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Electronic Supplementary Information

Paper Spray: A Simple and Efficient Means of Analysis of Different Contaminants in Foodstuffs

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1. Procedure for preparation of different foodstuffs

(1) **Pork and beef**: The pork/beef homogenate was prepared as follows: 0.3 g of the pork/beef was placed in a 15 mL centrifuge tube and 1 mL deionized water was added. The samples were homogenized using a digital homogenizer for at least half an hour until homogenized. After thoroughly cooling, the homogenized solution was transferred into a fresh tube and stored at -20^{o}C for later use.

(2) **Formula powder**: An exactly measured amount of infant formula powder was placed in a 15 mL centrifuge tube, and deionized water was added to obtain a concentration of 0.3 g mL^{-1}. After sonicating for about 10 min, the formula solution was ready for use.

(3) **Chili powder**: Dried chili powder was sieved through a medical gauze. An exactly measured amount of the sieved chili powder was placed in a 15 mL centrifuge tube, and deionized water added to make a chili powder solution with a concentration 0.3 g mL^{-1}. After sonicating for about 10 min the chili powder solution was ready for use.

(4) **Milk and sport juice**: Milk and sport juice were used without further treatment. Note: the foods used were purchased from local supermarkets.

2. Experimental Conditions for Paper Spray Mass Analysis

The procedure for paper spray experiment has been described in previous reports.[1-5] Briefly, the standards (Sigma-Aldrich, St. Louis, MO) used for these experiments were prepared as follows: illicit additive solutions were prepared by dilution of stock solutions into 1:1 methanol/water for melamine, clenbuterol, terbutaline, salbutamol and ractopamine, and into 1:1 chloroform/isopropanol for Sudan red II, III, IV and G, and into 2:8 dichloromethane/isopropanol for Bis(2-ethylhexyl) Phthalate (DEHP) and bis(2-ethylhexyl) adipate (DEHA). The standard solutions were then spiked into different food samples by pipetting 5 μL of the standard into 495 μL of sample food solution. The food samples of lower concentrations of additives were prepared with a series of dilutions, each with a 40 μL

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solution of higher concentration of an additive mixed with 360 μL of food solution. The concentrations of the additives in the final food solution samples were 0.1, 1, 5, 10, 20, 50, 100 and 1000 ng mL^{-1} or as otherwise indicated in the figures. Food samples were prepared by spotting a fixed volume (5 μL) of food sample solution onto Whatman SG81 silica coated paper substrate (Maidstone, England) and drying for at least 4 h at room temperature. The foodstuff solution sample (5 μL) was dropped onto the middle of the paper. The samples were stored at room temperature in a sealed plastic bag containing desiccant. For paper spray, the silica-coated paper spotted with sample was cut into a triangle with a height of 10 mm and a base width of 5 mm. A copper clip was used to hold the paper and to apply a high voltage (3.5 kV). The distance between the paper triangle tip and the mass spectrometer inlet was about 5 mm. Spray solvent of 25 μL was then added to the base of the paper triangle followed by application of a high voltage. The selection of solvent for paper spray was based on our experience from previous studies[2, 6] and other references on food safety[7]. Quantitation of the contaminants in those foodstuffs was carried out using a triple quadrupole mass spectrometer (TSQ Quantum Access Max, Thermo Scientific Inc., San Jose, CA). The signal intensity was averaged over the entire period of the selected reaction monitoring (SRM) with the paper spray.

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Supplementary Table S1 Selected reaction monitoring (SRM) conditions

Analyte	Parent ion *m/z*	Fragment ion *m/z*	Tube lens (V)	q2 offset (V)
Melamine	127, $(M + H)^+$	85	75	18
Clenbuterol	277, $(M + H)^+$	203	67	16
Terbutaline	226, $(M + H)^+$	152	70	16
Salbutamol	240, $(M + H)^+$	148	63	18
Ractopamine	302, $(M + H)^+$	164	69	15
Sudan Red II	277, $(M + H)^+$	121	54	22
Sudan Red III	353, $(M + H)^+$	197	70	20
Sudan Red IV	381, $(M + H)^+$	91	71	31
Sudan Red G	279, $(M + H)^+$	123	53	18
Bis(2-ethylhexyl) Phthalate (DEHP)	391, $(M + H)^+$	149	46	27
Bis(2-ethylhexyl) Adipate (DEHA)	371, $(M + H)^+$	129	81	14

