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

Hans Mol, Ruud Cj van Dam, Paul Zomer, Patrick P J Mulder

S

nts - alkaloids

plements, Honey

ith full scan high resolution (Orbitrap)
ted for the simultaneous detection of a
ariety of food and feed matrices. For a
resenting various chemical classes, the
d LC-MS conditions. Ion suppression
uated using generic extracts from
rices (food supplement, honey, silage,

could be measured as positive ions
power of 100,000 FWHM, a reliable high
spite 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
rsely affected the sensitivity.
f the substances the detection limits were
. Since non-selective sample preparation
n is performed, the presence of plant
uring data review, can be investigated

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

Hans G.J. Mol[*)], Ruud C.J. van Dam, Paul Zomer, Patrick P.J. Mulder

RIKILT Institute of Food Safety, Wageningen University and Research Centre, Akkermaalsbos 2, 6708 WB, Wageningen, The Netherlands

[*)] Corresponding author. E-mail address: hans.mol@wur.nl, tel.: +31 317 480 318

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

Introduction

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

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

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

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

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

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

At this moment, there is a lack of routine methods for determination of plant toxins. When methods are available, they are usually dedicated to a specific (group of) substance(s) in a certain commodity. With the above analytical challenge in mind, the availability of a generic method suited for a wide variety of plant toxin/matrix combinations would be highly beneficial. The high potential of LC-MS for detection of plant toxins was early recognized (Verpoorte and Niessen 1994), and today many methods based on LC-MS/MS have been reported (e.g. Holstege et al 2001, Josephs et al 2010, McIlhenny et al 2009, Kuo et al 2010,

Sproll et al 2006, Ye et al 2007, Zhou et al 2010). Full scan high resolution TOF-MS has also been applied (e.g. Li et al 2010, Yan et al 2010, Zhang et al 2009, Zhou et al 2008, Zhou et al 2009) which is especially interesting, because it allows searching for substances for which no reference standard is available which is an issue in the field of plant toxin analysis. It also enables the analyst to retrospectively re-evaluate the raw data when new toxins become known. Given the fact that over 200,000 secondary plant metabolites exist (Hartmann 2007) and that all these substances are primarily composed of the elements C, H, N and O, selectivity requirements in the detection of plant toxins in complex matrices are high. In LC with full scan MS this means that a high mass resolving power is needed to separate co-eluting compounds with similar exact masses.

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

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

Materials and methods

Chemicals and reagents

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

PRISNA (Leiden, The Netherlands). The pyrrolizidine metabolites retrorsine-N-oxide, senecionine-N-oxide, and seneciphylline-N-oxide, jacobine-N-oxide and erucifoline-N-oxide were prepared by N-oxidation of the corresponding alkaloid with 30% hydrogen peroxide in ethanol according to the method described by (Chou et al. 2003). Other reference standards were obtained from Sigma Aldrich (Zwijndrecht, The Netherlands). From the pure standards, a stock solution of 100-2000 µg/mL was prepared in methanol. The different stock solutions were combined into mixed standard solutions of 1 µg/ml in methanol. The solutions were stored at 2-10° C until use.

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

Samples and pretreatment

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

Sample preparation

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

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

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

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

Instrumentation

HPLC-Orbitrap MS

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

The LC mobile phases were water (A) and methanol:water 95:5 (B) both containing 2 mM ammonium formate and 0.5 mM formic acid (pH 5). The first minute of LC gradient was isocratic at 100% A, then a linear gradient to 55% B after 3 min and a linear gradient to 100% B after 9 min. For complete elution of all matrix compounds, the final composition was held for 11 min. In 1 min the initial conditions were restored and then equilibrated for 4.5 min before the next injection. The LC flow rate was 300 µl/min. The temperature of the column oven was 35 °C.

The electrospray source was operated in positive and negative mode, using the following parameters: electrospray voltage 2.5 kV; sheath gas 30 arbitrary units; auxiliary gas 10 arbitrary units; sweep gas 5 arbitrary units. The heater in the source was set at 300 °C and the heated capillary in the mass spectrometer was operated at 300 °C (positive mode) or 360°C (negative mode).

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

Results and discussion

Sensitivity of full scan Orbitrap-MS for detection of plant toxins

Plant toxins belong to a wide variety of chemical classes, including various types of alkaloids, terpenes and glycosides. The number of substances is enormous. In the selection of substances included in this work the following aspects were considered: listed in the EFSA compendium, coverage of various chemical (sub)classes, inclusion of substances known to be highly toxic or of high concern, and, last but not least, commercial availability of reference standards. The list of substances studied is provided in Table 1.

