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Evaluation of a generic multi-analyte method for detection of >100 representative compounds correlated to emergency events in 19 food types by ultrahigh-pressure liquid chromatography–tandem mass spectrometry

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ABSTRACT

A generic extraction procedure combined with triple quadrupole mass spectrometric detection was evaluated for multi-residue analysis in 19 different foods. Measurable peaks could be obtained at relevant concentrations for 108 out of a total of 127 targeted compounds representing a wide range of physico-chemical properties and compound classes related to emergency situations. Recoveries were determined for all 19 foods spiked with the 108 compounds. Seventy-five percent of the compounds had extraction recoveries of 70% or higher, with no compound below 46%. Suppression or enhancement effects on the MS response of the compounds dissolved in the extracts were low, as more than 80% of them had matrix effects between −35% and +20% and no compound was below −44% compared to matrix-free standard. In a validation, all compounds could be quantified at 200 μg/kg and 400 μg/kg food sample and 81% of the compounds at 40 μg/kg. It is concluded that the method is useful for the detection of various types of organic chemical toxicants at levels generally well below concentration thresholds for severe acute intoxication.

1. Introduction

The awareness of threats of various types in the food supply is increasing worldwide. This includes not only antagonistic threats but also the increased risk of contamination of raw materials and food items by various chemicals and biotoxins due to catastrophes, accidents and exposure to toxins such as mycotoxins due to climatic changes.

Numerous international CBRN (Chemical Biological RadioNuclear) cases have been documented throughout the years. In a recent report based on different databases and supported by the U.S. Department of Homeland Security, 431 CBRN incidents worldwide from 1950 to 2006 were listed [1]. The number of biological incidents was 91, of which 14 were anthrax attacks. The anthrax-letters in the early 2000s, mainly targeting media and government personnel, became well known to the public [2,3]. Not only did they cause several human fatalities and injuries but also wide public anxiety, which was increased by numerous cases where persons received envelopes containing white powder. The precautions taken against these "copycat crimes" caused significant economic burden in USA [4].

The most common incident was related to CTA (Chemical Threat Agents): 233 of the 431 CBRN cases reported in [1] involved elemental, inorganic, gaseous and organic compounds. A particularly extensive attack took place in 1978 when a group called "The Arab Revolutionary Group" injected mercury into oranges which were distributed to six western European countries [5]. In the years 1990–1995 the Japanese apocalyptic sect Aum Shinrikyo performed at least ten attacks using both chemical and biological agents [6]. The sarin attack targeting the subway system in Tokyo in 1995 was the most devastating one as 12 people died and over 1000 people were injured. Earlier that year the group attempted a similar attack using botulinus toxin.

Several types of low molecular (molecular weight <1200) organic compounds have been used as CTAs in small-scale incidents, such as insecticides, rat poisons, sedatives, toxins and narcotic compounds, involving a wide range of foodstuff and water resources [1,7]. One recent case concerns two Taiwanese siblings that were poisoned by the highly toxic rat poison TETS [8]. It has been banned from the market since 1984 but due to continuing demand and its ease of production it is still readily, although illegally, available in China and severe cases of intentional poisoning have occurred [9,10]. The marine biotoxin saxitoxin produced by dinoflagellates is associated with paralytic shellfish poisoning and numerous outbreaks have been reported [11,12]. Although they were considered to be unintentional, saxitoxin has been regarded

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as a high risk CTA and is, together with the plant protein ricin, the only naturally occurring toxin covered by the Chemical Weapons Convention [13]. Another class of natural toxins considered to be potential antagonistic agents are the mycotoxins [14]. The *Fusarium* trichothecene mycotoxins have a history as harmful toxins [15,16]. For example, in 1975 aircraft of the communist governments in Laos and Cambodia dropped a yellow oily liquid over Hmong tribes, an incident that is referred to as 'yellow rain'. The liquid was supposedly T-2 toxin, although it has been under debate [17].

Modern analytical techniques such as GC and LC coupled to tandem mass spectrometry (MS/MS) allow hundreds of compounds to be analyzed in just a single analysis. In the field of food analysis, there are numerous published multi-residue methods for screening of pesticides in fruits and vegetables and of veterinary drugs in animal products that often are used in routine analysis in control programs [18–20]. These methods are aimed at detecting and reliably quantifying legally regulated compounds at concentration levels which are often in the low μg/kg range. In order to meet such high requirements, sample preparation procedures often have to be optimized for the analytes in question. Extraction methods involving multiple clean-up steps usually produce 'clean' extracts but are time-consuming and might result in low recoveries. Single step solvent extractions, sometimes referred to as 'dilute and shoot', are fast and usually give high recoveries but at the cost of 'dirty' extracts and increased risk of significant matrix effects.

In an emergency situation, fast screening capability is essential in the sense that quick identification is important in order to take necessary action to avoid the additional spread of contaminated food, withdraw food from the market or warn the general population when needed. The more time-consuming forensic investigation using chemical analysis in order to retrieve attribution information (profiles) will then proceed with this first detection as a starting point. A recent study presented a method where 172 compounds, many of which are included in food control programs, were analyzed in one single multi-method using acetonitrile/water/formic acid extraction followed by tandem mass spectrometry detection [21]. In the present work, a similar approach but with some important modifications was applied: Firstly, the number of matrices was expanded. Secondly, compounds related to emergency purposes were focused on rather than compounds included in control programs. Thirdly, a deeper understanding of compound behavior was sought in the method, i.e. distinguishing between matrix effects and extraction recovery. The overall aim was to develop a single LC–MS/MS screening method applicable to a majority of food types and organic chemical toxicants. Over 100 representative compounds from groups such as drugs, laxatives, mycotoxins, narcotic compounds, pesticides, plant/mushroom/marine toxins and toxic industry chemicals, including compounds relevant from an antagonistic/forensic and catastrophic point of view, were selected. Possibilities and limitations of the method were explored by selecting compounds with as much physicochemical variation as possible, (polarity, molecular weight and the presence of different functional groups). In order to extract all compounds, the same single-step acetonitrile/water/formic acid extraction as used in [21] was used. The method was evaluated by extraction and LC–MS/MS analysis of 19 different foods and beverages spiked with standard mixtures.

2. Materials and methods

2.1. Reagents, standards and food samples

The matrices used in the study consisted of 19 beverage and food products as listed in Table 1.