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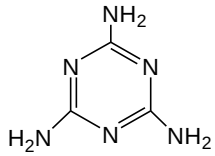
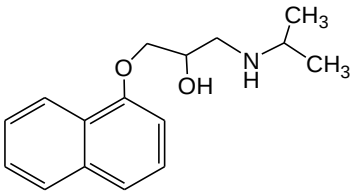
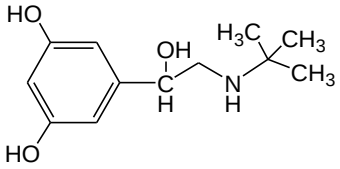
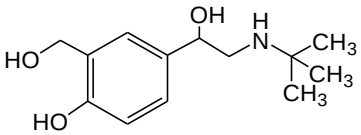
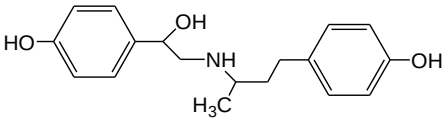
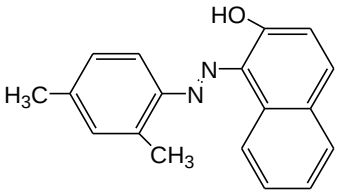
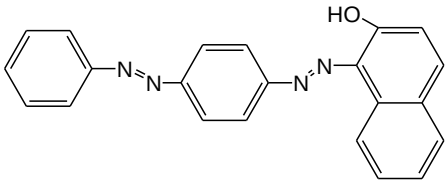
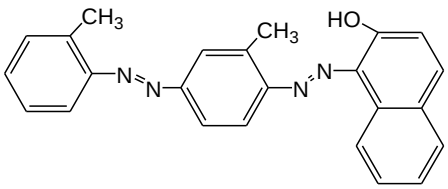
Supplementary Table S2 Lower limits of detection for contaminants with a wide range of chemical properties in various foodstuffs

Compound	Chemical structure	Lower limit of detection (ng/mL or ng/g)
Melamine		20
Clenbuterol		1
Terbutaline		1
Salbutamol		5
Ractopamine		1
Sudan Red II		50
Sudan Red III		50
Sudan Red IV		50

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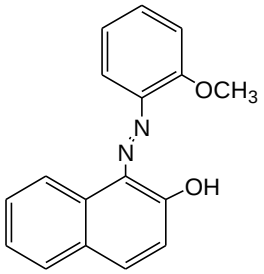
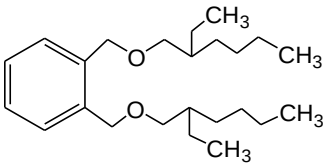
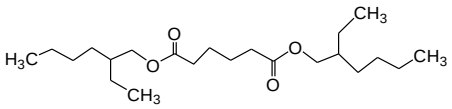
Sudan Red G		100
Bis(2-ethylhexyl) Phthalate (DEHP)		200
Bis(2-ethylhexyl) Adipate (DEHA)		50

Supplementary Table S3 RSD values for melamine in milk and clenbuterol in pork determined by paper spray (n = 4)

Melamine Concentration in Milk (ng/mL)	RSD	Clenbuterol Concentration in Pork (ng/g)	RSD
1	21.8%	1	9.4%
5	11.9%	5	11.3%
10	11.0%	10	7.4%
20	11.8%	20	7.7%
50	7.9%	50	10.6%
100	11.5%	100	12.8%
1000	8.2%	1000	3.7%

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Supplementary Table S4 LODs of the tested contaminants in different matrices using LC/MS-MS or GC/MS (from literature data)