The aim of this work was to evaluate the use of LC-high resolution MS for screening of plant toxins in various matrices, i.e. the detection of as many as possible substances by one method. Since the optimum conditions for the LC-MS measurement will vary for the different substances, compromises have to be made. In the past we evaluated the effects of chromatographic column, eluent composition, and source conditions in the frame of multi-analyte detection of pesticides and mycotoxins (results not published). Based on this experience an end-capped C18 column was selected for retaining polar substances and robust chromatography. Regarding the eluent, methanol was chosen as modifier due to its better MS sensitivity for most substances compared to acetonitrile, and ammonium formate was added to suppress sodium adduct formation. The pH of 5 was a compromise between retention and peak shape for basic alkaloids (best at neutral/basic conditions), and detection limits for

certain substances (e.g. pyrethrins and THC, better under more acidic conditions). MS source parameters were set as recommended by the manufacturer to be favorable for the majority of small molecules.

Reference standards totaling 150 substances were injected into the HPLC-Orbitrap MS system and measured in positive and negative mode. Accurate masses obtained were matched against exact masses of plausible ions. For positive ions, the tendency of formation of ammonium and sodium adducts was verified. The results are included in Table 1. The far majority of the substances could be measured as positive ions, mostly protonated. For several substances multiple adducts were obtained. Despite the addition of ammonium formate, the sodium adduct was the most abundant for a number of cardiac glycosides. Where sodium adducts were observed, the relative abundance of $[M+H]^+$, $[M+NH_4]^+$ and $[M+Na]^+$ was not always consistent over a longer period of time. Several substances also yielded a response in negative mode, but in most cases with lower sensitivity compared to positive mode. Glycerrhizic acid and the anthraquinones aloe-emodin, emodin and chrysophanic acid could only be measured as negative ion. For 17 substances no response was obtained under the applied conditions (see Supplemental Information Table S3). As was to be expected, the majority of these were alkenylbenzenes and monoterpenes. In contrast to earlier measurements by LC-MS (triple quadrupole), both in our laboratory and reported by others (Ye 2007), here no response was obtained for sennoside B. For glycerrhizic acid, chrysophanic acid, methyllycaconitine, oenin, and vincristine, a response was obtained upon injection of individual reference standards, but no or an inconsistent response was observed later on after preparation of mix-standard solutions. They were therefore excluded for further evaluation.

For the remaining substances, the detection limits of the instrumental measurement were determined by injection of solvent standards and manually reviewing extracted ion chromatograms (XICs) using a mass extraction window of ±5 ppm. In the XICs obtained this way, noise was typically absent and only the peak of the analyte of interest stood out. Therefore, establishment of the limit of detection (LOD) based on a signal-to-noise ratio was not really feasible. Instead, the determination of the LOD was done by reviewing stick plots showing the response as vertical lines for each individual scan, rather than (smoothed) peaks, for a series of dilutions of solvent standards. The requirement set was that at least three scans should be still be present at the retention time of the analyte. At the lower response levels, spikes were often observed in the XICs. In such cases, a second requirement was that the

response of the scans of the peak of interest should be at least three times that of the spikes present within one minute of the retention time of the target analyte. The lowest concentration injected was 0.5 µg/L, corresponding to 2.5 pg on-column. The LODs derived this way are included in Table 1. For almost 60% of the substances the system LOD was 2.5 pg or lower, important plant toxins such as pyrrolizidine alkaloids and tropane alkaloids were amongst the ones most sensitively detected.

Detection limits in real samples

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

Effect of matrix on selectivity

Especially at low levels of plant toxins, peaks from other substances may interfere in the qualitative and quantitative determination. To avoid this, a high degree of selectivity is required in the instrumental analysis. In full scan MS, a high mass resolving power / high mass accuracy is essential, as has been demonstrated elsewhere (Kellmann et al 2009). This is especially true for the analysis of plant toxins amongst thousands of other secondary plant metabolites. Here both target analytes and background interferences are small molecules in the 100-400 Da range with the same elemental composition (C, H, O and/or N). As described earlier (Nielen et al 2007) it can be calculated that to resolve co-eluting substances, differing in only one CO *vs* N_2 (i.e. 11.2 mDa), a mass resolving power of 17,800, 35,600,