The standards were either purchased from LGC standards (Borås, Sweden) or donated by the Swedish Defense Research Agency (FOI), Sahlgrenska University Hospital and the Swedish National Laboratory of Forensic Science (SKL). All standards were dissolved in MeOH or acetonitrile and mixed to a single standard solution which was used for all experiments.

Acetonitrile was of HPLC grade (Rathburn), MeOH of gradient grade (LiChrosolv) and formic acid of pro analysi grade (VWR international).

2.2. UHPLC–MS/MS

All LC–MS/MS experiments were performed on an Agilent 1290 Infinity LC coupled to an Agilent 6460 triple quadrupole instrument. The drying gas temperature was set to 300 °C and the sheath gas temperature to 250 °C. The capillary voltage was set to 3.5 kV and the nozzle voltage to 0.5 kV. An Agilent Eclipse Plus C18 RRHD (2.1 mm × 50 mm, 1.8 μm) UHPLC column was used for all experiments.

The MS-parameters (precursor/products ions, fragmentor voltage and collision energy) were individually optimized for all compounds and the multiple reaction monitoring (MRM) transitions are presented in Table 2.

The compounds were analyzed in two separate methods. In positive ionization mode, eluent A consisted of 4 mM ammonium formate (aq, pH 4.1) and eluent B of MeOH with formic acid (0.1%) and in negative ionization mode, eluent A consisted of water and eluent B of MeOH. The compounds were eluted using a linear gradient: 0–5 min, 10–30%B (v/v); 5–15 min, 30–95%B; 15–17 min, 95%B; 17–22 min, 10%B. The column temperature was set to 55 °C and the injection volume was 5 μl. The eluents were filtered through 0.45 μm filters and ultrasonicated before use.

2.3. Extraction

Homogenized food sample (0.125 ± 0.05 g) was placed in a 15 ml test tube and 0.25 ml of water was added for hydration of dry sample. After 15 min, 0.75 ml of acetonitrile/formic acid (1%) was added, rigorously mixed, left on a shaking table for 1 h at room temperature and finally centrifuged (10 min, 3200 × *g*). All extracts were analyzed as they were, except milk, dry milk and sour milk, which were further filtered using centrifuge tube filters (10 min, 16,000 × *g*).

In a scaled-up experiment, 8 of the foods were selected (olive oil, pan-pizza, milk, orange, baby food 1, ketchup, Coca-cola and salami) and subjected to the extraction described above but in 50 ml test tubes with 1 ± 0.05 g matrix, 2 ml water and 6 ml acetonitrile/formic acid (1%).

The recoveries were calculated by compensating for the water content in the food types (Table 1).

2.4. Determination of extraction recoveries and matrix effects

Triplicates of all food types listed in Table 1 were spiked with 70 μl standard solution in MeOH to give a concentration of 200 μg/kg in the food sample. For the blank extracts, 70 μl of MeOH was added. The samples were left for 15 min before the hydration and extraction described above. In the scaled-up experiments, food samples were spiked to 400 μg/kg. The latter experiment also included additional compounds (amanita toxins, domoic and okadaic acid, abamectin, 15-Ac-deoxynivalenol, acepromazine, cocaine, fusarenon X, MDMA, methylphenidate, nonylphenol, noscapine and papaverine).

After the extraction, the blank extracts were spiked with standard and the extraction recoveries calculated by comparing peak areas for the samples spiked before and after extraction, respectively. The matrix effects were determined by comparing peak areas

Table 1
Description and nutrient composition[a] of food types.

Matrix	Origin and comments	Fat (%)	Carbohydrates (%)	Protein (%)	Water (%)
Almond	Bought in Croatia	47	7.0	20	4.5
Banana	Fresh fruit	0.5	22	1.0	74
Bread	Rye bread, Aktiv Flerkorn Råg (flour from wheat and rye, rapeseed oil, flax seed, syrup) (Fazer)	6.5	40	9	37
Coca-cola	Original Coca-cola from a 50-cl plastic bottle.	0.0	11	0.0	89
Coffee	Instant coffee, prepared according to instructions (ICA)	0.0	0.4	0.1	99
Coffee whitener	Whitener for coffee and tea (glucose syrup, vegetable fat, milk protein, emulsifiers) (Nestle)	35	57	1.3	0.0
Baby food 1	Cod with mashed potato, jarred, ready-to-eat (Nestle)	2.7	6.2	3.0	87
Baby food 2	Pureed apple and pear, jarred, ready-to-eat (Semper)	0.1	15	0.2	22
Ketchup	Tomato ketchup (Felix)	0.5	24	1.5	70
Meat	Minced bovine muscle	4.6	0.0	27	66
Milk	3% fat (Arla Foods)	3.0	4.8	3.4	88
Olive oil	Extra virgin (Coop Forum)	100	0.0	0.0	0.0
Orange	Juice obtained by squeezing one orange	0.1	10	0.9	87
Pizza	Original Billy's pan pizza (pork, tomato purée, cheese, rapeseed oil, fat from pork). Prepared in microwave oven according to instructions (Gunnar Dafgård AB)	11	29	12	43
Red wine	Infinitus Tempranillo	0.0	1.1	0.0	89
Salami sausage	Grilled pepper salami (pork, beef) (Grilstad)	37	1.5	19	48
Sandwich caviar	Trad. Swedish "Lättrökt kaviar" (roe from cod, rapeseed oil, potato flakes, tomato purée) (ICA)	16	23	9.0	45
Sausage	Trad. Swedish "Falukorv" (pork, beef, potato flour, fat from pork) (Scan)	23	3.7	9.9	61
Soured milk	Fermented milk, 1.5% fat (Arla Foods)	1.5	5.0	3.5	89

[a] The nutrient composition was taken from the product description if available, otherwise from the Swedish food database which is available free of charge at http://www7.slv.se/Naringssok/SokLivsmedel.aspx.

from the spiked blank extracts with peak areas from spiked acetonitrile/water/formic acid (75:25:1). All samples were in triplicate.

2.5. Limit of detection

A solution of acetonitrile/water/formic acid (75:25:1) was spiked with standard to 200 ng/ml, which would correspond to 1600 μg/kg food sample, from which a nine-point 1:4 dilution series was constructed (1600, 400, 100, 25, 6.25, 1.56, 0.39, 0.098 and 0.025 ng/ml). The limit of detection (LOD) was defined as the concentration where the signal to noise ratio for the most intense MRM-transition exceeded 4.0.