Analyte	Matrix	LOD (LC/MS-MS)	LOD (GC/MS)	Reference
Clenbuterol	Pork	0.12 ng/g	-	[8]
Terbutaline	Pork	0.05 ng/g	-	[9]
Salbutamol	Pork	0.11 ng/g	-	[8]
Ractopamine	Pork	0.07 ng/g	-	[8]
Melamine	Milk and Formula	25 ng/g	-	[10]
Sudan Red II	Pepper Sauce	0.4 ng/g	-	[11]
Sudan Red III	Pepper Sauce	1.0 ng/g	-	[11]
Sudan Red IV	Pepper Sauce	1.2 ng/g	-	[11]
Sudan Red G	Pepper Sauce	0.4 ng/g	-	[11]
Bis(2-ethylhexyl) Phthalate (DEHP)	Jelly, Bacon, Cheese, Biscuit	-	2.0 ng/g	[12]
Bis(2-ethylhexyl) Adipate (DEHA)	Jelly, Bacon, Cheese, Biscuit	-	2.0 ng/g	[12]

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Supplementary Table S5 Comparison of the LODs of melamine in milk or formula determined using different ambient ionization sources coupled to mass spectrometers

Ambient Ionization Source	Matrix	LOD	Reference
Desorption Atmospheric Pressure Chemical Ionization (DAPCI)	Milk	6.6 ng/mL	[13]
Low-Temperature Plasma (LTP)	Milk	15 ng/mL	[14]
Paper Spray Ionization (PSI)	Milk	20 ng/mL	Present Study
	Formula	50 ng/g	
Ultrasound-Assisted Extractive Electrospray Ionization (EESI)	Milk	500 ng/mL	[15]
Nanoextractive Electrospray Ionization (nanoEESI)	Milk	≤200 ng/mL	[16]
Direct Analysis in Real Time (DART)	Milk Powder	170 ng/g	[17]

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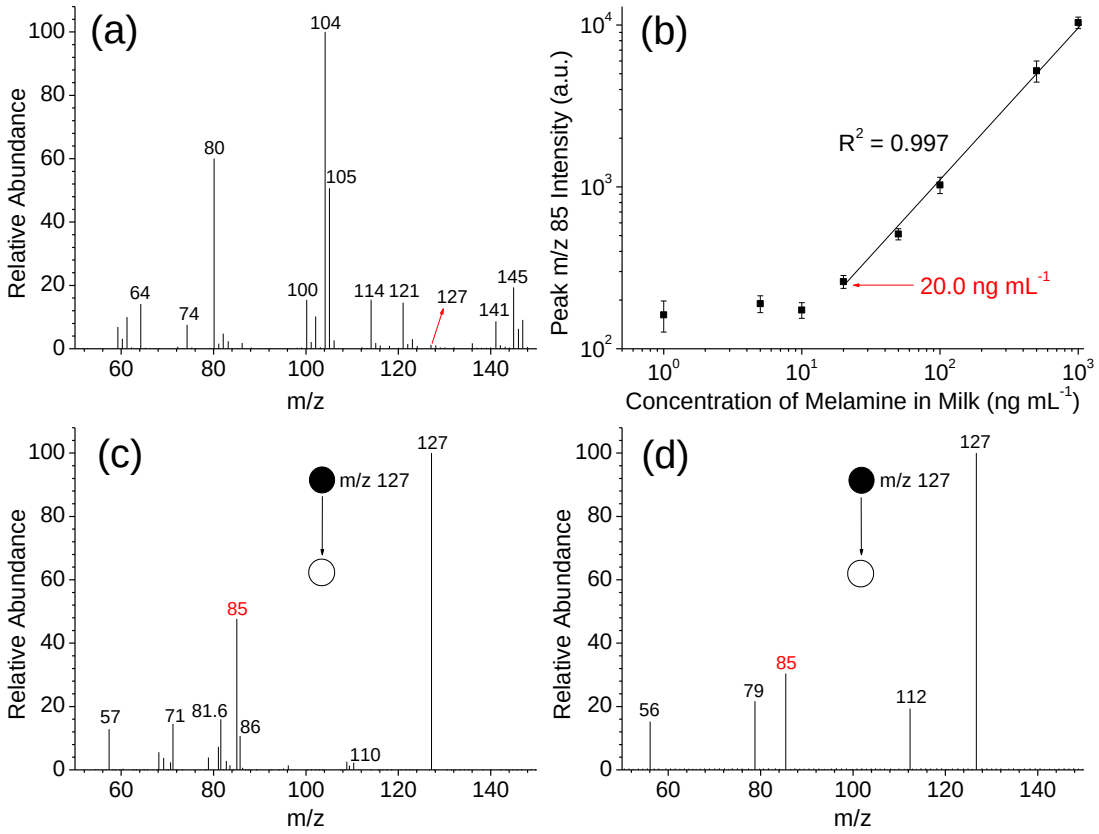