53,400 and 71,200 (FWHM) is required at m/z 100, 200, 300 and 400, respectively. For this reason we used the highest resolving power that could be set with the Orbitrap MS used in this work: 100,000 (FWHM) at m/z 200. This corresponds to 70,700 at m/z 400, since with Orbitrap MS the resolving power is inversely proportional with the square root of m/z. To evaluate the selectivity, generic extracts were prepared for four matrices: honey, a food supplement (mixture of dried aromatic plants, 'blood purifier'), silage, and a compound feed (complete pig feed). The extracts were spiked with plant toxins at three concentrations levels, corresponding to 0.01, 0.05 and 0.20 mg/kg in the sample, and analyzed. The selectivity was evaluated as follows: for each analyte, the XIC of the lowest concentration for which a peak was still obtained, was manually examined. In that XIC, the presence of other peaks was checked. Spikes, typically observed in the lower response range, were ignored. Furthermore, peaks from isobaric compounds that were present in the mix-reference standard used for spiking, were not regarded here as interference. In most cases, no significant interfering peaks were observed near the retention time of the targeted substances, in many cases not even in the entire chromatogram. In a limited number of cases (<10%), interfering peaks were observed. In rare cases, background noise across a larger part of the XIC occurred. Examples of all three situations are shown in Figure 2. Based on these observations, we conclude that selectivity limitations do occur for certain analyte/matrix combinations, but in general are not a major issue.

Effect of matrix on sensitivity

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

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

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

From the above it is clear that ion suppression negatively affects the sensitivity and higher LODs are obtained in samples than might be expected from the solvent standards. The LODs for the individual analytes in each of the four matrices tested are included in Table 1. Assuming quantitative recovery during extraction, for honey 70% of the analytes could still be detected down to the 0.01 mg/kg level. For the other matrices higher LODs were obtained, but still 70% of the analytes could be detected at 0.05 mg/kg or lower, while for 10-15% the LOD was higher than 0.2 mg/kg. Whether such higher LODs are acceptable or not depend on the final application. For the few substances for which maximum concentration limits have been established (see Supplemental Information Table S1), this seems acceptable. Also in case of analysis of individual aromatic plants or samples related to intoxications, the toxins are often present at the mg/kg level. On the other hand, the situation is different when the aim is to detect minor contamination with toxic plants, or to detect low levels of carcinogenic substances such as certain pyrrolizidine alkaloids and aristolochic acids. Furthermore, at this moment legislation in The Netherlands (Staatsblad 2001) as well as in other countries state that herbal preparations ‘should not contain materials originating from certain plants’. This is a very qualitative description and translation into maximum levels of the corresponding plant toxins has not been done so far. The same is true for the EU directive (EU 2002/32) that regulates undesirable substances in animal feed. For most plant toxins limits are set in mg plant/kg feedingstuff, or it is stated that the plant ‘or their processed derivatives may only be present in feedingstuffs in trace amounts not quantitatively determinable’. This has been set with visual/microscopic methods in mind. From various publications by others and this paper it is clear that chemical methods are available to measure the actual toxins. Therefore, it would make more sense to set limits for the toxins rather than for the plant, especially because

the relationship between plant parts present in the feed and the toxin concentrations is unlikely to be linearly correlated.

It is clear that more information on concentrations of toxins in the plants and in the final food or feed product is needed to translate mg toxic plant/kg into mg toxin(s)/kg and to see whether the LODs of the currently proposed screening method are fit-for-purpose.

Example applications

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

Plant toxins in food

Honey

Honey is an important food product in which plant toxins may occur due to transfer through nectar or pollen collected by bees foraging on areas with high abundance of toxic plants. Such transfer has been described for pyrrolizidine alkaloids (Edgar et al 2002) which are present in a wide variety of plants, and grayanotoxins with occur in the plant family *Ericaceae* (e.g. rhododendron) (Gunduz 2008). Figure 3a shows the analysis result of a honey sample from beehives intentionally placed at the Veluwe region in the Netherlands with high abundance of ragwort (*Jacobaea vulgaris*). Several pyrrolizidine alkaloids typical for this plant were detected based on accurate mass and retention time. In addition, traces of jacoline and jaconine and their N-oxides could be provisionally identified, based on accurate mass and lack of other peaks in the XIC. For jaconine, one of the rare examples of a plant toxin containing chlorine, the presence of the ^{37}Cl isotope supported its identification (see Figure

3b). For the pyrrolizidine alkaloids for which a reference standard was available, an estimation of the concentrations could be made based on solvent standards, i.e. not taking ion suppression into account. Levels were in the range of 0.05-0.6 mg/kg, with a total of 1.7 mg/kg. This is high in relation to the very low maximum limit of 1 µg/kg for toxic pyrrolizidine alkaloids in herbal preparations that has been established in Dutch legislation (Staatsblad 2001). In another sample from beehives located in the dunes near Vogelzang (The Netherlands), an area with a high abundance of Vipers bugloss (*Echium vulgare*), lycopsamine was detected.