2.6. Validation

The extraction was performed as described above using 0.25 ± 0.05 g baby food 1 and 2 ml acetonitrile/water/formic acid (75:25:1) in 15 ml test tubes. Prior to the extraction procedure, the food was spiked at 0, 10, 27, 89, 267 and 800 μg/kg (calibration points) and control samples at 40, 200 and 400 μg/kg in triplicate. The validation accuracy was calculated as the ratio between $\text{concentration}_{\text{found}}$ and $\text{concentration}_{\text{spiked}}$, expressed as a percentage. $\text{Concentration}_{\text{found}}$ was obtained using a matrix/recovery-included calibration curve.

2.7. Screening and semiquantification of naturally occurring toxins

Blank extracts of all food samples were first analyzed using UHPLC–MS/MS. After identification of toxins present in the extracts (amygdaline, chaconine, dehydrotomatine, solanine and tomatine), a 7-point 1:3 dilution series of these compounds in acetonitrile/water/formic acid (75:25:1) were constructed (100, 33, 11, 3.7, 1.2, 0.41 and 0.014 ng/ml). The concentrations were calculated by compensating for the recoveries and matrix effects.

3. Results and discussion

In terms of organic non-radioactive compounds, some of the most acute-toxic CTAs known have been included in the present study, such as alfa- and beta-amanitin, the microcystins, the second-generation rodenticides and saxitoxin I, the latter with an estimated oral lethal dose in humans as low as 10 μg/kg body weight [22] (Table 2). For an adult of 70 kg consuming up to 1 kg of a contaminated food this would correspond to a concentration in food of 700 μg/kg. Health effects are of course anticipated to be observed at levels below the lethal dose, and a food sample taken for analysis might be less contaminated than the food item that was causing the poisoning. On the other hand, most known CTAs do not exhibit an oral toxicity as low as that discussed above, but rather 100–10,000 times higher (LD50 values are given in Table 2). Despite the toxicity issue there might be a need to verify false threats at a lower level. Based on this, working levels of 200 and 400 μg/kg were chosen and the method was validated down to 40 μg/kg food sample.

3.1. LC–MS/MS method

The compounds were analyzed in two separate methods employing positive (97 compounds) and negative (11 compounds) electrospray ionization (ESI). Out of the 127 compounds originally included, 19 compounds did not give any results at relevant concentrations (Table 2). Ten of these did not ionize in the ESI-interface, including the halogenated pesticides toxaphen, irgasan and alachlor, which are normally analyzed by gas chromatography [20]. Although it was expected that these compounds would not ionize in the MS-interface, they were included in the study to make the range of target compounds as broad as possible.

The high molecular weight toxins digitonin and microcystin RR did not produce product ions in MS/MS-mode (this compound produces a double charged precursor ion [23] which was not targeted). The polar toxins deoxynivalenol-3-O-glucoside, bufotenine, nicotine, nivalenol, monoliformin and orellanine did not retain on the

Table 2
Compound description, MS/MS parameters, results from extraction experiments and limit of detection in acetonitrile/water/formic acid (75:25:1).