Fig. S1 Determination of melamine in milk using silica coated paper for paper spray: (a) full MS spectrum of 1000 ng mL^{-1} of melamine in milk, (b) linear dynamic range of melamine [$(M+H)^+$, m/z 127, product ion, m/z 85], (c) MS/MS spectrum of melamine (m/z 127) in milk at 1000 ng mL^{-1} and (d) 20 ng mL^{-1}. Sample amount: 5.0 µL; Solvent: 99:1 acetonitrile/water containing 26 µM citric acid and 5.0 mM ammonium acetate; solvent volume: 50 µL; voltage: 3.5 kV.

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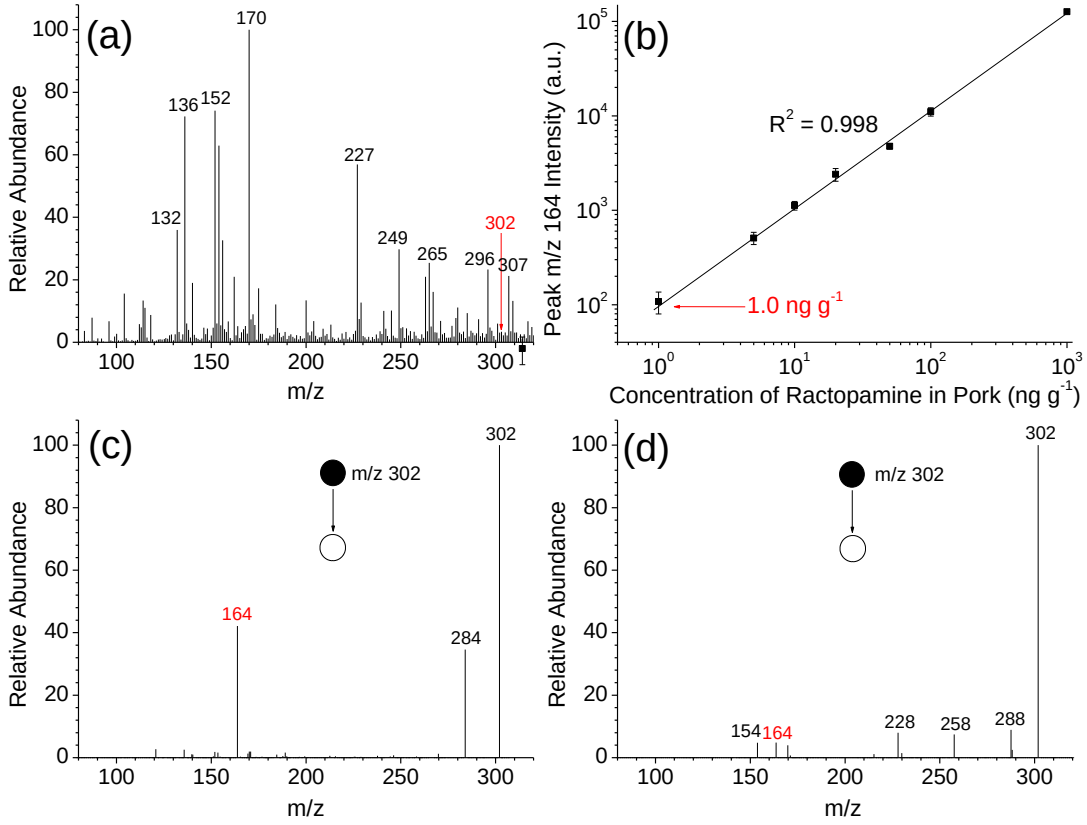

Fig. S2 Determination of ractopamine in pork homogenate sample using silica coated paper for paper spray: (a) full MS spectrum of 1000 ng g^{-1} of ractopamine in pork sample, (b) linear dynamic range of ractopamine [$(M+H)^+$, m/z 302, product ion, m/z 164], (c) MS/MS spectrum of ractopamine in pork at 1000 ng g^{-1} and (d) 1 ng g^{-1}. Sample amount: 5.0 µL; Solvent: 9:1 methanol/water; solvent volume: 50 µL; voltage: 3.5 kV.