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

Poppy seeds

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

Herbal tea

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

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

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

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

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

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

Several Traditional Chinese Medicines (TCMs) may contain highly toxic substances, besides the substances to which the therapeutic action is being attributed. Aconitine-type alkaloids are known to be present in the roots of *Aconitum carmichaeli* (Yue 2009). The TCM *Chuan Wu* was analyzed and aconitine was found at a level of approximately 0.1 mg/kg. Related substances such as hypaconitine and mesaconitine were also detected based on the exact mass of the protonated molecules. In all three cases, the substance targeted for through their XIC was the only major peak present (see Supplemental Information Figure S1). The pyrrolizidine alkaloids senecionine, its N-oxide and senkirkine were found at levels of approximately 1.4, 1.7 and 60 mg/kg, respectively, in *Kuan Dong Hua* (*Tussilago farfara*) which was in the same range as reported by (Jiang et al 2009) using a more dedicated method based on LC-MS/MS. In *Chuan Xiong Cha Tiao Wan* aristolochic acid I was detected at approximately0.3 mg/kg (without correction for ion suppression), and confirmed by LC-MS/MS. On one hand this was unexpected, since the toxicity of aristolochic acids is well known and plants containing them (*Aristolochia* and *Asarum*) have been banned for use in herbal preparations for many years in many countries. The label of the product purchased listed eight plant ingredients, none of them belonging to the genera just mentioned. On the other hand, this product has previously been shortlisted as a multi-ingredient TCM possibly containing aristolochic acids (Martena et al 2007). Apparently, despite all warnings, bans and

enforcement activities, herbal preparations containing aristolochic acids are still around, either as contaminant or intentionally added.

Plant toxins in feed and feed additives

Feed

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

Feed additives

Since the ban on the use of antibiotic growth promoters as feed additive in the EU in 2006, herbal preparations are increasingly being used as alternative to improve growth, feed conversion and for prophylactic purposes. Relatively little is known about the efficacy and safety of these additives which has been subject to EFSA concerns (EFSA 2010b). In contrast to food supplements, plant extracts for use as feed ingredients are typically admixtures and hardly standardized, which complicates safety evaluation of such products. Meanwhile, herbal products are being marketed, not rarely without proper labeling of composition. In other cases by-products from food or pharmaceutical industry end up as 'beneficial' feed additive or ingredient. In 2010, 69 out of a group of 650 calves died in the Netherlands after being fed with a feed ingredient labeled as 'parsley by-product'. Samples were taken and screened for

plant toxins, with specific attention to cardiac glycosides (e.g. digitoxin, oleandrin) based on diagnostic information from the veterinarian. Digoxin, digitoxin and digitoxigenin were found based on their accurate mass and retention time match. In addition, the accurate mass of protonated lanatoside (B/C) was found. The combined finding of these four related plant toxins provided evidence that the feed ingredient had been mixed up or exchanged with *Digitalis* (foxglove) most probably as by-product from pharmaceutical industry. In a subsequent quantitative analysis, the concentrations for digitoxin and digoxin were 180 and 1700 mg/kg and considered to be the cause of death.

Concluding Remarks

A generic method for simultaneous detection of various classes of plant toxins in a variety of food, feed and botanicals was set up. Inherent to such method, sample preparation is non-selective, complex raw extracts are obtained, and generic fixed LC-MS conditions are used for analysis. At an individual analyte level this is not always optimal and sensitivity is partly sacrificed for extended scope. Selectivity on the other hand was not compromised due to the ability to use very narrow mass extraction windows (±5 ppm) to extract the target analytes from the raw data. This was achieved by measuring at a very high mass resolving power (100,000 FWHM), resulting in a reliable high mass accuracy (mostly within 2 ppm) even in cases of higher levels of co-eluting matrix. Furthermore, the mass accuracy was not affected across a wide response range of the analytes (~4 orders). The latter is very relevant because the concentration range of plant toxins can vary from trace levels in the µg/kg range in cases of contamination, to high mg/kg levels in certain plant species or in cases of adulteration and intoxications.

In the current method, untargeted full scan MS acquisition was applied. Fragmentation, was not performed in this work which means that detection relied on the accurate mass and retention time for as far reference standards were available. Although in many cases only one peak was observed in the XIC over the entire run time, this is not sufficient for unambiguous identification. However, plant toxins are often present with other plant-specific secondary metabolites (toxic or not) which may provide additional confirmatory information. In a way the presence or absence of other compounds known to co-occur with certain plant toxins could be used in a similar way as additional accurate masses from adducts or fragments. For a

part of the detects obtained by the screening method, confirmatory LC-MS/MS analysis was performed. By doing so, 44 out of 51 detects were confirmed.

