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Screening of plant toxins in food, feed and botanicals using full scan high resolution (Orbitrap) mass spectrometry

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with full scan high resolution (Orbitrap) ed for the simultaneous detection of a ariety of food and feed matrices. For a representing various chemical classes, the d LC-MS conditions. Ion suppression ated using generic extracts from rices (food supplement, honey, silage, ould be measured as positive ions power of 100,000 FWHM, a reliable high pite the high abundance of co- ts. This enabled the use of ± 5 ppm mass n resulted in a high method selectivity. oney, strong ion suppression effects were sely affected the sensitivity. f the substances the detection limits were . Since non-selective sample preparation n is performed, the presence of plant sing data review can be investigated

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3 1 **Screening of plant toxins in food, feed and botanicals using full scan high**
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Abstract

A generic method based on LC with full scan high resolution (Orbitrap) mass spectrometry (MS) was systematically investigated for the simultaneous detection of a wide range of plant toxins in a variety of food and feed matrices. For a selection of 150 substances, representing various chemical classes, the limit of detection was established using fixed LC-MS conditions. Ion suppression effects and selectivity were evaluated using generic extracts from representative and relevant matrices (food supplement, honey, silage, compound feed). The majority of the substances could be measured as positive ions after electrospray ionization (ESI⁺). Using a mass resolving power of 100,000 a reliable high mass accuracy was obtained despite the high abundance of co-extractants in the sample extracts. This enabled the use of ± 5 ppm mass extraction windows which in turn resulted in a high degree of selectivity. On the other hand, except for honey, strong ion suppression effects were frequently observed which adversely affected the detection limits. Nevertheless, for the majority of the substances the detection limits were in the range of 0.01-0.05 mg/kg. Since non-selective sample preparation and non-targeted data acquisition was performed, the presence of plant toxins initially not targeted for during data review, can be subsequently investigated, which is a very useful option because for many known toxins no analytical reference standards are yet available. The applicability of the method was demonstrated by analysis of a variety of real-life samples, purchased on the market or from cases of intoxication. These included honey, herbal tea, food supplements, poppy seeds, traditional Chinese medicines (TCM), compound feed, silage and herb-based feed additives. Plant toxins that were detected included various pyrrolizidine alkaloids, grayanotoxins, opium alkaloids, strychnine, ricinine (marker for ricin), aconitine, aristolochic acid and cardiac glycosides (e.g. digitoxin, digoxin).

Keywords: Plant toxins, Alkaloids, Food, Food supplements, Feed, Feed additives, Botanicals, Herbal preparations, Traditional Chinese Medicines, Contaminants, Quality and Safety, High resolution mass spectrometry, Orbitrap

37 Introduction

38 Plant toxins, or phytotoxins, are secondary plant metabolites that exhibit acute or chronic
39 toxicity or have anti-nutritional effects. They may act as chemical defense to protect the plant
40 from herbivores, bacteria and fungi. We can distinguish between inherent plant toxins, which
41 are present in edible crops, and plant toxins entering the food and feed chain due to
42 contamination with non-edible plants. Examples of inherent plant toxins in major food and
43 feed commodities are glycoalkaloids in potatoes and cyanogenic glycosides in cassava
44 (Speijers et al 2010), and glucosinolates in species from the *Brassicaceae* family (EFSA
45 2008a). Aromatic plants used as food ingredient (e.g. herbs, spices), as raw materials for
46 flavors and fragrances (e.g. essential oils), or as (traditional) herbal medicinal products are
47 examples of minor products in terms of volume, but in which inherent plant toxins can be
48 very abundant and significant in terms of intake (Salgueiro et al 2010, Khan 2005). In the case
49 of herbal medicinal products, plant toxins can be the same substances as those to which the
50 health benefits are attributed, the difference between toxin and pharmaceutical obviously
51 being the dose. Aromatic plant products used as food ingredient, food supplement, feed
52 additive or as medicine are also referred to as botanicals or botanical preparations.

53 Contamination is another route of exposure of humans and livestock to plant toxins. Weeds
54 or weed seeds may be co-harvested with food and feed crops and end-up in the food/feed
55 chain. Animals may graze on contaminated pastures or resort to eating toxic plants in case of
56 lack of edible plants. This can directly affect animal health and productivity, or result in
57 indirect human exposure through contaminated animal products (e.g. milk, eggs). Bees
58 foraging on flowers of toxic plants may result in contamination of honey. For botanicals, it is
59 common that the raw plant materials are collected in the wild. Non-targeted species may be
60 included either by accidental substitution or by adulteration. Finally, plant toxins are being
61 used as crop protection product (Dayan et al 2009) which may leave residues on the crops at
62 the time of harvest.

63 Publications on toxic effects of plant toxins through food and feed are often initiated by, or
64 related to, severe cases of intoxication. One of the most extensively described classes of plant
65 toxins are pyrrolizidine alkaloids, some of which are hepatotoxic, carcinogenic, genotoxic and
66 teratogenic. They are of increasing concern due to their high world-wide abundance and many
67 cases of food and feed contamination, and the occurrence as inherent plant toxin in herbal
68 medicines (Wiedenfeld and Edgar 2011, and references therein). Many other intoxications

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3 69 have been reported, some with very serious health or even fatal consequences. One infamous
4 70 example is the development of renal failure and cancer in over 100 women in Belgium upon
5 71 treatment with slimming pills prepared from herbs containing aristolochic acid (Cosyns
6 72 2003). Another example concerned 20 people getting epileptic seizures after consumption of
7 73 herbal tea (Johanns et al 2002). The ingredient Chinese star anise (*Illicium verum*) had been
8 74 replaced by Japanese star anise (*Illicium anisatum*) which contains the neurotoxin anisatin.

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13 75 Despite the serious acute or chronic toxic effects of plant toxins, hardly any legislation has
14 76 been established, especially when compared to other toxicants such as pesticides, veterinary
15 77 drugs and environmental contaminants. In addition, the available legislation is not
16 78 harmonized and varies by country or region. A summary of plants and plant toxins which
17 79 have been regulated in the European Union (EU) is provided in the Supplementary
18 80 Information (Table S1- available on-line). This includes both specific toxins as such and
19 81 botanical impurities (i.e. plant material). In the latter case, the relationship between maximum
20 82 content of plant material and the actual toxin(s) is not obvious because levels of secondary
21 83 plant metabolites are known to vary considerably depending on species, geographical and
22 84 seasonal conditions, development stage of the plant, part of the plant ending up in food or
23 85 feed commodities, storage, and processing into the final product that is consumed.

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32 86 The European Food Safety Authority (EFSA) has increased concerns regarding plant
33 87 toxins. A number of scientific opinions have been issued and a compendium has been
34 88 compiled of botanicals that have been reported to contain toxic, addictive, psychotropic or
35 89 other substances of concern (EFSA 2009). More than 600 substances have been mentioned.
36 90 Some occur only in specific plant genera or are even species-specific, others are present in
37 91 several plant families. For risk assessment and quality and safety control, there is a need for
38 92 analysis methods to determine these plant toxins in a wide variety of complex matrices.
39 93 Especially in case of contamination, where it is often not *a priori* known what to look for, this
40 94 is a very challenging task.

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48 95 At this moment, there is a lack of routine methods for determination of plant toxins. When
49 96 methods are available, they are usually dedicated to a specific (group of) substance(s) in a
50 97 certain commodity. With the above analytical challenge in mind, the availability of a generic
51 98 method suited for a wide variety of plant toxin/matrix combinations would be highly
52 99 beneficial. The high potential of LC-MS for detection of plant toxins was early recognized
53 100 (Verpoorte and Niessen 1994), and today many methods based on LC-MS/MS have been
54 101 reported (e.g. Holstege et al 2001, Josephs et al 2010, McIlhenny et al 2009, Kuo et al 2010,
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3 102 Sproll et al 2006, Ye et al 2007, Zhou et al 2010). Full scan high resolution TOF-MS has also
4 103 been applied (e.g. Li et al 2010, Yan et al 2010, Zhang et al 2009, Zhou et al 2008, Zhou et al
5 104 2009) which is especially interesting, because it allows searching for substances for which no
6 105 reference standard is available which is an issue in the field of plant toxin analysis. It also
7 106 enables the analyst to retrospectively re-evaluate the raw data when new toxins become
8 107 known. Given the fact that over 200,000 secondary plant metabolites exist (Hartmann 2007)
9 108 and that all these substances are primarily composed of the elements C, H, N and O,
10 109 selectivity requirements in the detection of plant toxins in complex matrices are high. In LC
11 110 with full scan MS this means that a high mass resolving power is needed to separate co-
12 111 eluting compounds with similar exact masses.

13 112 With the introduction of a bench top Orbitrap mass spectrometer in 2008 (Bateman et al
14 113 2009), ultra-high resolving power (100,000 FWHM (full width at half maximum)) has
15 114 become an option for routine analysis. Previously the benefits of this in residue and
16 115 contaminant analysis in complex food and feed matrices have been reported (Kellmann et al
17 116 2009).

18 117 In this work, for the first time, a generic method based on LC with full scan high resolution
19 118 (Orbitrap) MS aiming at the simultaneous detection of a high number of plant toxins from
20 119 various chemical classes in a variety of food and feed matrices, is systematically investigated.
21 120 For a selection of 150 substances mentioned in the EFSA Compendium, representing various
22 121 toxin classes, the sensitivity was tested using fixed LC-MS conditions. Ion suppression effects
23 122 and selectivity were evaluated using crude extracts from representative and relevant matrices
24 123 (food supplement, honey, silage, compound feed). The applicability of the generic method is
25 124 demonstrated by qualitative analysis of a variety of products known or expected to contain
26 125 plant toxins.

27 126

28 127 **Materials and methods**

29 128 *Chemicals and reagents*

30 129 Reference standards: retrorsine, senecionine, seneciphylline and senkirkine were obtained
31 130 from Phytoplan (Heidelberg, Germany). Heliotrine was obtained from Accurate Chemical
32 131 (Westbury, NY, USA). Lycopsamine and echimidine were obtained from Phytolab
33 132 (Vestenbergsgreuth, Germany). Jacobine and erucifoline were isolated from plant material by

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3 133 PRISNA (Leiden, The Netherlands). The pyrrolizidine metabolites retrorsine-N-oxide,
4 134 senecionine-N-oxide, and seneciophylline-N-oxide, jacobine-N-oxide and erucifoline-N-oxide
5 135 were prepared by N-oxidation of the corresponding alkaloid with 30% hydrogen peroxide in
6 136 ethanol according to the method described by (Chou et al. 2003). Other reference standards
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8 137 were obtained from Sigma Aldrich (Zwijndrecht, The Netherlands). From the pure standards,
9 138 a stock solution of 100-2000 $\mu\text{g/mL}$ was prepared in methanol. The different stock solutions
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11 139 were combined into mixed standard solutions of 1 $\mu\text{g/ml}$ in methanol. The solutions were
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13 140 stored at 2-10° C until use.

141 Chemicals: methanol, acetonitrile and LC-MS grade water were purchased from Biosolve
142 (Valkenswaard, The Netherlands). Acetic acid, sodium chloride and magnesium sulfate were
143 obtained from Merck (Darmstadt, Germany), and formic acid and ammonium formate were
144 from Sigma-Aldrich.

145 *Samples and pretreatment*

146 Samples of a food supplement, silage, compound feed, and a feed ingredient were supplied
147 by the Dutch Food and Consumer Safety Authority. Three of the honey samples originated
148 from previous studies. All other samples were purchased in local stores and internet stores
149 based in the Netherlands. Details on sample composition, as far as available, are provided in
150 the Supplemental Information (Table S2). Dry samples were homogenized by milling into a
151 powder (<0.5 mm). Capsules were opened and only the powder was used for analysis. Honey
152 was used as such.

153 *Sample preparation*

154 Sample preparation was based on extraction with water and acetonitrile with subsequent
155 salt-induced phase partitioning (acetate-buffered QuEChERS, Lehotay et al 2010).
156 Homogenized sample (2.5 g) was weighed into a polypropylene tube, water (10 ml) was
157 added and the sample was thoroughly shaken. Acetonitrile (10 ml) containing 1% of acetic
158 acid was added and the tube was shaken end-over-end for 30 min. Sodium acetate (1 g) and
159 magnesium sulfate (4 g) were added, the tube was shaken by hand to induce phase separation
160 and partitioning, and then centrifuged at 3500 rpm for 10 min. No clean-up was performed,
161 i.e. the dispersive SPE step(s) from the QuEChERS procedure was omitted. An aliquot of the

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3 162 acetonitrile phase was diluted 1:1 with water and then filtered (0.45 μ m filter), resulting in an
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5 163 extract containing 0.125 g sample equivalent/ml.

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7 164 For evaluation of matrix effects, the mix-standard solution was spiked to the extract at 1.25,
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9 165 6.25 and 25 ng/ml (corresponding to 0.01, 0.05 and 0.20 mg/kg sample). More diluted
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11 166 extracts were prepared by diluting the 25 ng/ml extracts five times with acetonitrile:water 1:1
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13 167 (0.025 g sample equivalent/ml extract).

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15 168 To demonstrate the applicability of the method, samples were extracted as described
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17 169 above. The selected samples were known or expected to contain plant toxins and analyzed
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19 170 without fortification. The only exception in this respect was silage; this sample was fortified
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21 171 with the mix standard. The extracts were analyzed as such or after an additional 10-fold
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23 172 dilution.

24 173 ***Instrumentation***

25 26 174 *HPLC-Orbitrap MS*

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28 175 LC-Orbitrap analysis: An Accela HPLC (Thermo Fisher Scientific, San Jose, CA, USA)
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30 176 was coupled to an Exactive single stage Orbitrap system also from Thermo Fisher Scientific,
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32 177 fitted with a HESI II electrospray source. A 100 x 3 mm ID, 3 μ m Atlantis T3 LC column
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34 178 from Waters (Milford, MA, USA) was used.