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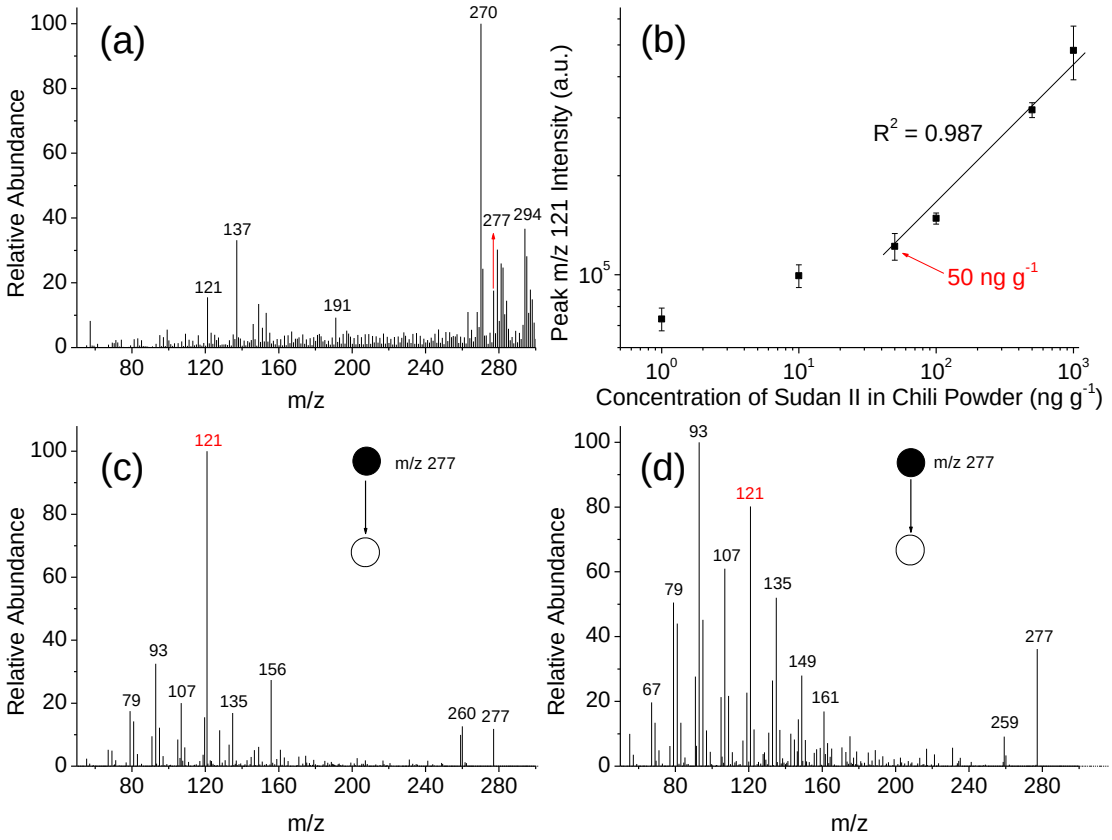

Fig. S3 Determination of Sudan red II in chili powder homogenate using silica coated paper for paper spray: (a) full MS of chili powder with 1000 ng g^{-1} of Sudan red II, (b) linear dynamic range of Sudan red II [$(M+H)^+$, m/z 277, product ion, m/z 121], (c) MS/MS spectrum of Sudan red II (m/z 277) in chili powder at 1000 ng g^{-1} and (d) 50 ng g^{-1}. Sample amount: 5.0 µL; solvent: 9:1 dichloromethane/isopropanol containing 0.5% water; solvent volume: 25 µL; voltage: 3.5 kV.