Compound (n = 127)	Description	Oral LD50 (mg/kg body-weight)	ESI	Precursor ion (m/z)	Product ions (m/z)	Extraction recovery (%)[a] n = 108)	Matrix effect (%)[a] n = 108)	LOD[b] (μg/kg)	Validation accuracy (%)[c]		
									40 g/kg	200 μg/kg	400 μg/kg
15-Ac-deoxynivalenol	Mycotoxin	No reference	–	337.0	219/150	*70*	*−43*	13	124	115	113
3-Ac-deoxynivalenol	Mycotoxin	34 (mouse) [33]	–	337.0	307.1/173.1	76	−16	13	108	89	86
Abamectin	Pesticide	10 (rat) [34]	+	895.4	751.3/449.2	*63*	*−24*	24	n/a[i]	109	125
Acepromazine	Drug	10 (rat) [35]	+	327.0	222/178.1	*73*	*−5*	1.6	93	110	127
Afla toxin B1	Mycotoxin	4.8 (rat) [36]	+	313.0	285.1/241.1	77	+3	1.2	105	109	121
Afla toxin B2	Mycotoxin	4.8 (rat) [36]	+	315.0	259.1/243.1	76	+2	1.2	125	117	116
Afla toxin G1	Mycotoxin	No reference	+	329.0	283.1/243.1	78	+6	0.3	110	119	116
Afla toxin G2	Mycotoxin	No reference	+	331.0	217.1/189.1	79	−3	1.2	110	117	118
Alachlor[d]	Pesticide	930 (rat) [37]									
Alfa-amanitin	*Amanita* toxin	0.3 (human) [38]	+	941.3	923.3/746.2	*101*	*−12*	29	88	95	93
Altenuene	Mycotoxin	No reference	+	293.0	257.1/115.1	81	+15	104	n/a[j]	98	96
Alternariol	Mycotoxin	No reference	–	257.0	215.1/212.1	65	−28	1.6	128	104	98
Altertoxin I	Mycotoxin	No reference	–	351.0	315.1/263.1	70	−22	1.6	105	97	87
Amphetamine	Narcotic compound	30 (rat) [39]	+	136.0	119.1/91.1	85	−3	1.6	101	98	96
Amygdaline	Almond toxin	No reference	+	480.2	374.1/347.1	99	−1	104	n/a[j]	96	92
Atenolol	Drug	2 (rat) [40]	+	267.0	145.1/74.1	99	−20	0.4	96	97	92
Atrazin	Drug/pesticide	672 (rat) [41]	+	216.0	104.1/68.1	71	−3	1.6	92	112	129
Beauvericin	Mycotoxin	No reference	+	801.6	244.1/134.1	58	+13	6.4	130	115	106
Benzyl butyl phthalate[e]	Industry chemical	2330 (rat) [42]	+	313.3	149.2/91.1						
Beta-amanitin	*Amanita* toxin	0.5 (human) [38]	+	920.3	902.3/86.1	*107*	*−6*	29	88	115	115
Bis-(4-amino-phenyl)-methane	Industry chemical	517 (rat) [43]	+	199.0	106.1/89.1	81	+46	6.4	94	93	95
Bisacodyl	Laxative	4320 (rat) [44]	+	362.0	184.1/167.1	67	−2	0.8	96	118	117
Bromociclen[d]	Flame retardant	12,500 (rat) [43]									
Bromodiolon	Rodenticide	0.49 (rat) [43]	–	525.0	263.1/250.1	52	−13	24	116	88	95
Bufotenin[f]	*Amanita* toxin	No reference	+	205.2	160/115						
Cabergoline	Drug	400 (mouse) [45]	+	452.0	381.5/167.1	79	+10	1.6	122	112	109
Chaconine	Solanaceae toxin	No reference	+	852.7	706.7/398.6	106	+1	0.4	n/a[k]	n/a[k]	n/a[k]
Chlorhexidine	Antiseptic agent	2515 (mouse) [43]	+	505.4	170.1/125.1	75	−2	1.6	95	119	109
Chlormequat chloride[d]	Pesticide	600 (rat) [43]									
Citrinin	Mycotoxin	112 (mouse) [43]	+	251.0	233.1/115.1	74	−6	104	n/a[j]	95	107
Cocaine	Narcotic compound	99 (mouse) [46]	+	304.0	182.1/82.1	*79*	*0*	0.06	105	112	116
Codeine	Narcotic compound	427 (rat) [47]	+	300.0	152.1/115.1	91	−1	1.6	93	108	103
Coumatetralyl	Rodenticide	30 (rat) [43]	–	291.0	247.1/142.1	63	−15	1.6	100	107	96
Cyclopiazonic acid	Mycotoxin	36 (rat) [48]	+	337.0	196.1/182.1	66	−7	49	n/a[j]	95	108
Cyhalothrin	Pesticide	144 (rat) [43]	+	467.0	225.1/208.1	56	+184	52	n/a[j]	97	118
Dehydrotomatine	Tomato toxin	No reference	+	1032.8	126.1/85.1	*116*	*+28*	10	n/a[j]	136	111
Δ^9-THC	Narcotic compound	666 (Rat) [49]	+	315.3	193.1/123.1	46	+391	104	n/a[j]	110	148
Deoxynivalenol	Mycotoxin	46 (Mouse) [33]	–	295.0	265/247.1	70	−44	28	108	89	86
Deoxynivalenol-3-O-glucoside[g]	Mycotoxin	No reference	+	457.2	427/246.8						
Dextropropoxifen	Drug	135 (rat) [50]	+	340.0	266.4/128.1	75	−3	6.4	82	105	118
Diacetoxyscirpenol	Mycotoxin	7 (rat) [51]	+	384.0	128.1/105.1	79	+4	24	113	117	127
Diclofenac	Drug	62.5 (rat) [52]	+	297.0	215.1/151.1	69	−1	104	n/a[j]	119	91
Difenacoum	Rodenticide	0.68 (rat) [43]	+	443.0	293.1/135.1	55	−12	6.4	121	94	95
Digitonin[h]	Digitalis toxin	50 (rat) [53]	+	1260.2							
Dimethenamid	Pesticide	No reference	+	276.0	244.1/168.1	76	−1	0.4	104	107	123
Diuron	Pesticide	1017 (rat) [54]	+	233.0	160/72.1	75	0	1.7	81	112	116
Domoic acid	Marine toxin	No reference	+	312.0	91/77	*71*	*−16*	28	n/a[j]	90	87
Doxorubicin	Drug	570 (mouse) [55]	+	544.3	397.1/130.1	90	+10	6.4	125	101	102
Enniatin A	Mycotoxin	No reference	+	699.6	228.1/210.1	61	+49	1.7	108	108	139
Enniatin A1	Mycotoxin	No reference	+	685.6	210.1/196.1	60	+43	0.8	104	113	139
Enniatin B	Mycotoxin	No reference	+	657.6	214.1/196.1	62	+24	0.8	94	107	116