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36 179 The LC mobile phases were water (A) and methanol:water 95:5 (B) both containing 2 mM
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38 180 ammonium formate and 0.5 mM formic acid (pH 5). The first minute of LC gradient was
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40 181 isocratic at 100% A, then a linear gradient to 55% B after 3 min and a linear gradient to 100%
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42 182 B after 9 min. For complete elution of all matrix compounds, the final composition was held
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44 183 for 11 min. In 1 min the initial conditions were restored and then equilibrated for 4.5 min
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46 184 before the next injection. The LC flow rate was 300 μ l/min. The temperature of the column
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48 185 oven was 35 $^{\circ}$ C.

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50 186 The electrospray source was operated in positive and negative mode, using the following
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52 187 parameters: electrospray voltage 2.5 kV; sheath gas 30 arbitrary units; auxiliary gas 10
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54 188 arbitrary units; sweep gas 5 arbitrary units. The heater in the source was set at 300 $^{\circ}$ C and the
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56 189 heated capillary in the mass spectrometer was operated at 300 $^{\circ}$ C (positive mode) or 360 $^{\circ}$ C
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58 190 (negative mode).

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3 191 Acquisition was performed at a resolving power of 100,000 (FWHM at m/z 200). The scan
4 192 time was 0.8 seconds resulting in an overall scan rate of 1.2 Hz. The automatic gain control
5 193 target was set to 10^6 ions. The other parameters for the mass spectrometer were automatically
6 194 tuned to get the highest TIC signal. Before each batch of analysis the mass calibration of the
7 195 mass spectrometer was checked and optimized by the Exactive Tune v 1.1 software from
8 196 Thermo Fisher Scientific by direct infusion of calibration mixtures (MSCALx) from Supelco
9 197 (Bellefonte, PA, USA). A mass which was always present in the background (m/z 218.1387,
10 198 substance not further identified), was used as a lock-mass to automatically correct the mass
11 199 calibration for each scan. The LC and mass spectrometer were controlled by Xcalibur 2.1
12 200 software. Data processing was done using ToXID 1.2.1 and Xcalibur 2.1 (Thermo Fisher
13 201 Scientific).
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23 203 **Results and discussion**

24 204 *Sensitivity of full scan Orbitrap-MS for detection of plant toxins*

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27 205 Plant toxins belong to a wide variety of chemical classes, including various types of alkaloids,
28 206 terpenes and glycosides. The number of substances is enormous. In the selection of
29 207 substances included in this work the following aspects were considered: listed in the EFSA
30 208 compendium, coverage of various chemical (sub)classes, inclusion of substances known to be
31 209 highly toxic or of high concern, and, last but not least, commercial availability of reference
32 210 standards. The list of substances studied is provided in Table 1.
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41 211 The aim of this work was to evaluate the use of LC-high resolution MS for screening of
42 212 plant toxins in various matrices, i.e. the detection of as many as possible substances by one
43 213 method. Since the optimum conditions for the LC-MS measurement will vary for the different
44 214 substances, compromises have to be made. In the past we evaluated the effects of
45 215 chromatographic column, eluent composition, and source conditions in the frame of multi-
46 216 analyte detection of pesticides and mycotoxins (results not published). Based on this
47 217 experience an end-capped C18 column was selected for retaining polar substances and robust
48 218 chromatography. Regarding the eluent, methanol was chosen as modifier due to its better MS
49 219 sensitivity for most substances compared to acetonitrile, and ammonium formate was added
50 220 to suppress sodium adduct formation. The pH of 5 was a compromise between retention and
51 221 peak shape for basic alkaloids (best at neutral/basic conditions), and detection limits for
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3 222 certain substances (e.g. pyrethrins and THC, better under more acidic conditions). MS source
4 223 parameters were set as recommended by the manufacturer to be favorable for the majority of
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6 224 small molecules.
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8 225 Reference standards totaling 150 substances were injected into the HPLC-Orbitrap MS
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10 226 system and measured in positive and negative mode. Accurate masses obtained were matched
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12 227 against exact masses of plausible ions. For positive ions, the tendency of formation of
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14 228 ammonium and sodium adducts was verified. The results are included in Table 1. The far
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16 229 majority of the substances could be measured as positive ions, mostly protonated. For several
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18 230 substances multiple adducts were obtained. Despite the addition of ammonium formate, the
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20 231 sodium adduct was the most abundant for a number of cardiac glycosides. Where sodium
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22 232 adducts were observed, the relative abundance of $[M+H]^+$, $[M+NH_4]^+$ and $[M+Na]^+$ was not
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24 233 always consistent over a longer period of time. Several substances also yielded a response in
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26 234 negative mode, but in most cases with lower sensitivity compared to positive mode.
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28 235 Glycerrhizic acid and the anthraquinones aloe-emodin, emodin and chrysophanic acid could
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30 236 only be measured as negative ion. For 17 substances no response was obtained under the
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32 237 applied conditions (see Supplemental Information Table S3). As was to be expected, the
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34 238 majority of these were alkenylbenzenes and monoterpenes. In contrast to earlier
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36 239 measurements by LC-MS (triple quadrupole), both in our laboratory and reported by others
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38 240 (Ye 2007), here no response was obtained for sennoside B. For glycerrhizic acid,
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40 241 chrysophanic acid, methyllycaconitine, oenin, and vincristine, a response was obtained upon
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42 242 injection of individual reference standards, but no or an inconsistent response was observed
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44 243 later on after preparation of mix-standard solutions. They were therefore excluded for further
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46 244 evaluation.
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48 245 For the remaining substances, the detection limits of the instrumental measurement were
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50 246 determined by injection of solvent standards and manually reviewing extracted ion
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52 247 chromatograms (XICs) using a mass extraction window of ± 5 ppm. In the XICs obtained this
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54 248 way, noise was typically absent and only the peak of the analyte of interest stood out.
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56 249 Therefore, establishment of the limit of detection (LOD) based on a signal-to-noise ratio was
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58 250 not really feasible. Instead, the determination of the LOD was done by reviewing stick plots
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60 251 showing the response as vertical lines for each individual scan, rather than (smoothed) peaks,
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253 252 for a series of dilutions of solvent standards. The requirement set was that at least three scans
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255 253 should be still be present at the retention time of the analyte. At the lower response levels,
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257 254 spikes were often observed in the XICs. In such cases, a second requirement was that the

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3 255 response of the scans of the peak of interest should be at least three times that of the spikes
4 256 present within one minute of the retention time of the target analyte. The lowest concentration
5 257 injected was 0.5 µg/L, corresponding to 2.5 pg on-column. The LODs derived this way are
6 258 included in Table 1. For almost 60% of the substances the system LOD was 2.5 pg or lower,
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8 259 important plant toxins such as pyrrolizidine alkaloids and tropane alkaloids were amongst the
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10 260 ones most sensitively detected.
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13 14 261 *Detection limits in real samples*

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17 262 The detection limit of the plant toxins in samples is influenced by the matrix, sample
18 263 preparation (recovery, concentration of final extract), the injection volume, and the effect of
19 264 the matrix on the MS response. The matrices in plant toxin analysis are often highly complex,
20 265 e.g. dried aromatic plants, herbal mixtures and extracts, compound feed, hay and silage.
21 266 Sample preparation in generic methods is straightforward with little or no clean up in order to
22 267 avoid losing substances of interest. Procedures described for multi-analyte methods are
23 268 water/acetonitrile partitioning from the field of pesticides (QuEChERS, Lehotay et al 2010),
24 269 and extraction/dilution with acidified aqueous acetonitrile (mycotoxins, e.g. Sulyok et al
25 270 2010) and other water miscible solvents (pesticides, veterinary drugs, natural toxins; Mol et al
26 271 2008). These approaches have proven to be effective and efficient and are equally attractive
27 272 for use in a wide scope screening method for plant toxins. Inevitably, the extracts generated
28 273 this way contain many co-extractants, as is illustrated in Figure 1. This may have adverse
29 274 effects on the selectivity and the sensitivity of the LC-MS analysis.
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39 275 40 41 276 *Effect of matrix on selectivity*

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43 277 Especially at low levels of plant toxins, peaks from other substances may interfere in the
44 278 qualitative and quantitative determination. To avoid this, a high degree of selectivity is
45 279 required in the instrumental analysis. In full scan MS, a high mass resolving power / high
46 280 mass accuracy is essential, as has been demonstrated elsewhere (Kellmann et al 2009). This is
47 281 especially true for the analysis of plant toxins amongst thousands of other secondary plant
48 282 metabolites. Here both target analytes and background interferences are small molecules in
49 283 the 100-400 Da range with the same elemental composition (C, H, O and/or N). As
50 284 described earlier (Nielen et al 2007) it can be calculated that to resolve co-eluting substances,
51 285 differing in only one CO vs N₂ (i.e. 11.2 mDa), a mass resolving power of 17,800, 35,600,
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3 286 53,400 and 71,200 (FWHM) is required at m/z 100, 200, 300 and 400, respectively. For this
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5 287 reason we used the highest resolving power that could be set with the Orbitrap MS used in
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7 288 this work: 100,000 (FWHM) at m/z 200. This corresponds to 70,700 at m/z 400, since with
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9 289 Orbitrap MS the resolving power is inversely proportional with the square root of m/z . To
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11 290 evaluate the selectivity, generic extracts were prepared for four matrices: honey, a food
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13 291 supplement (mixture of dried aromatic plants, 'blood purifier'), silage, and a compound feed
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15 292 (complete pig feed). The extracts were spiked with plant toxins at three concentrations levels,
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17 293 corresponding to 0.01, 0.05 and 0.20 mg/kg in the sample, and analyzed. The selectivity was
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19 294 evaluated as follows: for each analyte, the XIC of the lowest concentration for which a peak
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21 295 was still obtained, was manually examined. In that XIC, the presence of other peaks was
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23 296 checked. Spikes, typically observed in the lower response range, were ignored. Furthermore,
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25 297 peaks from isobaric compounds that were present in the mix-reference standard used for
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27 298 spiking, were not regarded here as interference. In most cases, no significant interfering peaks
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29 299 were observed near the retention time of the targeted substances, in many cases not even in
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31 300 the entire chromatogram. In a limited number of cases (<10%), interfering peaks were
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33 301 observed. In rare cases, background noise across a larger part of the XIC occurred. Examples
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35 302 of all three situations are shown in Figure 2. Based on these observations, we conclude that
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37 303 selectivity limitations do occur for certain analyte/matrix combinations, but in general are not
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39 304 a major issue.

305 *Effect of matrix on sensitivity*

306 It is well known that high concentrations of co-extracted matrix can affect ionization of the
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308 307 analytes in the ion source. This sometimes results in an enhancement, but more often in a
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310 308 suppression of the response of the analyte in an extract compared to that of the same analyte
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312 309 in a solvent standard. Previous work (Mol et al 2008) showed that suppression is most
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314 310 pronounced in dry commodities, especially those of complex composition (e.g. compound
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316 311 feed). Such types of matrices are relevant for plant toxin analysis and therefore matrix effects
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318 312 were studied for three complex dry matrices (herbal food supplement, silage, complete pig
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320 313 feed). In addition, one less complex matrix (honey) was also included. Generic extracts
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322 314 containing 0.125 g matrix equivalent/ml extract were prepared and spiked with the analytes at
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324 315 the level corresponding to 0.20 mg/kg. These extracts were analyzed as such and also after an
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326 316 additional five-fold dilution. The response of the analytes in the extracts relative to the
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328 317 response of solvent standards of the same concentration was calculated. The results are
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330 318 summarized in Table 2. For honey, no or only modest (less than factor of 2) suppression

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3 319 occurred. Suppression was more pronounced for the other three matrices in increasing order:
4 320 complete pig feed, silage, food supplement. For the food supplement, a mixture of dried
5 321 herbs, suppression resulted in a loss of sensitivity of more than a factor of five for 39% of the
6 322 substances (5 μ L injection of 0.125 g/ml extracts). This reduced to 5% of the substances for
7 323 the five-fold diluted extracts (0.025 g/ml).

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11 324 For analytes that can be sensitively detected by the instrument, dilution is an option to
12 325 allow a better estimation of the concentration based on solvent standards. For other analytes,
13 326 if the required detection limits cannot be met, matrix suppression needs to be reduced by
14 327 clean up or use of more optimum LC-MS conditions which will compromise scope.

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19 328 From the above it is clear that ion suppression negatively affects the sensitivity and higher
20 329 LODs are obtained in samples than might be expected from the solvent standards. The LODs
21 330 for the individual analytes in each of the four matrices tested are included in Table 1.
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23 331 Assuming quantitative recovery during extraction, for honey 70% of the analytes could still
24 332 be detected down to the 0.01 mg/kg level. For the other matrices higher LODs were obtained,
25 333 but still 70% of the analytes could be detected at 0.05 mg/kg or lower, while for 10-15% the
26 334 LOD was higher than 0.2 mg/kg. Whether such higher LODs are acceptable or not depend on
27 335 the final application. For the few substances for which maximum concentration limits have
28 336 been established (see Supplemental Information Table S1), this seems acceptable. Also in
29 337 case of analysis of individual aromatic plants or samples related to intoxications, the toxins
30 338 are often present at the mg/kg level. On the other hand, the situation is different when the aim
31 339 is to detect minor contamination with toxic plants, or to detect low levels of carcinogenic
32 340 substances such as certain pyrrolizidine alkaloids and aristolochic acids. Furthermore, at this
33 341 moment legislation in The Netherlands (Staatsblad 2001) as well as in other countries state
34 342 that herbal preparations 'should not contain materials originating from certain plants'. This is
35 343 a very qualitative description and translation into maximum levels of the corresponding plant
36 344 toxins has not been done so far. The same is true for the EU directive (EU 2002/32) that
37 345 regulates undesirable substances in animal feed. For most plant toxins limits are set in mg
38 346 plant/kg feedingstuff, or it is stated that the plant 'or their processed derivatives may only be
39 347 present in feedingstuffs in trace amounts not quantitatively determinable'. This has been set
40 348 with visual/microscopic methods in mind. From various publications by others and this paper
41 349 it is clear that chemical methods are available to measure the actual toxins. Therefore, it
42 350 would make more sense to set limits for the toxins rather than for the plant, especially because
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3 351 the relationship between plant parts present in the feed and the toxin concentrations is
4 352 unlikely to be linearly correlated.