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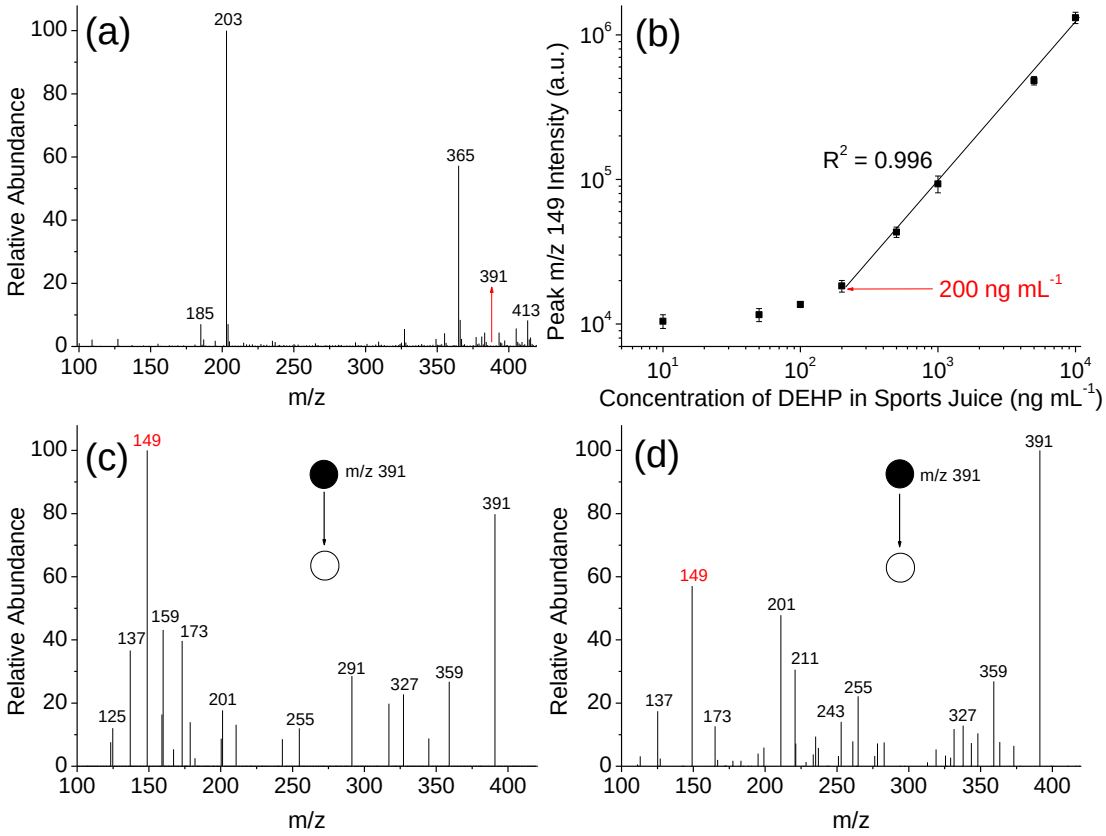

Fig. S4 Determination of DEHP in sports drink using silica coated paper for paper spray: (a) full MS spectrum of 1000 ng mL^{-1} of DEHP in sports drink, (b) linear dynamic range of DEHP [$(M+H)^+$, m/z 391, product ion, m/z 149], (c) MS/MS spectrum of DEHP, m/z 391, in sports juice at 1000 ng mL^{-1} and (d) 20 ng mL^{-1}. Sample amount: 5.0 µL; Solvent: 9:1 methanol/water containing 0.2% formic acid; solvent volume: 50 µL; voltage: 3.5 kV