Enniatin B1	Mycotoxin	No reference	+	671.6	210.1/196.1	63	+32	0.8	102	102	130
Ephedrine	Narcotic compound	689 (mouse) [43]	+	166.0	148.1/117.1	84	+2	6.4	86	102	101
Ergocornine	Mycotoxin	No reference	+	562.3	223.1/208.1	77	−22	0.4	91	108	118
Ergocristine	Mycotoxin	No reference	+	610.5	223.1/208.1	71	−16	1.6	120	113	145
Ergocryptine	Mycotoxin	No reference	+	576.3	223.1/208.1	86	−15	0.4	101	103	122
Ergometrine	Mycotoxin	No reference	+	326.0	223.1/208.1	87	+104	0.4	86	105	102
Ergosine	Mycotoxin	No reference	+	548.3	268.1/208.1	76	−13	0.4	95	112	114
Ergotamine	Mycotoxin	No reference	+	582.2	223.1/208.1	73	−9	0.4	113	115	127
Ethacridine lactate	Antiseptic agent	No reference	+	254.0	226.1/197.1	77	−8	104	n/a[j]	123	126
Ethylene brassylate[d]	Industry chemical	No reference									
Ethylmorphine	Narcotic compound	810 (rat) [56]	+	314.0	152.1/115.1	85	−3	0.4	112	112	107
Felodipine	Drug	1050 (rat) [43]	+	385.0	353.1/339.1	67	0	24	147	132	126
Fenoterol	Drug	No reference	+	304.3	135.1/107.1	87	−1	1.6	95	119	111
Flocoumafen	Rodenticide	0.25 (rat) [43]	–	541.3	382.1/161.1	51	−16	6.4	128	93	85
Flumetasone	Drug	No reference	+	411.0	253.1/121.1	78	+1	24	114	96	109
Flunitrazepam	Drug	415 (rat) [43]	+	314.0	268.1/239.1	73	+2	1.6	109	100	116
Fusarenon X	Mycotoxin	4 (rat) [33]	–	353.0	187/59	*79*	*+183*	49	n/a[j]	n/a[g]	n/a[g]
γ-Butyrolactone[f]	Narcotic precursor	1540 (rat) [43]	+	87.0	43						
HT-2-toxin	Mycotoxin	3.8 (mouse) [33]	+	447.0	345.1/285.1	76	0	104	n/a[j]	124	115
Hydroxyzine	Drug	840 (rat) [43]	+	375.0	201.1/165.1	75	−3	0.4	102	116	134
Hyoscyamine	Solanaceae toxin	95 (rat) [57]	+	290.0	124.1/93.1	86	−2	0.4	102	104	98
Ibuprofen[h]	Drug	636 (rat) [58]									
Irgasan/Trichlosan[d]	Pesticide	3700 (rat) [43]									
Levomepromazin	Drug	1150[43]	+	329.0	100.1/58.1	68	+6	0.4	106	108	117
Lornoxicam	Drug	5.7 (rat) [59]	+	372	120.9/95	66	+86	104	n/a[j]	97	121
MDMA (ecstasy)	Narcotic compound	No reference	+	194.0	163.1/105.1	*83*	*−2*	0.06	102	107	109
Methadone	Narcotic compound	86 (rat) [60]	+	310.0	265/105	74	+4	1.6	96	118	120
Methyl alternariol	Mycotoxin	No reference	+	271.0	256.1/228.1	65	−10	1.2	119	111	107
Methylphenidate	Drug	367 (rat) [43]	+	234.0	115.1/84.1	*82*	*+1*	0.06	101	107	101
Metoprolol	Drug	3470 (rat) [61]	+	268.0	133.1/116.1	85	+4	1.6	105	105	96
Microcystin LR	Marine toxin	0.5 (rat) [62]	+	995.7	135.1/70.1	76	+7	28	64	102	85
Microcystin YR	Marine toxin	No reference	+	1045.8	135/107	77	+12	28	n/a[j]	113	85
Microcystin RR[h]	Marine toxin	No reference	+	1038.8							
Monoliformin[f]	Mycotoxin	41 (rat) [63]	–	97.1	41.0						
Moxidectin	Pesticide	No reference	+	640.6	528.6/498.6	91	+741	24	117	118	143
Na picosulfate	Laxative	11,300 (rat) [43]	+	438.1	184.1/167.1	81	+2	90.5	n/a[j]	119	109
Narasin	Veterinary drug	18.5 (rat) [64]	+	787.3	531.4/431.4	63	+476	24	116	111	141
Neosolaniol	Mycotoxin	No reference	+	400.3	185.1/105.1	93	+9	24	80	105	94
Nicotine[f]	Tobacco toxin	50 (rat) [43]	+	163.3	130/117						
Nitrazepam	Drug	825 (rat) [65]	+	282.0	236.1/180.1	72	+60	6.4	105	97	117
Nonylphenol	Industry chemical	580 (rat) [43]	–	219.0	133/116.7	*50*	*−30*	6.4	93	90	99
Noscapine	Narcotic compound	1520 (rat) [43]	+	414.2	220/205	*80*	*+11*	0.4	92	112	116
Ochratoxin A	Mycotoxin	20 (rat) [66]	+	404.3	358.1/239.1	69	+5	64	n/a[i]	108	138
Okadaic acid	Marine toxin	No reference	+	827.5	809.1/723.1	*68*	*+50*	6.5	92	96	106
Olanzapine	Drug	No reference	+	313.0	256.1/198.1	107	+5919	44	n/a[j]	107	99
Orellanine[f]	Mushroom toxin	33 (mouse) [67]	+	253.1	219/191						
Oxfendazole	Pesticide	No reference	+	316.3	159.1/131.1	78	+3	0.4	103	113	119
Papaverine	Drug	325 (rat) [43]	+	340.0	324/202	*79*	*+4*	0.4	129	122	113
Paracetamol	Drug	1944 (rat) [43]	+	152.0	110.1/93.1	75	−1	4	108	109	109
Penicillic acid	Mycotoxin	600 (mouse) [43]	+	171.0	125.1/97.1	83	−2	104	n/a[j]	117	112
Penitrem A	Mycotoxin	10 (rat) [43]	+	634.5	616.5/558.5	66	+159	160	n/a[j]	100	141
Phallacidin	*Amanita* toxin	1.5 (Human) [38]	+	847.3	811.2/156.9	*121*	*+13*	19	108	104	94
Phalloidin	*Amanita* toxin	2 (Human) [38]	+	811.3	783.2/767.2	*109*	*+52*	26	91	89	87
Phenolaftalein	Drug	No reference	+	319.3	225.1/141.1	72	+8	24	52	120	145

Table 2 (*Continued*)

Compound (n = 127)	Description	Oral LD50 (mg/kg body-weight)	ESI	Precursor ion (m/z)	Product ions (m/z)	Extraction recovery (%)[a] n = 108)	Matrix effect (%)[a] n = 108)	LOD[b] (μg/kg)	Validation accuracy (%)[c]		
									40 g/kg	200 μg/kg	400 μg/kg
Propiomazine	Drug	No reference	+	341.0	86.1/71.1	73	+1	0.4	88	119	141
Prothiofos[d]	Pesticide	875 (rat) [43]									
Ropinirole	Drug	396 (rat) [43]	+	261.3	132.1/114.1	83	+1	1.6	90	105	105
Roquefortine C	Mycotoxin	No reference	+	390.0	193.1/108.1	75	−2	1.7	82	100	119
Saxitoxin I	Marine toxin	0.01 (human) [22]	+	300.3	60/55	93	+4	8	100	104	98
Solanine	Solanaceae toxin	560 (rat) [68]	+	868.7	722.6/398.5	106	−9	0.4	n/a[k]	n/a[k]	n/a[k]
Solasonine	Solanaceae toxin	No reference	+	884.7	866.7/253.4	104	+6	0.4	95	88	83
Sterigmatocystin	Mycotoxin	120 (rat) [69]	+	325.3	310.1/281.1	68	−2	24	136	119	132
Strychnine	*Strychnos* toxin	2.3 (rat) [70]	+	335.0	184.1/156.1	84	−7	0.4	81	109	112
Sulphadoxine	Drug	5200 (rat) [63]	+	311.3	156.1/108.1	80	+6	1.6	107	123	126
T-2-toxin	Mycotoxin	1 (Human) [71]	+	484.5	215.1/105.1	73	−3	6.4	106	107	118
Tetrabrombisphenol A[d]	Flame retardant	No reference									
Tomatine	Tomato toxin	500 (mouse) [72]	+	1034.8	1016.8/85.1	119	+52	6.4	126	94	91
Toxaphene[d]	Pesticide	50 (rat) [73]									
Tramadol	Drug	228 (rat) [65]	+	264.0	77.1/58.1	83	0	0.4	106	109	106
Trans-chlordane[d]	Pesticide	275 (rat) [74]									
Triazolam	Drug	7500 (rat) [43]	+	343.3	308.1/239.1	76	+3	1.6	95	111	118
Tulobuterol	Drug	No reference	+	228.3	154.1/118.7	80	−1	0.06	133	127	120
Verapamil	Drug	163 (rat) [75]	+	455.5	165.1/150.1	74	+4	0.4	110	103	121
Warfarin	Rodenticide	1.6 (rat) [76]	+	309.0	251.1/163.1	74	−4	6.4	91	115	136
Zearalenone	Mycotoxin	16 (rat) [43]	−	317.2	175.2/131.1	65	−22	3	108	93	94
Zolpidem	Drug	No reference	+	308.3	263.1/235.1	79	+5	0.4	98	112	123

[a] Values for compounds only evaluated in the up-scaled experiment are in *italics.*

[b] A solution of acetonitrile/water/formic acid (75:25:1) was spiked with standard to 200 ng/ml, which would correspond to 1600 μg/kg food sample, from which a nine-point 1:4 dilution series was constructed. The limit of detection (LOD) was defined as the concentration where the signal to noise ratio for the most intense MRM-transition exceeded 4.0.