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6 353 It is clear that more information on concentrations of toxins in the plants and in the final
7 354 food or feed product is needed to translate mg toxic plant/kg into mg toxin(s)/kg and to see
8 355 whether the LODs of the currently proposed screening method are fit-for-purpose.
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13 14 15 357 *Example applications*

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18 358 To demonstrate the potential and applicability of the screening method, a variety of
19 359 products in which plant toxins might be present were analysed. The XICs were manually
20 360 reviewed for presence of the analytes based on retention time, exact mass and isotopic pattern.
21 361 Substances from the scope of this work were detected in most of the samples. Obviously,
22 362 retention time and accurate mass alone do not provide an unambiguous identification. That
23 363 requires a further confirmation through fragment ions. For 34 of the detected substances an
24 364 LC-MS/MS method was available in our laboratory and a confirmatory analysis performed.
25 365 Out of the 51 detects that were verified, 44 were confirmed. An overview of the screening
26 366 results is provided in Table S4 of the supplemental information. A selection of the findings is
27 367 discussed and put in context in more detail below.

28 29 30 31 32 33 368 *Plant toxins in food*

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39 370 Honey is an important food product in which plant toxins may occur due to transfer through
40 371 nectar or pollen collected by bees foraging on areas with high abundance of toxic plants. Such
41 372 transfer has been described for pyrrolizidine alkaloids (Edgar et al 2002) which are present in
42 373 a wide variety of plants, and grayanotoxins which occur in the plant family *Ericaceae* (e.g.
43 374 rhododendron) (Gunduz 2008). Figure 3a shows the analysis result of a honey sample from
44 375 beehives intentionally placed at the Veluwe region in the Netherlands with high abundance of
45 376 ragwort (*Jacobaea vulgaris*). Several pyrrolizidine alkaloids typical for this plant were
46 377 detected based on accurate mass and retention time. In addition, traces of jacoline and
47 378 jacoline and their N-oxides could be provisionally identified, based on accurate mass and
48 379 lack of other peaks in the XIC. For jacoline, one of the rare examples of a plant toxin
49 380 containing chlorine, the presence of the ³⁷Cl isotope supported its identification (see Figure
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3 381 3b). For the pyrrolizidine alkaloids for which a reference standard was available, an
4 382 estimation of the concentrations could be made based on solvent standards, i.e. not taking ion
5 383 suppression into account. Levels were in the range of 0.05-0.6 mg/kg, with a total of 1.7
6 384 mg/kg. This is high in relation to the very low maximum limit of 1 µg/kg for toxic
7 385 pyrrolizidine alkaloids in herbal preparations that has been established in Dutch legislation
8 386 (Staatsblad 2001). In another sample from beehives located in the dunes near Vogelzang (The
9 387 Netherlands), an area with a high abundance of Vipers bugloss (*Echium vulgare*),
10 388 lycopsamine was detected.

11 389 Grayanotoxins could easily be detected in Nepalese honey causing intoxication.
12 390 Grayanotoxin III was present at an estimated level of 30 mg/kg. Based on the exact mass of
13 391 the sodium adduct, also grayanotoxin I was provisionally identified. Recently concerns about
14 392 poisoning through grayanotoxins in rhododendron honey originating from the Turkish Black
15 393 Sea region were raised by the German Federal Institute for Risk Assessment (BfR 2010)
16 394 which calls for more systematic control of honey from certain specific regions.
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18 396 Poppy seeds

19 397 Poppy seeds are commonly used as food ingredient in central Europe. During harvest the
20 398 seeds can get contaminated with the latex of the plant that contains opium alkaloids. Levels
21 399 can vary widely (sub mg/kg to >100 mg/kg) and have increased in recent years. This has
22 400 triggered a call for data on opium alkaloids in poppy seeds in order to assess the need for
23 401 regulatory measures (Battilani et al 2009, EFSA 2010a). As an example of the applicability of
24 402 the method described in this work, Figure 4 shows the presence of six alkaloids in samples of
25 403 white and blue poppy seeds purchased in Dutch shops. At the time of analysis, only a
26 404 morphine reference standard was available, but the other five alkaloids could easily be found
27 405 as they were the only peaks standing out in their respective XICs. As shown in Figure 4, the
28 406 relative abundance of the alkaloids differed remarkably. Since a full quantitative
29 407 determination was beyond the scope of the current work, a concentration estimate was made
30 408 based on the solvent standard; the levels of morphine found were approximately 8 and 20
31 409 mg/kg.
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33 411 Herbal tea

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3 412 Herbal teas are mixtures of a variety of dried aromatic plants. The substitution of toxic
4 413 Japanese star anise (*Illicium anisatum*) for the similar looking Chinese star anise (*Illicium*
5 414 *verum*) and its dramatic consequences has been mentioned in the introduction. Anisatin, the
6 415 responsible toxin, could easily be detected in a sample of herbal tea to which 10% of Japanese
7 416 star anise had been added to simulate a situation of misidentification or adulteration. This
8 417 demonstrates the potential of the screening method for quality control purposes, as alternative
9 418 option to TLC/LC-MS/MS described by (Lederer et al 2006).

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15 419 Several other substances were detected in the herbal tea sample that was analyzed. Of these,
16 420 the detection of ricinine was the most remarkable finding. Ricinine is an alkaloid found in
17 421 *Ricinus communis* (Castor plant). Since the alkaloid is specific for this plant, it has been used
18 422 as (bio)marker to reveal exposure to Castor plant material or derivatives (Johnson et al 2005,
19 423 Wang et al 2009). The seeds (beans), and to a lesser extend other parts of the plant, contain
20 424 ricin, a glycoprotein known as one of the most toxic natural poisons. Because of this high
21 425 toxicity *Ricinus communis* has been included in the list of prohibited plants in the Dutch act
22 426 on herbal preparations (Staatsblad 2001) and also in the EU directive regulating undesirable
23 427 substances in animal feed (EU 2002/32). Ricinine was also detected in another herbal
24 428 preparation, sold as food supplement ('stool plus'). A subsequent analysis by LC-MS/MS
25 429 resulted in confirmation of the identification (correct retention time, three transitions with
26 430 matching ion ratios). The estimated concentrations were 0.07 and 0.14 mg/kg. An attempt was
27 431 made to correlate this to the amount of *Ricinus* plant material, of which the beans are most
28 432 likely to be used. Based on a reported ricinine content of 0.3-0.8% in castor beans (Johnson et
29 433 al 2005), using the average of 0.55%, the amount of ricinine found would correspond to 13
30 434 and 25 mg castor seeds/kg sample. This would exceed the maximum limit of 10 mg seeds or
31 435 husks/kg set for animal feed. Interpretation for human consumption could not be done
32 436 because in the act it is only stated that *Ricinus* should not be used and no specific value for the
33 437 toxin itself or the plant material has been set.

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49 439 *Plant toxins in food supplements and Traditional Chinese Medicine (TCM)*

50 440 Various herbal preparations sold as food supplements or over-the-counter drugs were
51 441 analyzed. A range of targeted substances were detected, including expected ones such as the
52 442 bioactive substance lapachol/lapachone in Pau d'Arco and the fumocoumarins bergapten,
53 443 umbelliferone and psoralen in a food supplement containing lovage (*Levisticum officinale*).

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3 444 The detection of ricinine in one of the food supplements has been described in the previous
4 445 section. *Ricinus communis* had not been specified on the label and it is not known whether the
5 446 presence was a contamination or an adulteration; from the name of the product ‘stool plus’ an
6 447 intended laxative effect can be derived and *Ricinus* oil is known as a strong laxative.

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10 448 Strychnine was detected in a product sold as ‘testosterone booster’. The estimated level
11 449 based on calibration against a solvent standard was 0.02 mg/kg. In Figure 5 the XIC and the
12 450 profile mass spectra are shown. From this figure, the benefit of the high resolving power
13 451 becomes evident: several other ions originating from co-eluting substances, one only differing
14 452 15 mDa, were easily mass spectrometrically resolved. In a confirmatory quantitative analysis
15 453 by LC-MS/MS using standard addition a concentration of 0.04 mg/kg was found which was
16 454 in good agreement when taking into account that suppression was not corrected for in the LC-
17 455 Orbitrap MS screening. Strychnine is an alkaloid used as rodenticide. *Strychnos spp.* is the
18 456 host plant and is being applied in herbal medicine treatments. In the Netherlands, the use of
19 457 the species *Strychnos nux-vomica* in herbal preparations is prohibited.

20 458 Several Traditional Chinese Medicines (TCMs) may contain highly toxic substances,
21 459 besides the substances to which the therapeutic action is being attributed. Aconitine-type
22 460 alkaloids are known to be present in the roots of *Aconitum carmichaeli* (Yue 2009). The TCM
23 461 *Chuan Wu* was analyzed and aconitine was found at a level of approximately 0.1 mg/kg.
24 462 Related substances such as hypaconitine and mesaconitine were also detected based on the
25 463 exact mass of the protonated molecules. In all three cases, the substance targeted for through
26 464 their XIC was the only major peak present (see Supplemental Information Figure S1). The
27 465 pyrrolizidine alkaloids senecionine, its N-oxide and senkirkine were found at levels of
28 466 approximately 1.4, 1.7 and 60 mg/kg, respectively, in *Kuan Dong Hua (Tussilago farfara)*
29 467 which was in the same range as reported by (Jiang et al 2009) using a more dedicated method
30 468 based on LC-MS/MS. In *Chuan Xiong Cha Tiao Wan* aristolochic acid I was detected at
31 469 approximately 0.3 mg/kg (without correction for ion suppression), and confirmed by LC-
32 470 MS/MS. On one hand this was unexpected, since the toxicity of aristolochic acids is well
33 471 known and plants containing them (*Aristolochia* and *Asarum*) have been banned for use in
34 472 herbal preparations for many years in many countries. The label of the product purchased
35 473 listed eight plant ingredients, none of them belonging to the genera just mentioned. On the
36 474 other hand, this product has previously been shortlisted as a multi-ingredient TCM possibly
37 475 containing aristolochic acids (Martena et al 2007). Apparently, despite all warnings, bans and
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3 476 enforcement activities, herbal preparations containing aristolochic acids are still around, either
4 477 as contaminant or intentionally added.
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9 479 *Plant toxins in feed and feed additives*

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11 480 Feed

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13 481 In the current EU legislation on undesirable substances in animal feed (EU 2002/32) several
14 482 botanical impurities are regulated as plant material and not by the actual toxins. This
15 483 originates from the time that no chemical methods were available and microscopy was the
16 484 method of choice. Although these methods can be rapid and efficient, the recognition of toxic
17 485 plant materials is often lost in preserved feeds such as hay, silage and compound feeds.
18 486 Furthermore, large variations in patterns of the toxins in plant materials occur. Therefore a
19 487 chemical screening method would be highly desirable to complement the existing method and
20 488 to aid in the establishment of limits for the toxins rather than the amount of plant material.
21 489 This has been recognized and discussed in EFSA opinions on pyrrolizidine alkaloids (EFSA
22 490 2007) and *Datura* alkaloids (EFSA 2008b). To demonstrate the potential of the proposed
23 491 screening method, a silage sample was spiked at the 0.05 mg/kg level with various plant
24 492 toxins mentioned in the opinions. All spiked pyrrolizidine alkaloids, the *Datura* alkaloids
25 493 atropine and scopolamine as well as ricinine (marker for ricin) could be detected at this level
26 494 or even lower (see Figure 6 and Supplemental Information Figure S2).
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29 496 Feed additives

30 497 Since the ban on the use of antibiotic growth promoters as feed additive in the EU in 2006,
31 498 herbal preparations are increasingly being used as alternative to improve growth, feed
32 499 conversion and for prophylactic purposes. Relatively little is known about the efficacy and
33 500 safety of these additives which has been subject to EFSA concerns (EFSA 2010b). In contrast
34 501 to food supplements, plant extracts for use as feed ingredients are typically admixtures and
35 502 hardly standardized, which complicates safety evaluation of such products. Meanwhile, herbal
36 503 products are being marketed, not rarely without proper labeling of composition. In other cases
37 504 by-products from food or pharmaceutical industry end up as 'beneficial' feed additive or
38 505 ingredient. In 2010, 69 out of a group of 650 calves died in the Netherlands after being fed
39 506 with a feed ingredient labeled as 'parsley by-product'. Samples were taken and screened for
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3 507 plant toxins, with specific attention to cardiac glycosides (e.g. digitoxin, oleandrin) based on
4 508 diagnostic information from the veterinarian. Digoxin, digitoxin and digitoxigenin were found
5 509 based on their accurate mass and retention time match. In addition, the accurate mass of
6 510 protonated lanatoside (B/C) was found. The combined finding of these four related plant
7 511 toxins provided evidence that the feed ingredient had been mixed up or exchanged with
8 512 *Digitalis* (foxglove) most probably as by-product from pharmaceutical industry. In a
9 513 subsequent quantitative analysis, the concentrations for digitoxin and digoxin were 180 and
10 514 1700 mg/kg and considered to be the cause of death.
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19 516 **Concluding Remarks**

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22 517 A generic method for simultaneous detection of various classes of plant toxins in a variety
23 518 of food, feed and botanicals was set up. Inherent to such method, sample preparation is non-
24 519 selective, complex raw extracts are obtained, and generic fixed LC-MS conditions are used
25 520 for analysis. At an individual analyte level this is not always optimal and sensitivity is partly
26 521 sacrificed for extended scope. Selectivity on the other hand was not compromised due to the
27 522 ability to use very narrow mass extraction windows (± 5 ppm) to extract the target analytes
28 523 from the raw data. This was achieved by measuring at a very high mass resolving power
29 524 (100,000 FWHM), resulting in a reliable high mass accuracy (mostly within 2 ppm) even in
30 525 cases of higher levels of co-eluting matrix. Furthermore, the mass accuracy was not affected
31 526 across a wide response range of the analytes (~ 4 orders). The latter is very relevant because
32 527 the concentration range of plant toxins can vary from trace levels in the $\mu\text{g}/\text{kg}$ range in cases
33 528 of contamination, to high mg/kg levels in certain plant species or in cases of adulteration and
34 529 intoxications.
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44 530 In the current method, untargeted full scan MS acquisition was applied. Fragmentation, was
45 531 not performed in this work which means that detection relied on the accurate mass and
46 532 retention time for as far reference standards were available. Although in many cases only one
47 533 peak was observed in the XIC over the entire run time, this is not sufficient for unambiguous
48 534 identification. However, plant toxins are often present with other plant-specific secondary
49 535 metabolites (toxic or not) which may provide additional confirmatory information. In a way
50 536 the presence or absence of other compounds known to co-occur with certain plant toxins
51 537 could be used in a similar way as additional accurate masses from adducts or fragments. For a
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3 538 part of the detects obtained by the screening method, confirmatory LC-MS/MS analysis was
4 539 performed. By doing so, 44 out of 51 detects were confirmed.