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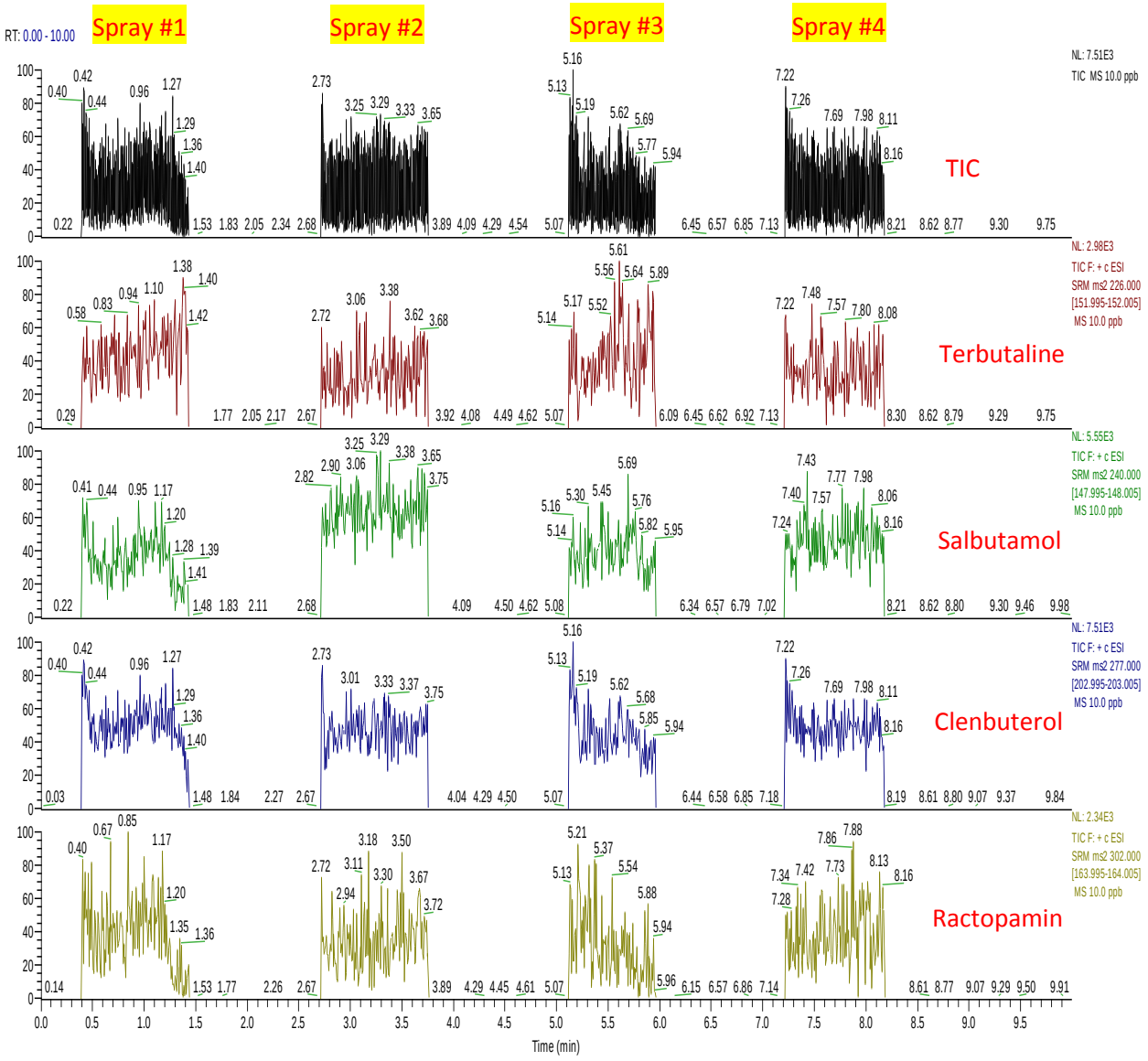

Fig. S5 Simultaneous determination of clenbuterol-like compounds. Ion chronograms for total ion curent (TIC) and terbutaline, salbutamol, clenbuterol, and ractopamin, each at 10 ng g^{-1} concentration in pork homogenate sample on silica coated paper for paper spray, selected reaction monitoring (SRM) mode of a Thermo TSQ. Sample amount: 5.0 µL; solvent: 9:1 methanol/water; solvent volume: 50 µL; voltage: 3.5 kV.

Note: Paper spray not only benefits from minimum sample preparation, but also from high throughput analysis. Fig. S5 shows the simultaneous determination of four samples spiked with 10 ng g^{-1} terbutaline, salbutamol, clenbuterol, ractopamin in a pork sample in less than 8.5 min. The time was mainly spent on the changing of

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sample and adding solvent. For each sample run the time was less than 1.0 min after applying a potential to the paper substrate.

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