[c] The validation accuracy was calculated as the ratio between $\text{concentration}_{\text{found}}$ and $\text{concentration}_{\text{spiked}}$, expressed as a percentage. $\text{Concentration}_{\text{found}}$ was obtained using a matrix-matched calibration curve.

[d] No ESI-ionization.

[e] This compound was present in the MS-instrument (frequently used in plastic products).

[f] No HPLC retention.

[g] Low chromatographic performance.

[h] No MS/MS fragmentation.

[i] Too low s/n value.

[j] Concentration below LOD.

[k] Compound naturally occurring in matrix.

C18 HPLC-column. Benzyl butyl phthalate, a compound frequently used in plastic materials, was present in the HPLC system. Injection of MeOH produced a large signal for this compound and could therefore not be included in the method.

ESI-mode, fragmentor voltage (cone voltage) and MRM-transitions were first determined for each individual compound. This was straightforward for most of the compounds, but some of them needed more optimization. In negative ionization mode, the trichothecenes type B mycotoxins deoxynivalenol, 3-Ac-deoxynivalenol, 15-Ac-deoxynivalenol and fusarenon X readily formed formate adducts which have been used as precursor ions in earlier MS/MS experiments [24]. However, in the instrument used in this study, the formate adduct of these compounds was unsuitable as precursor ions as the formate ion (m/z 45) was the only product ion that was formed. Hence, it was crucial to elute these compounds with eluents devoid of formic acid. This was achieved by using water and MeOH as eluents and flushing the column with 50% water/MeOH for 1 h before analysis in negative ionization mode to ensure a formate-free system.

When the MS/MS-parameters had been established, the LC-method was optimized. Several multi-step gradients were tested in order to obtain acceptable chromatographic performance with as short an analysis time as possible. In order to increase the number of time-windows that can be used for the MRM-transitions, and thereby lowering the LOD, it is important to obtain as wide distribution as possible of the analyzed compounds. By using a 17-min gradient, the compounds were spread out evenly over the analytical run. In Fig. 1, the base-peak chromatogram of the 97 compounds analyzed in ESI+, together with selected MRM-transitions, is shown. Overall, the chromatography on the C18 column used in the method produced sharp peaks for the majority of the compounds (widths ~0.1 min at half peak height). There were some early-eluting compounds that gave broad and split peaks. This occurred when crude extracts with a high acetonitrile concentration were injected, but it could be overcome by diluting the extracts with water. For deoxynivalenol and ephedrine, the peaks were sharpened and the signal to noise ratios doubled upon a five times dilution of baby food 1 extract (100 μl extract + 400 μl water) (Fig. 2). For the majority of the compounds the LOD decreased after dilution and some compounds could not be detected at spiking level 400 μg/kg after a one time dilution (100 μl extract + 100 μl water), for example penitrem A (Fig. 2). Based on these observations and the fact that the peaks could be integrated despite the poor peak shape, the extracts were analyzed as they were. However, if one of these compounds should be detected during screening of samples from a real case scenario, it would be useful to know that the analysis could be repeated with dilution of the extract to improve the quality of the data.

For 67% of the compounds an LOD below 10 μg/kg was obtained and 88% were below 50 μg/kg. These values are comparable to LODs reported from triple quadrupole MS/MS analysis of for example mycotoxins [25] and pesticides [26].

3.2. Screening of non-spiked foods

Prior to method evaluation, blank extracts of all 19 matrices were screened for possible background from any of the analytes in the food. Amygdaline (almond toxin), chaconine, dehydrotomatine, solanine and tomatine were all present at the ~1–32,000 μg/kg level in some of the food samples (Table 3). The solanaceae (e.g. potato) glycoalkaloids chaconine and solanine were present in baby food 1 (cod with mashed potatoes) but also in sausage and sandwich caviar, probably due to the presence of potato flakes and potato pulp suspension (Table 1) [27]. Tomatine and dehydrotomatine were present in ketchup, pizza and sandwich caviar, originating from tomato puree included in these products. It should however be noted that the toxin concentrations in mixed matrices such as sausage and pizza are likely to be different among different samples and that a higher number of samples would be needed for reliable quantification.

3.3. Extraction recoveries and matrix effects

The extraction recoveries were calculated by comparing the LC–MS/MS peak areas in the extracts with those from spiked control extracts. The average recovery in the matrices was 76% (80 or 108 compounds, see Table 4), ranging from 51% (bread) to 88% (coffee) (Table 4), while the average recoveries of individual compounds (8 or 19 food types, see Table 4) varied from 46% (Δ^9-THC) to 121% (phallacidin) (Table 2). The matrix effects were calculated by comparing peak areas from spiked control extracts with spiked acetonitrile/water/formic acid (75:25:1). The average matrix effects were +2% and ranged from −16% (salami) to +40% (olive-oil). These values are the average of all the individual compounds excluding the ones with enhancement effects of over +100% (Table 4). The coccidiostat narasin lost the observed enhanced response when the injection volume was decreased from 5 to 1 μl (+476% to −24%) but for the other compounds with enhanced response these effects persisted. The possible mechanism(s) behind the enhancement effect lies beyond the scope of this article as enhancement effects do not limit the use of the new screening method. No further studies were conducted in this matter. All other compounds had low variations in matrix effects: 78% of the compounds had values of between −20% and +20%, 31% had enhancement effects (>0%) and only 5.3% had values below −20%. The mycotoxin deoxynivalenol was the compound with the highest suppression effect, at −44% (Table 2).