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6 540 Inherent to analysis of crude extracts of dried herbal preparations was that strong ion
7 541 suppression effects were observed. Dilution is the solution to this, and an option when very
8 542 low detection limits are not required, e.g. intoxications and quality control focusing on the
9 543 main bioactive substances (toxic or therapeutic) in herbal preparations. For trace level
10 544 analysis and safety control, dilution is not an option and other approaches have to be
11 545 considered. . Nevertheless, LODs in the range of 0.01-0.05 mg/kg were obtained for 70% of
12 546 the substances investigated. Whether this is sufficient, will depend on establishment of safe
13 547 levels for the presence of plant toxins in food, feed, and botanicals. We believe that methods
14 548 such as described here, and further improvements thereof, will contribute to gain insight in
15 549 occurrence of expected and unexpected plant toxins and, through that, to risk assessment and
16 550 setting of maximum limits for the toxins. The need for such limits and the need for more
17 551 efforts in quality control of herbal preparations are evident. This can be derived both from the
18 552 existing literature and the detection of pyrrolizidine alkaloids, aristolochic acids, strychnine
19 553 and ricinine in real samples analyzed to demonstrate the applicability of the screening
20 554 method.

21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 555 **Acknowledgements**

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37 557 financially supporting this work (project #1217272001).

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704 **Table 1. Plant toxins and other natural substances of interest or concern included in this work (a)**

Substance	Molecular formula	RT (min)	Ion (+)	Exact mass	ESI ⁺ relative abundance			system LOD (pg)	Detectability in samples			
					M+H	M+NH ₄	M+Na		silage	honey	complete pig feed	food supplement
Aconitine	C34H47NO11	8.31	[M+H]	646.3222	100			2.5	10	10	10	10
Alloclptoptine	C21H23NO5	6.56	[M+H]	370.1599	100			2.5	10	10	10	10
Aloe-emodin (/emodin)	C15H10O5	10.60	[M-H]	269.0455				12.5	20	20	20	20
Aloin A/B	C21H22O9	8.49/8.66	[M+H]	419.1337	100			25	200	200	200	200
Amentoflavone	C30H18O10	10.06	[M+H]	539.0973	100			25	50	50	50	pos
Amygdalin	C20H27NO11	5.87	[M+NH ₄]	475.1922		100	20	25	50	50	50	50
Anisatine	C15H20O8	6.40	[M+NH ₄]	346.1496		100		50	na	na	na	na
Anthrone	C14H10O	11.78	[M+]	194.0726				12.5	200	50	50	50
Antiarin alpha	C29H42O11	6.56	[M+Na]	589.2619			100	5	>	50	>	>
Arbutin	C12H16O7	8.07	[M+H]	273.0969	100			125	>	>	>	>
Arecaidine	C7H11NO2	1.82	[M+H]	142.0863	100			2.5	10	10	10	10
Arecoline	C8H13NO2	4.36	[M+H]	156.1019	100			12.5	50	50	50	50
Aristolochia acid I	C17H11NO7	9.74	[M+NH ₄]	359.0874		100	10	2.5	50	10	10	50
Aristolochia acid II	C16H9NO6	9.34	[M+NH ₄]	329.0768		100	10	2.5	50	50	50	50
Asarone alpha	C12H16O3	10.54	[M+H]	209.1172	100			12.5	>	50	200	>
Atropine	C17H23NO3	5.84	[M+H]	290.1751	100			2.5	10	10	10	10
Berberine	C20H18NO4	7.09	[M+]	336.1230				2.5	10	10	10	10
Calycanthine	C22H26N4	5.49	[M+H]	347.2230	100			2.5	50	10	50	50
Canavanine L-	C5N4H12O3	1.47	[M+H]	177.0982	100			5	50	50	200	200
Chelidonine	C20H19NO5	7.32	[M+H]	354.1336	100			2.5	10	10	10	10
Cinchonidine (/cinchonine)	C19H22N2O	6.63	[M+H]	295.1805	100			2.5	10	10	10	50
Cinchonine (/cinchonidine)	C19H22N2O	6.63	[M+H]	295.1805	100			2.5	10	10	10	50
Colchicine	C22H25NO6	8.09	[M+H]	400.1755	100		8	2.5	10	10	10	50
Coniine	C8H17N	5.45	[M+H]	128.1434	100			2.5	10	10	10	10
Convallatoxin	C29H42O10	7.94	[M+Na]	573.2670			100	2.5	>	200	200	>
Corynanthine (yohimbine)	C21H26N2O3	6.76	[M+H]	355.2016	100			2.5	10	10	10	50
Cucurbitacin I	C30H42O7	9.79	[M-H2O+H]	497.2898	100			12.5	50	50	50	200
Curcumin	C21H20O6	10.58	[M+H]	369.1333	100		10	12.5	200	50	50	50

Substance	Molecular formula	RT (min)	Ion (+)	Exact mass	ESI* relative abundance			system LOD (pg)	Detectability in samples			
					M+H	M+NH4	M+Na		silage	honey	complete pig feed	food supplement
					LOD in µg/kg (b)							
Cymarín	C30H44O9	9.05	[M+Na]	571.2878	1	1	100	2.5	>	10	>	>
Cytisine	C11H14N2O	4.40	[M+H]	191.1179	100			2.5	50	10	50	50
Digitoxigenin	C23H34O4	9.86	[M+H]	375.2530	100	20	60	2.5	50	10	10	50
Digitoxin	C41H64O13	11.34	[M+Na]	787.4239		10	100	25	na	na	na	na
Digoxin	C41H64O14	9.57	[M+H]	781.4369		20	100	25	na	na	na	na
Echimidine	C20H31NO7	6.58	[M+H]	398.2173	100			2.5	10	10	10	10
Emetine	C29H40N2O4	5.61	[M+H]	481.3061	100			12.5	50	50	50	200
Emodin (/aloe-emodin)	C15H10O5	12.53	[M-H]	269.0455				12.5	20	20	20	20
Ephedrine	C10H15NO	5.47	[M+H]	166.1226	100			2.5	int	10	10	50
Erucifoline	C18H23NO6	5.02	[M+H]	350.1598	100			2.5	50	10	10	50
Erucifoline-N-oxide	C18H23NO7	5.35	[M+H]	366.1547	100			2.5	10	10	10	10
Eserine	C15H21N3O2	5.54	[M+H]	276.1707	100			2.5	10	10	10	10
Evodiamine	C19H17N3O	10.68	[M+H]	304.1444	100			5	10	10	10	10
Forskolin	C22H34O7	10.66	[M+NH4]	428.2643	10	7	100	2.5	>	10	50	200
Geranyloxypsoralen 5-	C21H22O4	13.99	[M+H]	339.1591	100			5	50	10	50	50
Gitoxigenin	C23H34O5	8.92	[M+Na]	413.2298			100	5	>	10	200	>
Gramine	C11H14N2	5.40	[M+H]	175.1230	100			2.5	10	10	10	10
Grayanotoxin III	C20H34O6	6.95	[M-2H2O+H]	335.2211				12.5	50	50	50	50
Harmaline	C13H14N2O	6.63	[M+H]	215.1179	100			2.5	50	10	10	200
Harmine	C13H12N2O	7.00	[M+H]	213.1022	100			2.5	10	10	10	10
Heliotrine	C16H27NO5	5.89	[M+H]	314.1962	100			2.5	10	10	10	50
Histamine	C5H9N3	1.63	[M+H]	112.0869	100			12.5	>	200	>	>
Huperzine A	C15H18N2O	5.60	[M+H]	243.1492	100		10	2.5	10	50	50	50
Hydrastine beta	C21H21NO6	7.39	[M+H]	384.1442	100			2.5	50	10	10	50
Hydroxylupanine 17α-	C15H24N2O2	4.50	[M+H]	265.1911	100			50	>	>	>	>
Hydroxytryptophan 5-	C11H12N2O3	4.62	[M+H]	221.0921				125	>	>	>	>
Imperatorin	C16H14O4	10.56	[M+H]	271.0965	100	30		2.5	50	10	50	50
Iodoresiniferatoxin 5'-	C37H39IO9	13.55	[M+H]	755.1712	100		8	5	50	50	50	200
Jacobine	C18H25NO6	5.28	[M+H]	352.1755	100			2.5	10	10	10	10
Jacobine-N-oxide	C18H25NO7	5.69	[M+H]	368.1704	100			2.5	50	10	10	10
Khellin	C14H12O5	9.07	[M+H]	261.0758	100			2.5	10	10	10	10

Substance	Molecular formula	RT (min)	Ion (+)	Exact mass	ESI* relative abundance			system LOD	Detectability in samples			
					M+H	M+NH4	M+Na	(pg)	silage	honey	complete pig feed	food supplement
					LOD in µg/kg (b)							
Lapachol (/lapachone, beta)	C15H14O3	10.35	[M+H]	243.1016	100			2.5	10	10	10	10
Lapachone beta (/lapachol)	C15H14O3	10.35	[M+H]	243.1016	100			2.5	10	10	10	10
Lupanine	C15H24N2O	4.80	[M+H]	249.1961	100			2.5	10	10	10	10
Lupinine	C10H19NO	4.55	[M+H]	170.1539	100			5	10	10	10	50
Lycopsamine	C15H25NO5	5.35	[M+H]	300.1805	100			2.5	10	10	10	10
Lycorine	C16H17NO4	4.80	[M+H]	288.1230	100			2.5	50	10	50	10
Methoxypsoralen 5- (Bergapten)	C12H8O4	9.37	[M+H]	217.0495	100			2.5	10	50	50	pos
Methoxypsoralen 8-	C12H8O4	8.68	[M+H]	217.0495	100	2	2	5	10	50	50	10
Monocrotaline	C16H23NO6	4.90	[M+H]	326.1598	100			2.5	10	10	50	10
Morphine	C17H19NO3	4.68	[M+H]	286.1438	100			2.5	50	10	10	10
Nicotine	C10H14N2	4.60	[M+H]	163.1230	100			25	200	50	50	50
Norharman	C11H8N2	6.90	[M+H]	169.0760	100			2.5	pos	10	pos	10
Oleandrin	C32H48O9	10.49	[M+H]	577.3371	100	20	25	2.5	200	10	50	200
Ouabain (Strophanthin G-)	C29H44O12	6.16	[M+H]	585.2906	100		50	2.5	200	50	50	200
Parthenolide	C15H20O3	9.54	[M+NH4]	266.1751	45	100	5	2.5	10	10	10	10
Physcion	C16H12O5	14.33	[M+H]	285.0758	100			250	>	>	>	>
Picrotin	C15H18O7	6.69	[M+NH4]	328.1391	2	100		2.5	50	10	10	200
Picrotoxinin	C15H16O6	7.26	[M+NH4]	310.1285		100		5	50	10	10	50
Piperine	C17H19NO3	10.74	[M+H]	286.1438	100			2.5	10	10	pos	10
Prenylnaringenin 8-	C20H20O5	10.66	[M+H]	341.1384	100			5	50	10	10	200
Pseudopelletierine	C9H15NO	2.59	[M+H]	154.1226	100			2.5	10	10	10	10
Psoralen	C11H6O3	8.64	[M+H]	187.0390	100			2.5	10	10	10	10
Pulegone	C10H16O	10.68	[M+H]	153.1274	100			25	200	50	200	200
Pyrethrins Cinerin I	C20H28O3	13.62	[M+H]	317.2111	100			12.5	> (int)	50	200	200
Pyrethrins Cinerin II	C21H28O5	11.89	[M+H]	361.2010	100			25	>	50	200	>
Pyrethrins Jasmolin I	C21H30O3	14.53	[M+H]	331.2268	100			25	>	200	200	200
Pyrethrins Jasmolin II	C22H30O5	10.66	[M+H]	375.2166	100			5	200	10	50	200
Pyrethrins Pyrethrin I	C21H28O3	13.63	[M+H]	329.2111	100			12.5	200	50	200	200
Pyrethrins Pyrethrin II	C22H28O5	11.95	[M+H]	373.2010	100			12.5	>	50	50	200
Quercetin	C15H10O7	8.71	[M+H]	303.0499	100			25	pos	pos	10	pos
Quercitrin	C21H20O11	7.86	[M+H]	449.1078	100		40	50	int	10	10	pos

