 were not performed due to low peak intensity).

Table S1. Selected reaction monitoring (SRM) conditions for all therapeutic drugs and their internal standards

Analyte	Parent Ion m/z	Fragment Ion *m/z*	Collision Energy (V)	Tube Lens (V)
Amitriptyline	278, $[M + H]^+$	84	25	83
D_3-Amitriptyline	281, $[M + H]^+$	87	23	83
Clozapine	327, $[M + H]^+$	270	21	89
D_8-Clozapine	335, $[M + H]^+$	275	21	91
Amisulpride	370, $[M + H]^+$	112	24	95
D_5-Amisulpride	375, $[M + H]^+$	117	25	96
Quetiapine	384, $[M + H]^+$	253	21	97
D_8-Quetiapine	392, $[M + H]^+$	258	23	98
Risperidone	411, $[M + H]^+$	191	26	90
D_4-Risperidone	415, $[M + H]^+$	195	26	91
Aripiprazole	448, $[M + H]^+$	285	25	109
D_8-Aripiprazole	456, $[M + H]^+$	293	25	106

Table S2. Selected reaction monitoring (SRM) conditions for all pesticides

Analyte	Parent Ion m/z	Fragment Ion *m/z*	Collision Energy (V)	Tube Lens (V)
Alachlor	270, $[M + H]^+$	162	20	70
Acetochlor	270, $[M + H]^+$	133	30	70
Metolachlor	312, $[M + H]^+$	252	15	91
Butachlor	312, $[M + H]^+$	162	20	91
S-Metolachlor	284, $[M + H]^+$	252	13	60
Benzeacetamide	239, $[M + H]^+$	163	15	86

Table S3. Figures of merit for the quantitative analysis of therapeutic drugs in 2 µL of different liquid samples (e.g., serum and urine) with isotope-labelled compounds as internal standards via SCIESI-MS.

analytes	samples	slope	intercept	R^2	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	linearity range (ng mL^{-1})
amitriptyline	serum	0.042	-0.124	0.9996	0.003	0.010	0.01 – 1,000
	urine	0.066	-0.126	0.9999	0.003	0.010	0.01 – 1,000
clozapine	serum	0.037	-0.033	0.9999	0.002	0.006	0.01 – 1,000
	urine	0.036	-0.074	0.9999	0.004	0.012	0.01 – 1,000
amisulpride	serum	0.049	0.148	0.9999	0.003	0.009	0.01 – 1,000
	urine	0.100	-0.078	0.9999	0.003	0.011	0.01 – 1,000
quetiapine	serum	0.112	0.128	1.0000	0.002	0.006	0.01 – 1,000
	urine	0.284	-0.062	1.0000	0.002	0.007	0.01 – 1,000
risperidone	serum	0.452	1.626	0.9997	0.003	0.009	0.01 – 1,000
	urine	0.157	0.487	0.9998	0.003	0.011	0.01 – 1,000
aripiprazole	serum	0.029	-0.049	0.9999	0.002	0.008	0.01 – 1,000
	urine	0.050	0.147	0.9999	0.003	0.011	0.01 – 1,000

Note: The LOD and LOQ values were calculated according to the 3σ/s and 10σ/s, in which σ is the standard deviation of the blank (n = 4), and s is the slope of related calibration curves.

Table S4. Figures of merit for the quantitative analysis of therapeutic drugs in 2 μL of different liquid samples (e.g., serum and urine) without isotope-labelled compounds as internal standards via SCIESI-MS.

analytes	samples	slope	intercept	R^2	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	linearity range (ng mL^{-1})
amitriptyline	serum	59.3	237.3	0.9998	0.004	0.013	0.01 – 1,000
	urine	32.9	-80.6	0.9998	0.002	0.008	0.01 – 1,000
clozapine	serum	77.1	299.7	0.9998	0.002	0.007	0.01 – 1,000
	urine	48.0	147.1	0.9999	0.003	0.009	0.01 – 1,000
amisulpride	serum	149.2	464.0	0.9999	0.003	0.010	0.01 – 1,000
	urine	210.5	-663.4	0.9998	0.002	0.007	0.01 – 1,000
quetiapine	serum	504.9	1360.9	0.9999	0.003	0.009	0.01 – 1,000
	urine	167.6	627.8	0.9997	0.003	0.011	0.01 – 1,000
risperidone	serum	584.8	1747.6	0.9998	0.003	0.010	0.01 – 1,000
	urine	136.0	-433.2	0.9998	0.004	0.013	0.01 – 1,000
aripiprazole	serum	78.2	233.1	0.9998	0.003	0.009	0.01 – 1,000
	urine	52.1	158.1	0.9999	0.002	0.008	0.01 – 1,000

Note: The LOD and LOQ values were calculated according to the 3σ/s and 10σ/s, in which σ is the standard deviation of the blank (n = 4), and s is the slope of related calibration curves.

Table S5. Figures of merit for the quantitative analysis of different pesticides in 2 µL of different liquid samples (e.g., milk and orange juice) without isotope-labelled compounds as internal standards via SCIESI-MS.

analytes	samples	slope	intercept	R^2	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	linearity range (ng mL^{-1})
Alachlor	milk	53.22	207.94	0.9998	0.003	0.012	0.01-1000
	orange juice	70.01	306.49	0.9998	0.002	0.008	0.01-1000
Acetochlor	milk	80.56	324.61	0.9998	0.003	0.009	0.01-1000
	orange juice	199.56	-180.76	0.9999	0.002	0.007	0.01-1000
Metolachlor	milk	239.85	-263.00	0.9999	0.003	0.011	0.01-1000
	orange juice	277.60	689.45	0.9998	0.002	0.006	0.01-1000
Butachlor	milk	53.41	223.98	0.9998	0.003	0.011	0.01-1000
	orange juice	279.22	1143.80	0.9997	0.002	0.007	0.01-1000
S-Metolachlor	milk	172.37	594.34	0.9998	0.003	0.013	0.01-1000
	orange juice	168.53	664.78	0.9998	0.002	0.007	0.01-1000
Benzeacetamide	milk	83.34	161.17	0.9999	0.004	0.014	0.01-1000
	orange juice	215.63	-292.86	0.9999	0.003	0.010	0.01-1000

Note: The LOD and LOQ values were calculated according to the 3σ/s and 10σ/s, in which σ is the standard deviation of the blank (n = 4), and s is the slope of related calibration curves.

References

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(3) Zheng, Y.; Wang, Q.; Wang, X.; Chen, Y.; Wang, X.; Zhang, X.; Bai, Z.; Han, X.; Zhang, Z., Development and Application of Zirconia Coated Paper Substrate for High Sensitivity Analysis of Therapeutic Drugs in Dried Blood Spots. *Anal. Chem.* **2016,** *88* (14), 7005-7013.