The potential of a simple solvent-extraction of over hundred compounds that is presented in this study has previously been demonstrated by Mol and co-workers where 172 pesticides, mycotoxins and plant toxins were extracted with acetonitrile/water/formic acid, which produced extracts with a low degree of matrix effects and high analyte recoveries [21]. Additional studies have demonstrated that solvent extraction using acetonitrile produces high recoveries for mycotoxins [28,29], veterinary drugs [30], pesticides [31] and drugs [32]. The present results show that this is valid for a wide range of different matrices, which indicates that this extraction technique combined with tandem mass spectrometry has the potential to be applicable for almost any given food sample. As shown in Table 4, the extraction recoveries in the small scale (0.125 g sample) and up-scaled (1 g sample) were similar for most of the food types. This demonstrates that the homogenization process is efficient enough for the use of such low sample amounts. The method is therefore reliable for semiquantitative purposes even though larger amounts of sample would be more representative and hence preferred in a real case scenario for a more accurate concentration determination.

3.4. Validation

After the extraction recoveries and matrix effects had been established the method was applied to an 'in-house' validation study. Baby food 1 was selected as the test food type as it is a mixed food type with several components representing several groups of food constituents. The experiment was designed to be representative of the previous experiments by using 0.25 g material and spiking the control samples at three levels (40, 200 and 400 μg/kg). For the calibration curve, 0.25 g of sample was spiked at six different concentrations. All samples were processed with the method and the concentrations of the control samples were calculated. The accuracy for each compound was determined by dividing the found concentration with the spiked concentration.

Including all 108 compounds at all three levels: 76% had an accuracy of between 80% and 120% (Table 2). For samples spiked

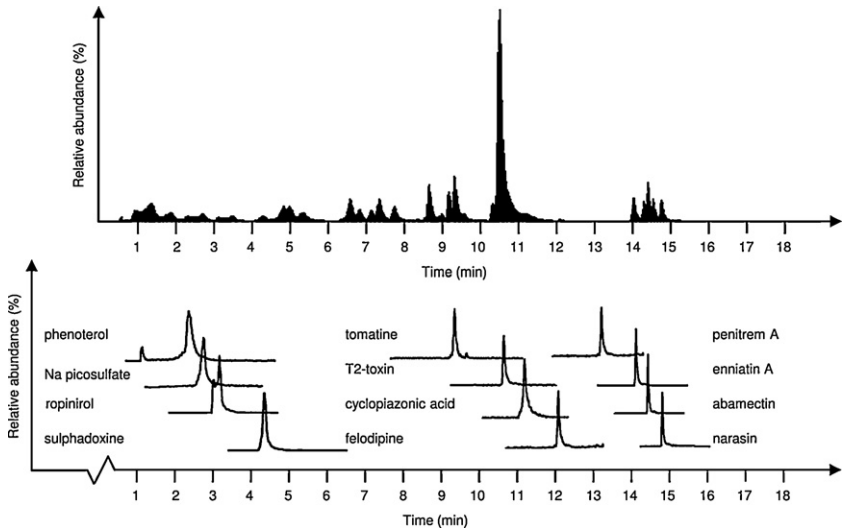

Fig. 1. Base peak chromatogram of the 97 compounds analyzed in positive mode together with 12 extracted MRM-traces. The compounds were analyzed in extraction blank at 50 ng/ml.

Table 3
Concentration of naturally occurring toxins in food types (μg/kg).

Matrix	Tomatine	Dehydrotomatine	Chaconine	Solanine	Amygdalin
Almond					106,00 ± 220
Baby food 1			8800 ± 170	3700 ± 270	
Ketchup	322,00 ± 1060	730 ± 10			
Pizza	200 ± 20		9100 ± 560	4400 ± 380	
Sandwich caviar	830 ± 80	300 ± 31			
Sausage			900 ± 20	320 ± 90	

at 40 μg/kg level, 21 compounds could not be validated due to low signal to noise ratio. At 200 and 400 μg/kg all compounds except fusarenon X (low signal to noise ratio due to bad chromatography) and the glycoalkaloids chaconine and solanine (food components) were included in the experiment. This demonstrates that the method is also useful for semiquantification purposes. In a real case scenario, this would be the next step after the initial identification of CTA in order to get a picture of the level of exposure and expansion.

3.5. Limitations and suggested further developments

One limitation of the method was the bad chromatographic performance for some of the most polar compounds. Although

Table 4
Mean extraction recoveries and matrix effects in different food types.

Food type	Mean extraction recovery and standard deviation (%)			Mean matrix effect and standard deviation[a]
	0.125 g	1 g	Average	
Almond	72 ± 10	–	72 ± 10	−3 ± 32
Baby food 1	71 ± 18	65 ± 31	68 ± 25	+2 ± 33
Baby food 2	79 ± 24	–	79 ± 24	+2 ± 28
Banana	83 ± 28	–	83 ± 28	−2 ± 31
Bread	51 ± 46	–	51 ± 46	−10 ± 34
Coca-cola	82 ± 19	70 ± 37	76 ± 28	+1 ± 30
Coffee	88 ± 26	–	88 ± 26	−14 ± 34
Dry milk	84 ± 18	–	84 ± 18	+16 + 41
Ketchup	57 ± 12	96 ± 21	77 ± 16	−7 ± 31
Meat	67 ± 8	–	67 ± 8	−3 ± 36
Milk (3%)	73 ± 22	101 ± 26	87 ± 24	−1 ± 34
Olive oil	83 ± 13	59 ± 15	71 ± 17	+40 ± 59
Orange	68 ± 9	71 ± 34	70 ± 21	+1 ± 37
Pizza	82 ± 19	91 ± 28	86 ± 23	−10 ± 37
Red wine	80 ± 12	–	80 ± 12	+9 ± 40
Salami	72 ± 13	90 ± 22	81 ± 17	−16 ± 40
Sandwich caviar	84 ± 18	–	84 ± 18	+8 ± 41
Sausage	69 ± 31	–	69 ± 31	+9 ± 37
Sour milk	69 ± 20	–	69 ± 20	+13 ± 38

[a] Signal strength compared to that of the compounds dissolved in acetonitrile/water/formic acid (75:25:1). The values were calculated excluding compounds with an average enhancement effect exceeding 2.0 (cyhalothrin, narasin, Δ^9-THC, moxidectin, fusarenon X and olanzapine). For almond, baby food 2, banana, bread, coffee, dry milk, meat, red wine, sandwich caviar, sausage and sour milk the mean matrix effects were calculated from 80 compounds (in *italic* in Table 2). For baby food 1, coca-cola, ketchup, milk, olive oil, orange, pizza and salami all 108 compounds were included.