Substance	Molecular formula	RT (min)	Ion (+)	Exact mass	ESI* relative abundance			system LOD (pg)	Detectability in samples			
					M+H	M+NH4	M+Na		silage	honey	complete pig feed	food supplement
					LOD in µg/kg (b)							
Quinidine	C20H24N2O2	7.09	[M+H]	325.1911	100			5	20	20	20	20
Quinine	C20H24N2O2	7.09	[M+H]	325.1911	100			5	20	20	20	20
Retrorsine	C18H25NO6	5.61	[M+H]	352.1755	100			2.5	10	10	10	50
Retrorsine-N-oxide	C18H25NO7	6.15	[M+H]	368.1704	100			2.5	10	10	10	50
Ricinine	C8H8N2O2	5.49	[M+H]	165.0659	100			2.5	50	10	10	50
Rotenone	C23H22O6	11.05	[M+H]	395.1489	100		15	2.5	10	10	10	50
Rutaecarpine	C18H13N3O	11.44	[M+H]	288.1131	100			2.5	10	10	10	10
Rutin	C27H30O16	7.35	[M+H]	611.1607	100			50	int	50	50	pos
Sanguinarine	C20H14NO4	10.91	[M+]	332.0917				5	10	10	10	10
Santonin	C15H18O3	8.41	[M+H]	247.1329	100			2.5	50	10	10	50
Scopolamine	C17H21NO4	5.47	[M+H]	304.1543	100			2.5	50	10	10	10
Scopoletin	C10H8O4	7.00	[M+H]	193.0495	100		4	2.5	pos	10	10	pos
Senecionine	C18H25NO5	6.28	[M+H]	336.1805	100			2.5	10	10	10	10
Senecionine-N-oxide	C18H25NO6	6.96	[M+H]	352.1755	100			2.5	50	10	10	50
Seneciophylline	C18H23NO5	5.83	[M+H]	334.1649	100			2.5	10	10	10	50
Seneciophylline-N-oxide	C18H23NO6	6.42	[M+H]	350.1598	100			2.5	10	10	10	50
Senkirkine	C19H27NO6	6.70	[M+H]	366.1911	100			2.5	10	10	10	50
Solanine alpha	C45H73NO15	8.08	[M+H]	868.5053	100			12.5	200	50	50	pos
Sparteine	C15H26N2	6.29	[M+H]	235.2169	100			2.5	10	10	10	10
Strophanthidin	C23H32O6	8.04	[M+Na]	427.2091	60	15	100	2.5	>	50	200	200
Strychnine	C21H22N2O2	5.77	[M+H]	335.1754	100			2.5	10	10	10	50
Synephrine	C9H13NO2	2.60	[M+H]	168.1019	100			2.5	pos	10	10	10
Tetrahydrocannabinol (THC)	C21H30O2	15.11	[M+H]	315.2319	100			25	50	10	50	50
Tetrandrine	C38H42N2O6	6.63	[M+H]	623.3116	100			5	50	10	50	200
Thapsigargin	C34H50O12	13.68	[M+NH4]	668.3641		100	16	2.5	50	50	50	50
Theobromine	C7H8N4O2	5.30	[M+H]	181.0720	100			25	>	200	200	200
Theophylline	C7H8N4O2	5.89	[M+H]	181.0720	100			50	>	50	50	200
Tinyatoxin	C36H38O8	12.66	[M+H]	599.2639		100	55	2.5	50	10	50	50
Trigonelline	C7H7NO2	1.85	[M+H]	138.0550	100			2.5	pos	10	pos	pos
Tropine	C8H15NO	2.04	[M+H]	142.1226	100			2.5	pos	10	10	10
Tryptamine	C10H12N2	5.61	[M+H]	161.1073	100			2.5	pos	10	pos	pos

Substance	Molecular formula	RT (min)	Ion (+)	Exact mass	ESI* relative abundance			system	Detectability in samples			
					M+H	M+NH4	M+Na	LOD	silage	honey	complete pig feed	food supplement
					LOD in µg/kg (b)							
Tubocurarine	C37H42N2O6	5.20	[M+H](2+)	305.1516				5	50	10	10	50
Umbelliferone	C9H6O3	7.14	[M+H]	163.0390	100	2		25	50	50	50	50
Vinblastine	C46H58N4O9	9.07	[M+H]	811.4277	100			5	50	50	50	50
Vindoline	C25H32N2O6	9.96	[M+H]	457.2333	100			2.5	10	10	10	10
Visnagin	C13H10O4	9.32	[M+H]	231.0652	100		5	2.5	10	10	10	10
Withaferin A	C28H38O6	9.61	[M+H]	471.2741	100	50	25	12.5	200	50	50	50
Yohimbine (/corynanthine)	C21H26N2O3	6.76	[M+H]	355.2016	100			2.5	10	10	10	50

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706 (a) substances which were tested but for which no (consistent) MS response was obtained are listed in the Supplemental Information S3.

707 (b) extract 0.125 g/ml, 5 µl injection

708 int = partial co-elution with interference

709 pos = peak at retention time of analyte

710 > = LOD > 200 µg/kg

711 na = not analysed

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3 714 **Table 2.**
4 715 **Matrix-induced ion suppression for plant toxins in crude extracts of various matrices.**
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Sample	Extract (b)	MS response in extract relative to solv. std			
		>50%	33-50%	20-33%	<20%
		% of substances (c)			
food supplement (a)	0.125 g/ml	13	16	32	39
	0.025 g/ml	35	28	33	5
honey	0.125 g/ml	89	7	2	2
	0.025 g/ml	94	4	2	0
silage	0.125 g/ml	16	40	27	17
	0.025 g/ml	40	39	18	3
complete pig feed	0.125 g/ml	64	22	9	5
	0.025 g/ml	68	25	6	0

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20 (a) 'blood purifier' sample details see Supplemental Information Table S2

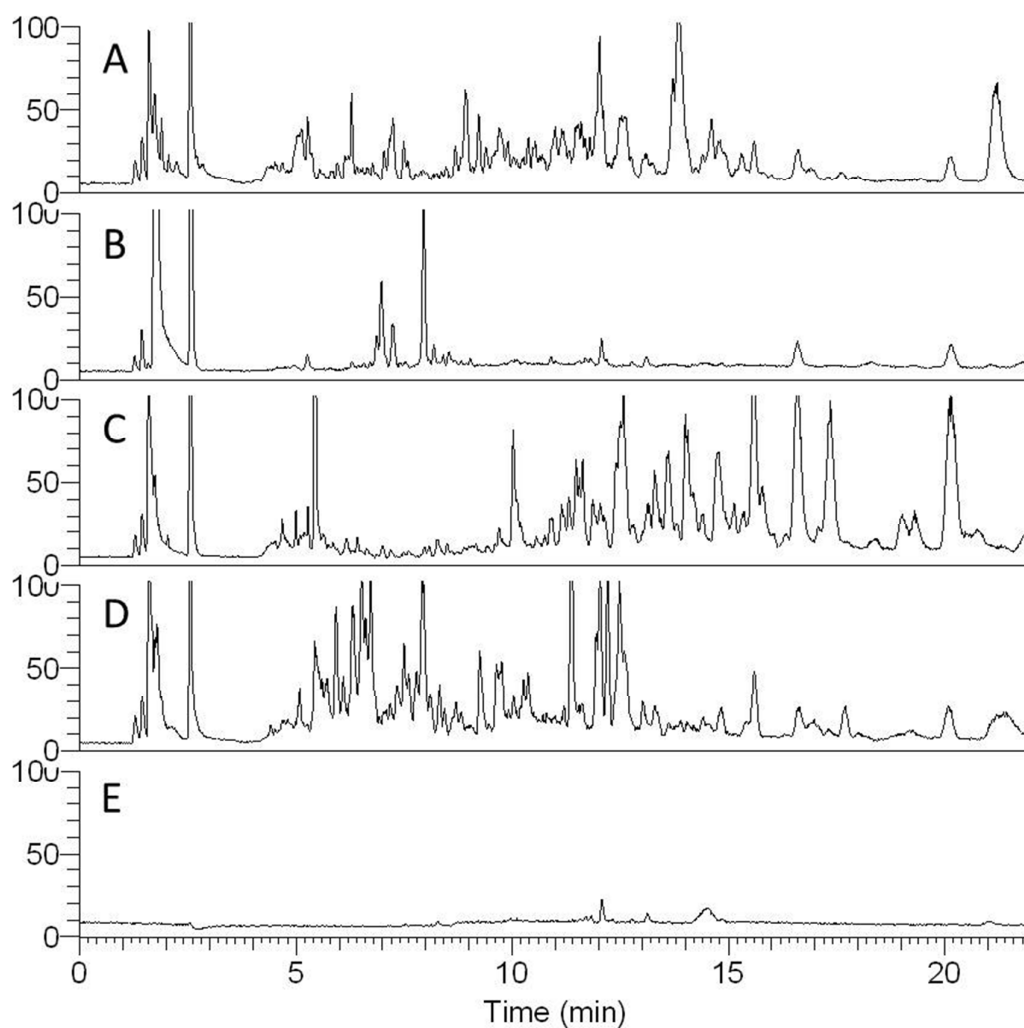
21 (b) g matrix equivalent per ml of final extract

22 (c) N= 98-122 substances

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718 **Figure 1.**

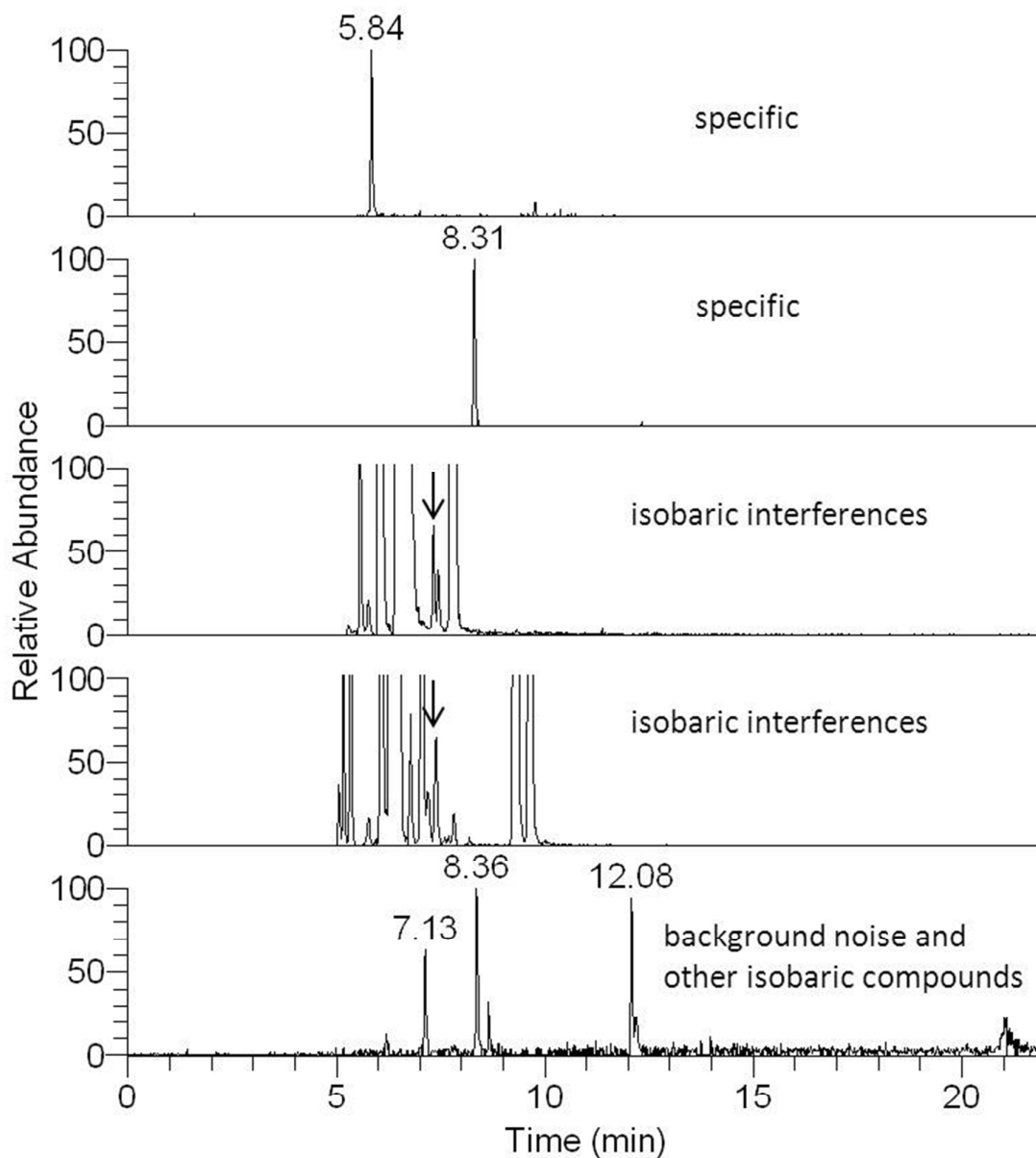
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721 Figure 1. Total ion current chromatograms (TIC, m/z 55-1,000) obtained after LC-full scan
722 MS analysis of crude extracts (0.125 g/ml extracts, 5 μ l injection) of four matrices relevant in
723 plant-toxin analysis. The scaling has been fixed to allow comparison of complexity. A)
724 silage, B) honey, C) compound feed (complete pig feed), D) food supplement (mixture of
725 dried aromatic plants 'blood purifier'), E) blank.