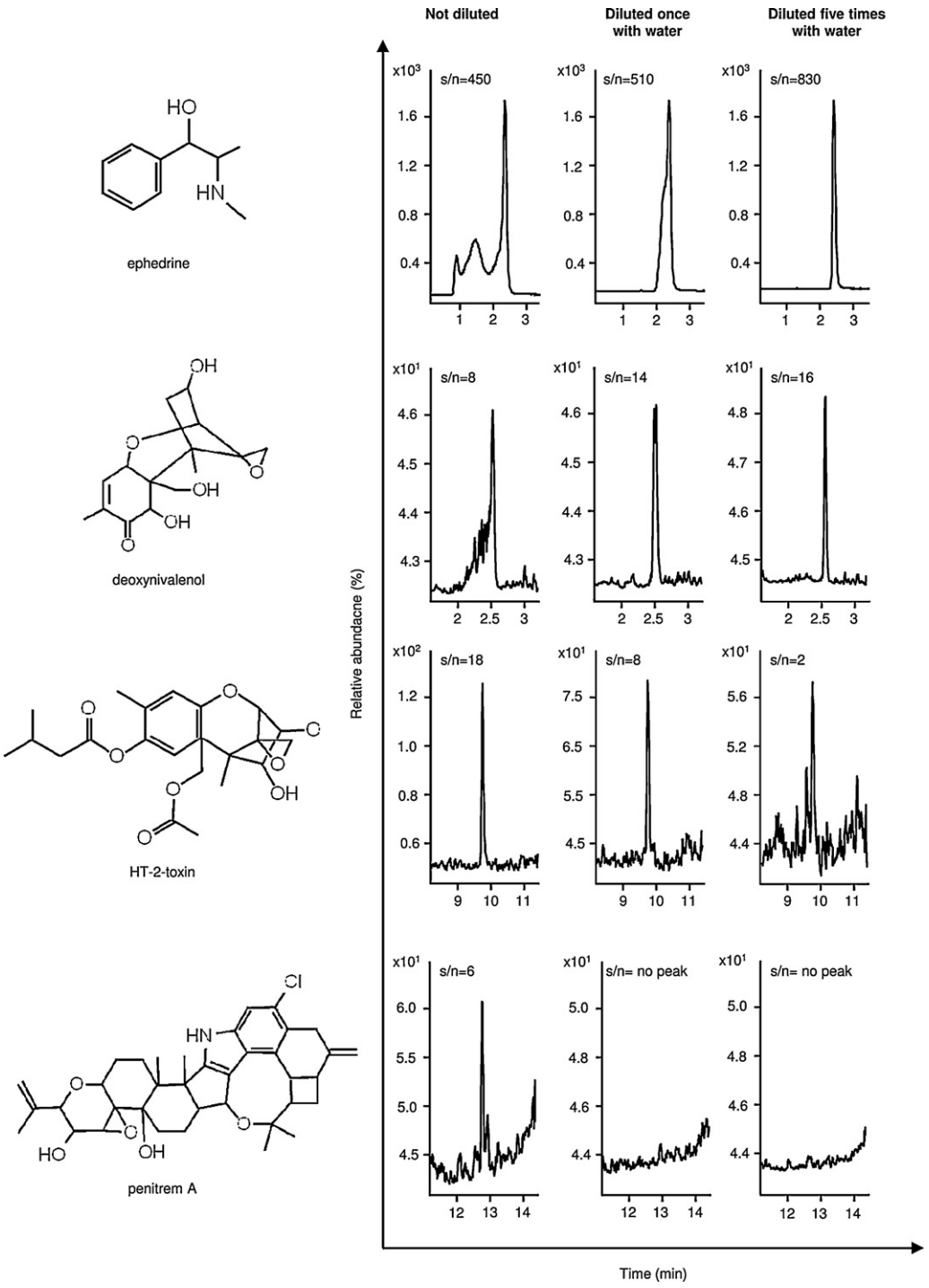

Fig. 2. Effect on chromatographic peaks and signal to noise ratio (s/n) of selected compounds upon diluting extracts of baby food 1 (400 μg/kg) with water.

this could partly be overcome by reducing the elution strength of the extracts by water dilution prior to injection, some compounds did not retain at all in the reversed phase system used. In order to improve the detection capability for polar compounds, the extracts could be analyzed by an additional LC–MS/MS run on a system employing aqueous normal phase (ANP) chromatography (i.e. hydrophobic interaction liquid chromatography, HILIC). Another method limitation is the general inability of the ESI to ionize some compounds. Further extension of the range of analytes could thus be achieved by additional analysis of the extracts on an LC–MS/MS system equipped with a different ion source, e.g. atmospheric pressure chemical ionization (APCI), or analysis on a GC–MS system.

It is practically impossible to develop methods targeting all compounds that could be used as terrorist agents. The present work intended to develop a method that could be used as an "add-on-method", i.e. allowing for new compounds to be constantly included. Since the method was demonstrated to work well for such a wide range of structurally non-related compounds, it is likely that additional compounds could be added without needing to modify the method. Ability for such quick method development is an important feature for laboratories responsible for preparedness and

response to food emergencies dealing with emergency situations, e.g. in the case of a threat situation involving a specific chemical agent or when suspected chemical agents are indicated by a non-targeted screening technique, such as full scan time-of-flight (TOF) mass spectrometry, and need to be confirmed.

4. Conclusion

This model study clearly demonstrates the feasibility of LC–MS/MS for multi-analyte detection of toxic organic compounds in food at concentrations relevant for food-scare events. A screening method, based on simple generic extraction combined with LC–MS/MS, was set up and evaluated for analysis of 19 different food types spiked with 108 representative compounds with high structural variation. A large majority of the selected model compounds could be detected at 10–100 μg/kg in all food types. It was concluded that signal reduction due to extraction recoveries and ion suppression in the electrospray interface was of minor importance for most compounds.

The method thus meets the requirements for fast response in a threat or emergency situation where it is essential to have quick identification in order to take necessary action in order to avoid the additional spread of contaminated food, withdraw food from the market or to warn the general population when needed.

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