726

727 **Figure 2.**

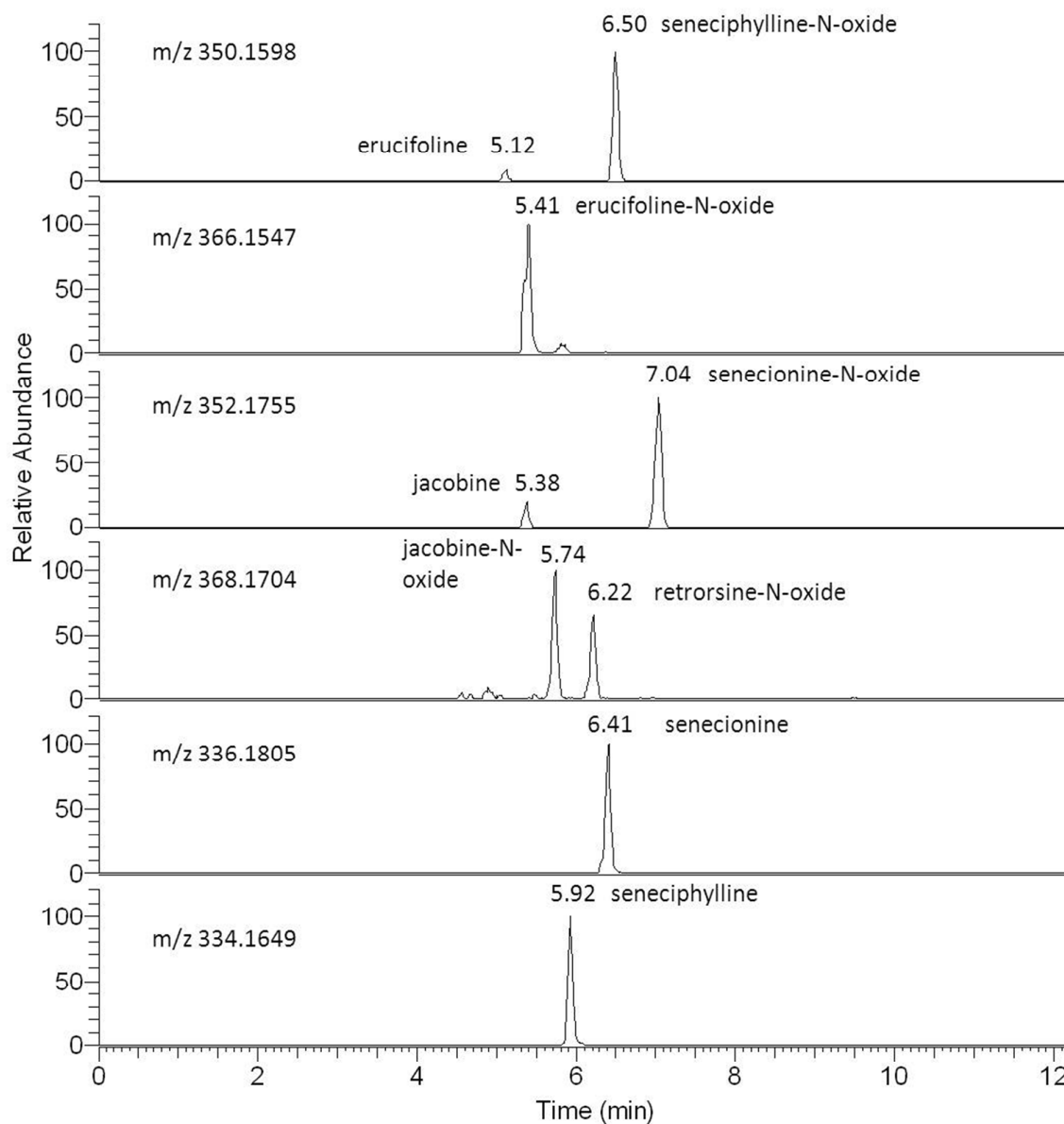
728

729 Figure 2. Example of extracted ion chromatograms of plant toxins (exact mass ± 5 ppm) in
 730 spiked crude extracts of various matrices. A) atropine (5.84 min, m/z 290.1751) in silage at
 731 the level of 0.05 mg/kg; B) aconitine (8.31 min, m/z 646.3222) in a herbal food supplement,
 732 0.05 mg/kg; C) chelidonine (7.32 min, m/z 354.1336) in herbal food supplement, 0.05 mg/kg;
 733 D) hydrastine (7.39 min, m/z 384.1442) in herbal food supplement, 0.05 mg/kg, E)
 734 umbelliferone (7.13 min, m/z 163.0390) in silage, 0.20 mg/kg.

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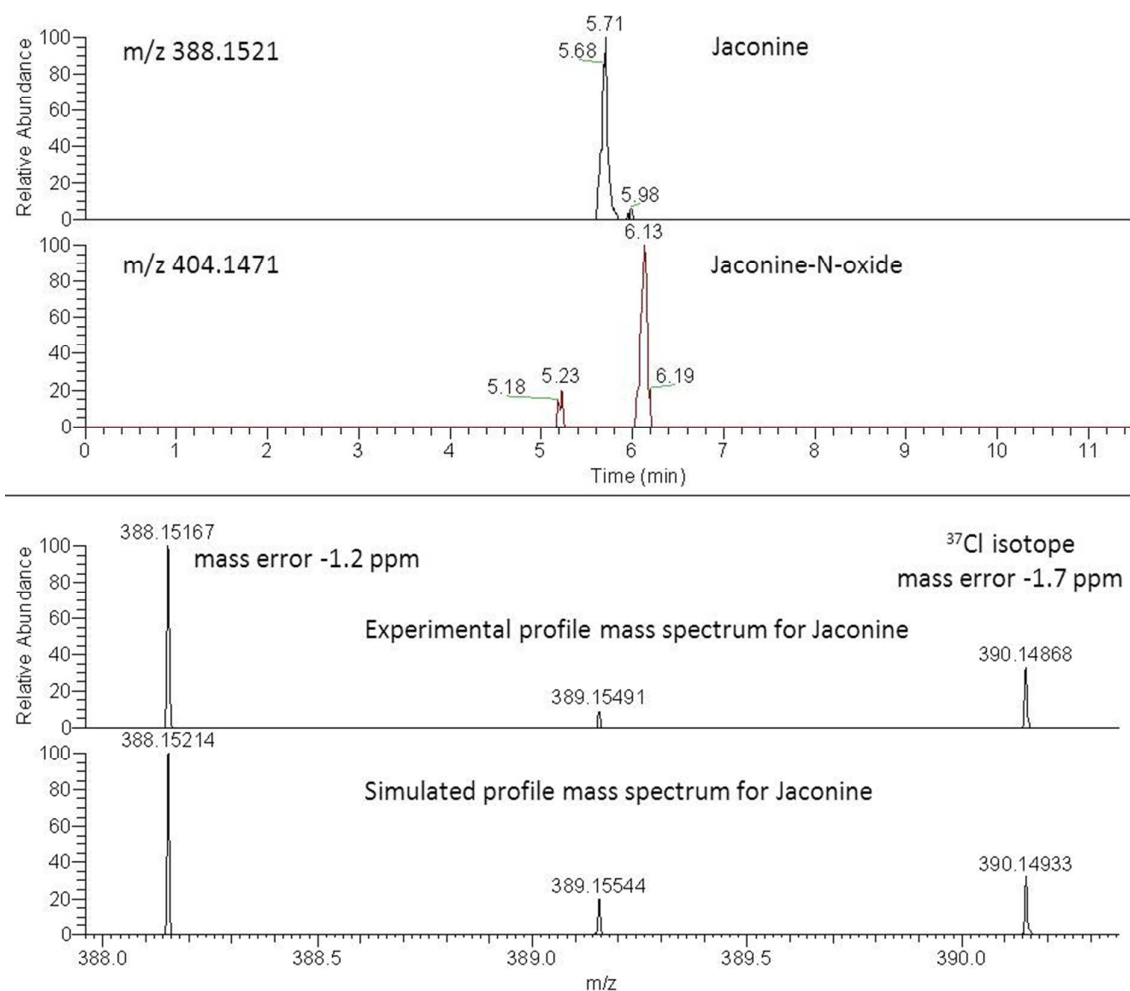
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740 **Figure 3a.**

741

742 Figure 3a. XICs of a honey sample contaminated with pyrrolizidine alkaloids. Estimated
743 levels range from 0.05 to 0.6 mg/kg (see Supplemental Information Table S4).

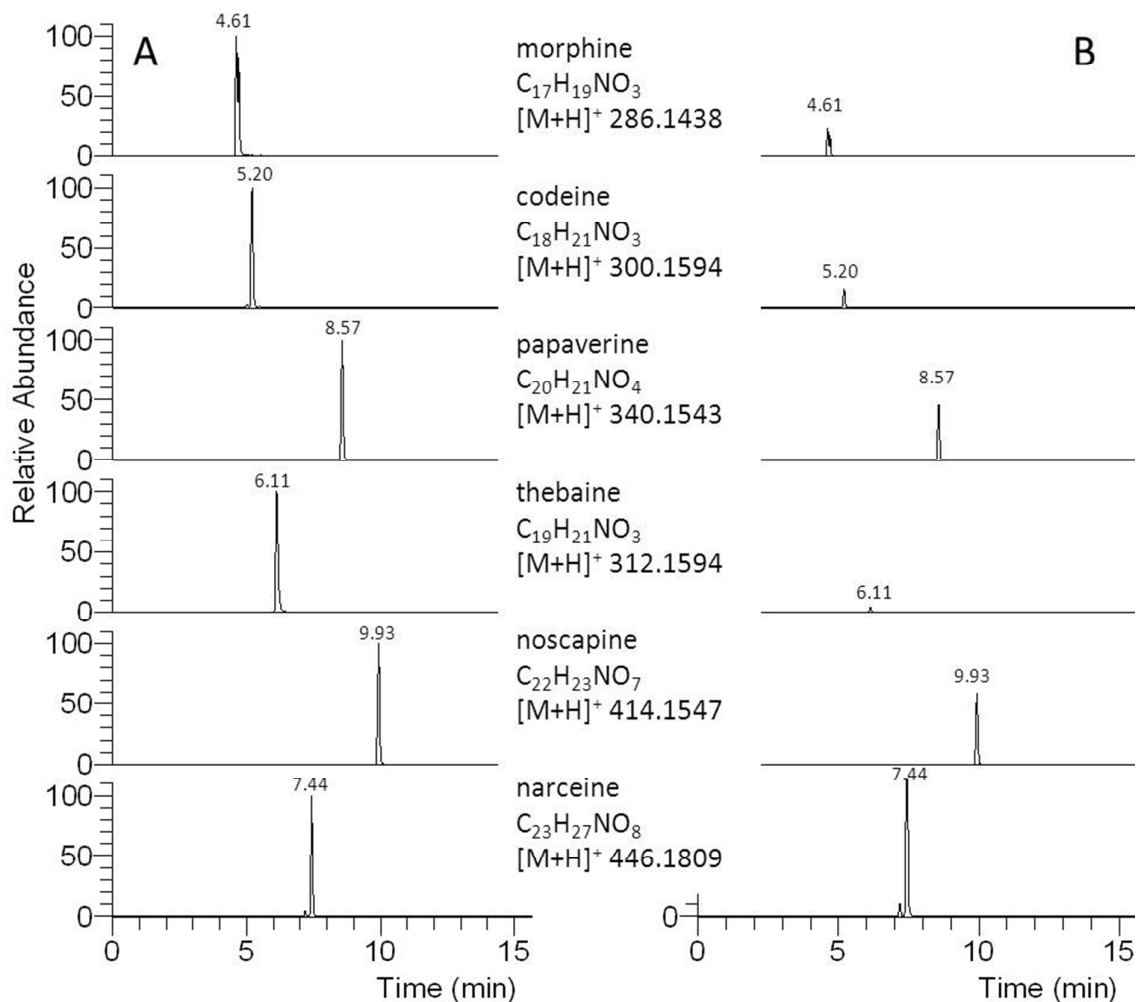
744

745 **Figure 3b.**

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747 Figure 3b. Upper two traces: XIC for jaconine ($C_{18}H_{26}ClO_6$ as $[M+H]^+$) and its N-oxide,
748 present at low levels (< 0.05 mg/kg) in honey. Lower two traces: experimental and theoretical
749 mass spectrum of jaconine

750

751 **Figure 4.**

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753 Figure 4. XICs of opium alkaloids after analysis of poppy seeds purchased as food ingredient.

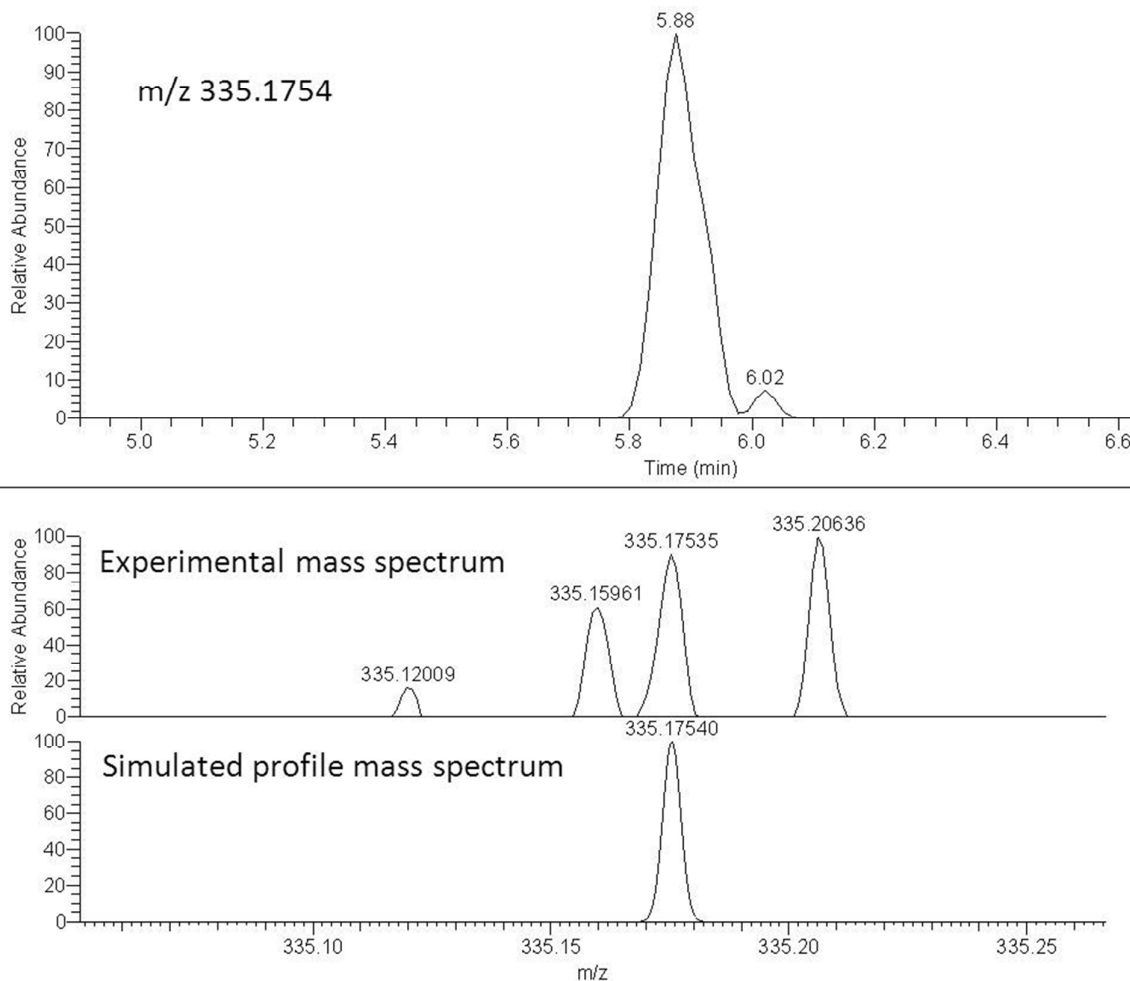
754 A) white poppy seeds, B) blue poppy seeds. Extracts were 10-fold diluted before analysis. For

755 each alkaloid, the Y-axis has been fixed to allow direct comparison of the levels in the two

756 samples.

757 **Figure5.**

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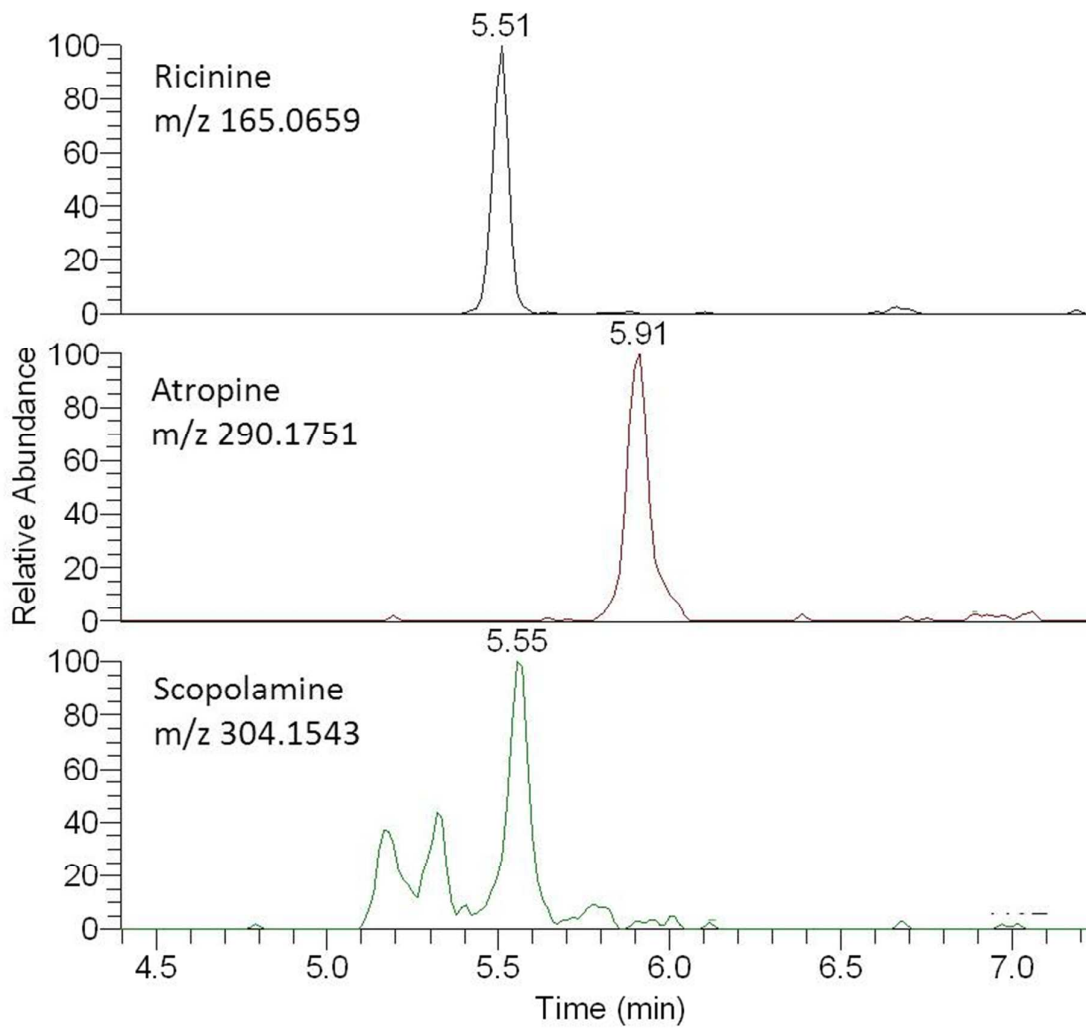


759

760 Figure 5. Top: XIC of strychnine (0.02 mg/kg) in a food supplement ('testosterone booster').

761 Bottom: experimental and theoretical mass spectrum.

762

763 **Figure 6.**

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766 Figure 6. Detection of ricinine (alkaloid marker for ricin), atropine and scopolamine which
767 were spiked to a silage sample at 0.05 mg/kg.

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SUPPLEMENTAL INFORMATION to the paper entitled:**Screening of plant toxins in food, feed and botanicals using full scan high resolution (Orbitrap) mass spectrometry**

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This document provides more detailed information to the main paper mentioned above.
The following information is included:

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Table S2	Sample details	4
Table S3	Additional MS details for plant toxins included in the evaluation	5
Table S4	Application of the LC-Orbitrap MS screening method to food and feed samples. Analysis results.	6
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Figure S2	XICs of pyrrolizidine alkaloids spiked to a silage	10

Table S1.
Overview of EU maximum levels for plant toxins or plant material in food and feed
(June 2011)

Plant toxin or plant species	product	maximum concentration
EU directive 2002/32/EC (undesirable substances in animal feed)		
hydrocyanic acid	various feed ingredients and complete feed	10-250 mg/kg*
free gossypol		20-5000 mg/kg*
theobromine		50-300 mg/kg*
volatile mustard oil		100-4000 mg/kg* expressed as allyl isothiocyanate
5-vinylloxazolidine-2-thione		500-1000 mg/kg*
Weed seeds and unground and uncrushed fruits containing alkaloids, glucosides or other toxic substances separately or in combination including:	all feeding stuffs	3000 mg/kg*
- <i>Datura</i> sp.	all feeding stuffs	1000 mg/kg*
- Seeds and husks from <i>Ricinus communis</i> L., <i>Croton tiglium</i> L. and <i>Abrus precatorius</i> L. as well as their processed derivatives (20), separately or in combination		10 mg/kg*
- <i>Crotalaria</i> spp.	all feeding stuffs	100 mg/kg*
- Unhusked beech mast — <i>Fagus silvatica</i> L. - <i>Purghera</i> — <i>Jatropha curcas</i> L. - Indian mustard — <i>Brassica juncea</i> (L.) Czern. And Coss. ssp. <i>Intergrifolia</i> (West.) Thell. - Sareptian mustard — <i>Brassica juncea</i> (L.) Czern. And Coss. ssp. <i>juncea</i> - Chinese mustard — <i>Brassica juncea</i> (L.) Czern. And Coss. ssp. <i>Juncea</i> var. <i>lutea</i> Batalin - Black mustard — <i>Brassica nigra</i> (L.) Koch - Ethiopian mustard — <i>Brassica carinata</i> A. Braun	all feeding stuffs	Seeds and fruit of the plant species listed opposite as well as their processed derivatives may only be present in feedingstuffs in trace amounts not quantitatively determinable
* relative to a feed with a moisture content of 12%. The maximum concentration depends on feed product and animal species		
EU regulation 396/2005 (food and feed of plant and animal origin)		
azadirachtin	fruits, vegetables, animal products	0.01-1 mg/kg
nicotin	mush rooms	0.04-2.3 mg/kg
pyrethrins (sum)	fruits, vegetables, animal products	0.05-3 mg/kg
rotenone	fruits, vegetables, animal products	0.01-0.02 mg/kg

EU regulation 1334/2008 (flavorings/food ingredients)		
beta-asarone	Alcoholic beverages	1 mg/kg
estagole	dairy products, various foods	10-50 mg/kg
hydrocyanic acid	various food products	5-50 mg/kg
menthofuran	confectionary/ beverages	200-3000 mg/kg
methyleugenol	dairy products, various foods/beverages	1-60 mg/kg
pulgone	confectionary/ beverages	20-2000 mg/kg
quassin	beverages, bakery wares	0.5-1.5 mg/kg
safrole	meat preparations, fish, soups, beverages	1-25 mg/kg
teucrin A	Alcoholic beverages	2-5 mg/kg
thujone (alpha/beta)	beverages	0.5-35 mg/kg
coumarin	bakery ware, breakfast cereals, desserts	5-50 mg/kg
EU regulation 37/2010 and amendments (foodstuffs of animal origin)		
<i>Aristolochia spp.</i> and preparations thereof	foodstuffs of animal origin	prohibited substance (MRL cannot be established)
Isoeugenol	fin fish	6 mg/kg

Table S2. Sample details

Category	Product name	Ingredients according to label specification	
		EN	Latin
food	honey	honey (NL, transfer study)	
food	honey	honey (NL)	
food	honey	honey (Nepal, intoxication)	
food	honey	honey ('Australian Honey')	
food	Hemp spaghetti	wheat flour hemp germ flour	<i>Triticum spp</i> <i>Cannabis</i>
food	'sterrenmix' (herbal tea) + Japanese star anise (10%)	chinese star anise, other herbs japanese star anise	<i>Illicium verum</i> <i>Illicium anisatum</i>
food ingredient	poppy seeds (blue)	poppy seeds	<i>Papaver somniferum</i>
food ingredient	poppy seeds khus khus	poppy seeds	<i>Papaver somniferum</i>
food supplement	'bloedzuiver' ('blood purifier')	elder (blossom) nettle plantains smilax nut tree (leaves) chicory anis common juniper fumewort	<i>Sambucus spp. (blossom)</i> <i>Urtica spp.</i> <i>Plantago</i> <i>Smilax spp</i> <i>Juglans</i> <i>Cichorium intybus</i> <i>Pimpinella anisum</i> <i>Juniperus communis</i> <i>Fumaria</i>
food supplement	Pau d'Arco immuunbast	pink lapacho	<i>Tabebuia impetiginosa</i>
food supplement	blaas en urine kruiden (('bladder/urine herbs'))	golden rod common juniper meadowsweet lovage bearberry	<i>Solidago virgaurea</i> <i>Juniperus communis</i> <i>Filipendula ulmaria</i> <i>Levisticum officinale</i> <i>Arctostaphylos uva-ursi</i>
food supplement	stoelgang plus (('stool plus'))	casacara buckthorn psyllium (seed husks) aloë vera	<i>Rhamnus purshiana</i> <i>Plantago ovata</i> <i>Aloë Vera</i>
food supplement	darmbalans ('gut balance')	aloë vera casacara buckthorn	<i>Aloë Vera</i> <i>Rhamnus purshiana</i>
food supplement	Testosterone booster	unknown	<i>unknown</i>
TCM	Chuan Xiong Cha Tiao Wan	chinese privet (root) wild angelica (root) nut grass (root) field mint japanese catmint liquorice	<i>Ligustrum sinense (root)</i> <i>Angelica dahurica (root)</i> <i>Cyperus rotundus (root)</i> <i>Puerariae</i> <i>Ledebouriella divaricata</i> <i>Mentha arvensis</i> <i>Nepeta subsessilis</i> <i>Glycyrrhiza glabra</i>
TCM	Chuan Wu	carmichael's monkshood	<i>Aconitum carmichaeli</i>
TCM	Kuan Dong Hua	coltsfoot	<i>Tussilago farfara</i>
feed	complete pig feed (NL)	unknown	
feed	silage (NL)	grass	
feed ingredient	'parsley' by-product	parsley	<i>Petroselinum spp.</i>

TCM = Traditional Chinese Medicine

Table S3. Additional MS details for plant toxins included in the evaluation

Substance	Molecular formula	RT (min)	Ion	Exact mass
Substances for which besides ESI+ also a response in ESI- was observed				
Aloin	C ₂₁ H ₂₂ O ₉	8.49/8.66	[M-H]	417.1191
Amygdalin	C ₂₀ H ₂₇ NO ₁₁	5.87	[M-H]	456.1511
Anisatine	C ₁₅ H ₂₀ O ₈	6.40	[M-H]	327.1085
Curcumin	C ₂₁ H ₂₀ O ₆	10.58	[M-H]	367.1187
Digitoxin	C ₄₁ H ₆₄ O ₁₃	11.34	[M+HCOOH-H]	809.4329
Digoxin	C ₄₁ H ₆₄ O ₁₄	9.57	[M+HCOOH-H]	825.4278
Evodiamine	C ₁₉ H ₁₇ N ₃ O	10.68	[M-H]	302.1299
Forskolin	C ₂₂ H ₃₄ O ₇	10.66	[M-H]	409.2232
Grayanotoxin III	C ₂₀ H ₃₄ O ₆	6.95	[M-H]	369.2283
Lapachol	C ₁₅ H ₁₄ O ₃	10.35	[M-H]	241.0870
Physcion	C ₁₆ H ₁₂ O ₅	14.33	[M-H]	283.0612
Picrotin	C ₁₅ H ₁₈ O ₇	6.69	[M-H]	309.0980
Picrotoxinin	C ₁₅ H ₁₆ O ₆	7.26	[M-H]	291.0874
Quercetin	C ₁₅ H ₁₀ O ₇	8.71	[M-H]	301.0354
Quercitrin	C ₂₁ H ₂₀ O ₁₁	7.86	[M-H]	447.0933
Rutin	C ₂₇ H ₃₀ O ₁₆	7.35	[M-H]	609.1461
Scopoletin	C ₁₀ H ₈ O ₄	7.00	[M-H]	191.0350
Substances for which response was inconsistent and/or issues related to multi-analyte standard or stability				
Chrysophanic acid	C ₁₅ H ₁₀ O ₄	13.16	[M-H]	253.0506
Glycyrrhizic acid	C ₄₂ H ₆₂ O ₁₆	10.06	[M-H]	821.3965
Methyllycaconitine	C ₃₇ H ₅₀ N ₂ O ₁₀	7.07	[M+H]	683.3538
Oenin (cyclamin)	C ₂₃ H ₂₅ O ₁₂	6.00	[M+]	493.1341
Vincristine	C ₄₆ H ₅₆ N ₄ O ₁₀	10.42	[M+H]	825.4069
Substances for which no response was obtained under the applied generic conditions				
Coumaric acid p-	C ₉ H ₈ O ₃		no response	
Epigallocatechin gallate	C ₂₂ H ₁₈ O ₁₁		no response	
Eucalyptol	C ₁₀ H ₁₈ O		no response	
Gossypol	C ₃₀ H ₃₀ O ₈		no response	
Hydroxycitric acid (HCA)	C ₆ H ₈ O ₈		no response	
Limonene	C ₁₀ H ₁₆		no response	
Menthofuran	C ₁₀ H ₁₄ O		no response	
Methyl eugenol	C ₁₁ H ₁₄ O ₂		no response	
Methyl salicylate	C ₈ H ₈ O ₃		no response	
Myristicin	C ₁₁ H ₁₂ O ₃		no response	
Safrole	C ₁₀ H ₁₀ O ₂		no response	
Sarsasapogenin	C ₂₇ H ₄₄ O ₃		no response	
Sennoside B	C ₄₂ H ₃₈ O ₂₀		no response	
Strophanthin K-	C ₃₆ H ₅₄ O ₁₄		no response	
Terpineol	C ₁₀ H ₁₈ O		no response	
Thujone	C ₁₀ H ₁₆ O		no response	
Thymol	C ₁₀ H ₁₄ O		no response	

Table S4. Application of the LC-Orbitrap MS screening method to food and feed samples. Analysis results.

	food							Food supplements						TCM			Feed/ingredients			
	honey (NL, transfer study a)	honey (NL)	honey (Nepal, intoxication)	honey ('Australian Honey')	Hemp Spaghetti	Sterrenmix (herbal tea) + Japanese staranise (10%)	Poppy seeds (blue)	Poppy seeds khus khus	bloedzuiver ('blood purifier')	Pau d'Arco	Blaas en urinekruiden ('bladder/urine herbs')	Stoelgang plus ('stool plus')	Darmbalans ('gut balance')	Testosterone booster	Chuan Xiong Cha Tiao Wan	Chuan Wu	Kuan Dong Hua	Complete pig feed (NL)	silage (NL)	parsley' by-product
Aconitine															0.1					
Alloctyptopine							+	+												
Aloe-emodin/emodin									+		+	+			+	+				
Aloin											+	+								
Amentoflavone						+		+		+										
Amygdalin						+									+					
Anisatine						+														
Arbutin																				+
Aristolochic acid I															0.3					
Berberine							0.03				1				0.5					
Chelidionine																				
Curcumin						+								+						
Digitoxigenin																				+
Digitoxin																				180
Digoxin																				1700
Ephedrine																				X
Erucifoline	0.05																			
Erucifoline-N-oxide	0.18																			
Geranyloxypsoralen 5-															0.5					0.04
Granyanotoxin III																				
Harmaline															0.06					
Harmine															+					
Hydrastine beta																				X
Imperatorin															9					0.4
Jacobine	0.16																			

Supplemental information

6

	food								Food supplements					TCM			Feed/ingredients			
	honey (NL, transfer study a)	honey (NL)	honey (Nepal, intoxication)	honey (Australian Honey)	Hemp Spaghetti	Sterrenmix (herbal tea) + Japanese staranise (10%)	Poppy seeds (blue)	Poppy seeds khus khus	bloedzuiver ('blood purifier')	Pau d'Arco	Blaas en urinekuiden ('bladder/urine herbs')	Stoelgang plus ('stool plus')	Darmbalans ('gut balance')	Testosterone booster	Chuan Xiong Cha Tiao Wan	Chuan Wu	Kuan Dong Hua	Complete pig feed (NL)	silage (NL)	parsley' by-product
Jacobine-N-oxide	0.07									+										
Lapachol/lapachone beta									+											
Lycopsamine		+		+																
Methoxypsoralen 5-(bergapten)						+		+		+	+			+						+
Methoxypsoralen 8-								+						+						+
Monocrotaline																				X
Morphine						8	20													
Norharmane										+				+	+	+	+	+	+	+
Oleandrin																				+
Parthenolide										+										
Physcion											+	+								
Piperine	0.02	< 0.01	< 0.01			0.03								X				+		0.1
Preynlaringenin 8-														+					+	
Psoralen						+				+				+						+
Pulegone						+								+						
Pyrethrins Cinerin I																	X			
Pyrethrins Jasmolin I																	X			
Quercetin	+	+	+	+	+	+		+		+				+	+	+	+	+	+	+
Quercitrin			+			+		+		+	+					+		+	+	+
Quinine											+									
Retrorsine	+																			
Retrorsine-N-oxide	0.05																			
Ricinine						0.07						0.14			0.01 (a)					
Rutin						+		+		+	+					+		+	+	
Sanquinarine							+													
Scopoletin	+				+	+		+		+				+	+	+	+	+	+	+

Supplemental information

	food								Food supplements					TCM			Feed/ingredients			
	honey (NL, transfer study a)	honey (NL)	honey (Nepal, intoxication)	honey (Australian Honey)	Hemp Spaghetti	Sterrenmix (herbal tea) + Japanese staranise (10%)	Poppy seeds (blue)	Poppy seeds khus khus	bloedzuiver ('blood purifier')	Pau d'Arco	Blaas en urinekruiden ('bladder/urine herbs')	Stoelgang plus ('stool plus')	Darmbalans ('gut balance')	Testosterone booster	Chuan Xiong Cha Tiao Wan	Chuan Wu	Kuan Dong Hua	Complete pig feed (NL)	silage (NL)	parsley' by-product
Senecionine	0.12																1.4			
Senecionine-N-oxide	0.57																1.7			
Seneciophylline	0.11																			0.02
Seneciophylline-N-oxide	0.31																			
Senkirkine																	60			
Solanine alpha								+												
Strychnine													0.02							
Synephrine													+	+	+					
Tetrahydrocannabinol					+															
Trigonelline					+	+	+	+	+	+			+++	+					+	
Tropine															X				0.03	
Tryptamine					+	+	+	+	+	+					+				+	+
Umbelliferone										+				+	+					

+ : peak detected; retention time $\leq \pm 0.1$ min, accurate mass $\leq \pm 5$ ppm

Green cell : identity confirmed by LC-MS/MS (2 transitions); number is estimated concentration in mg/kg (based on solvent standard, one-point calibration, and assuming 100% recovery)

Red cell (X) : peak detected by full scan LC-HRMS screening but not confirmed by LC-MS/MS

(a) : additionally found during LC-MS/MS confirmatory measurement

Figure S1.

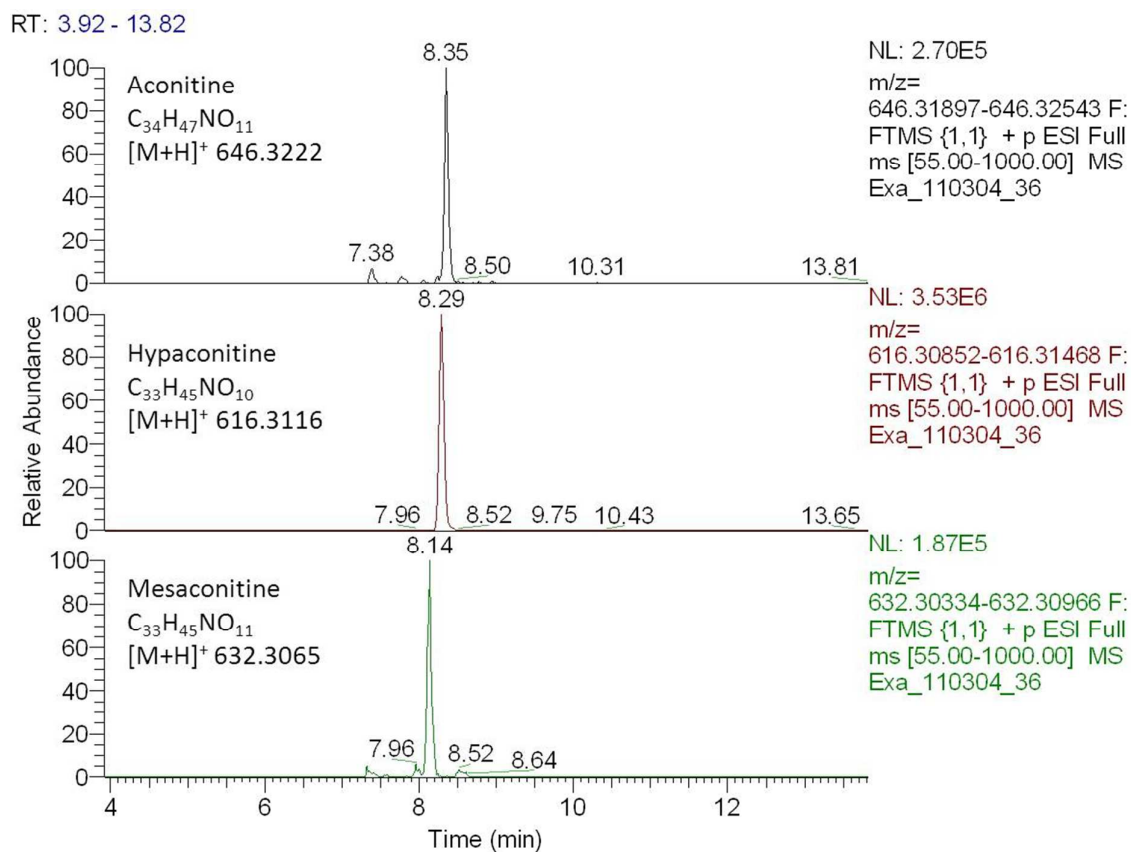


Figure S1. XICs of aconitine-alkaloids in the TCM Chuan Wu (*Aconitum carmichaeli*).
Estimated level of aconitine is 0.1 mg/kg.

Figure S2.

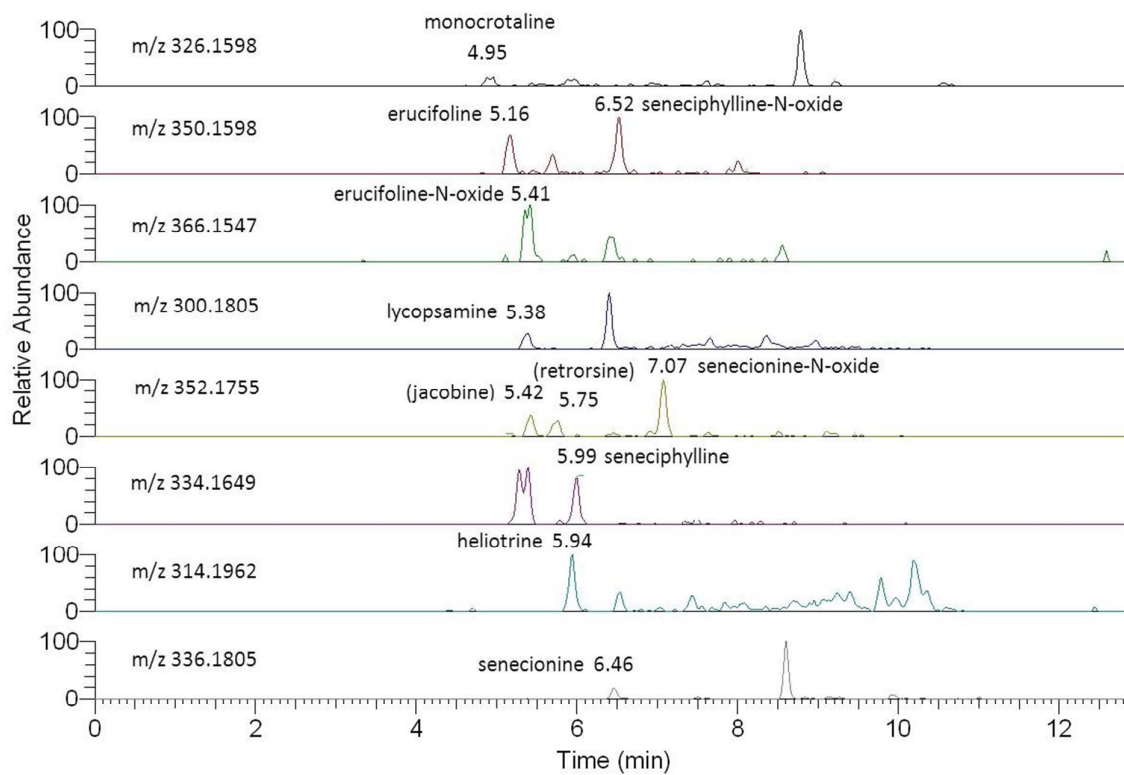


Figure S2. XICs of pyrrolizidine alkaloids spiked to a silage samples at 0.05 mg/kg.