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

Acknowledgements

The Dutch Ministry of Economic Affairs, Agriculture and Innovation is acknowledged for financially supporting this work (project #1217272001).

References

Bateman KP, Kellmann M, Muenster H, Papp R, Taylor L. 2009. Quantitative–Qualitative Data Acquisition Using a Benchtop Orbitrap Mass Spectrometer. J Am Soc Mass Spectrom 20: 1441–1450.

Battilani P, Costa LG, Dossena A, Gullino ML, Marchelli R, Galaverna G, Pietri A, Dall'Asta C, Giorni P, Spadaro D, Gualla A. 2009. Scientific information on mycotoxins and natural plant toxicants. Scientific/technical report submitted to EFSA. CFP/EFSA/CONTAM/2008/01.

BfR 2010. Cases of poisoning through grayanotoxins in rhododendron honey originating from the Turkish Black Sea Region. Bfr Opinion Nr. 043/2010, 3 September 2010

Chou MW, Wang YP, Yan J, Yang JC, Beger RD, Williams LD, Doerge DR, Fu PP. 2003. Riddelliine N-oxide is a phytochemical and mammalian metabolite with genotoxic activity that is comparable to the parent pyrrolizidine alkaloid riddelliine, Toxicology Letters 145: 239-247

Cosyns JP. 2003. Aristolochic acid and 'Chinese herbs nephropathy', a review of the evidence to date. Drug Safety, 26:33-48.

Dayan FE, Cantrell CL, Duke SO. 2009. Natural products in crop protection. Bioorganic & Medicinal Chemistry 17:4022–4034.

Edgar JA, Roeder E, Molyneux RJ. 2002. Honey from plants containing pyrrolizidine alkaloids: a potential threat to health. J. Agric. Food Chem. 50:2719-2730.

EFSA 2007. Scientific opinion. Pyrrolizidine alkaloids as undesirable substances in animal feed. The EFSA Journal (2007) 447, 1-51.

EFSA 2008a. Scientific opinion. Glucosinolates as undesirable substances in animal feed, The EFSA Journal (2008) 590, 1-76.

EFSA 2008b. Scientific opinion. Tropane alkaloids (from Datura sp.) as undesirable substances in animal feed. The EFSA Journal (2008) 691, 1-55

EFSA 2009. Compendium of botanicals that have been reported to contain toxic, addictive, psychotropic or other substances of concern on request of EFSA. EFSA Journal 2009; 7(9):281. [100 pp]

EFSA 2010a. EFSA call for scientific data on opium alkaloids in poppy seeds. http://www.efsa.europa.eu/en/dataclosed/call/datex101020.htm

EFSA 2010b. EFSA Panel on additives and products or substances used in animal feed (FEEPAP) Statement on the preparation of guidance for the assessment of plant/herbal products and their constituents used as feed additives. EFSA journal 2010; 8(7):1694 [7 pp].

EU 2002/32. EU directive 2002/32/EC on undesirable substances in animal feed. Consolidated document including amendments of the original directive (2002/32/EC, Official Journal L 140, 30.5.2002, p. 10). http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2002L0032:20100302:EN:PDF (accessed March 2011)

Gunduz A, Turedi SAC, Russell RM, Ayaz FA. 2008. Clinical review of grayanotoxin/mad honey poisoning past and present. Clinical Toxicology 46: 437-442

Hartmann T. 2007. From waste products to ecochemicals: Fifty years research of plant secondary metabolism. Phytochemistry 68: 2831–2846.

Holstege DM, Puschner B, Le T. 2001. Determination of Grayanotoxins in Biological Samples by LC-MS/MS. J. Agric. Food Chem. 49:1648-1651.

Jiang Z, Liu F, Goh JJL, Yu L, Li SFY, Ong ES, Ong CN. 2009. Determination of senkirkine and senecionine in *Tussilago farfara* using microwave-assisted extraction and pressurized hot water extraction with liquid chromatography tandem mass spectrometry. Talanta 79:539-546.

Johanns, ESD, van der Kolk LE, van Gemert, HMA, Sijben AE. 2002. An epidemic of epileptic seizures after consumption of herbal tea. Ned. Tijdschr. Geneeskd. 146:813-816.

Johnson RC, Lemire SW, Woolfitt AR, Ospina M, Preston KP, Olson CT, Barr JR. 2005. Quantification of ricinine in rat and human urine: a biomarker for ricin exposure. J. Anal. Toxicol. 29: 149-155.

Josephs RD, Daireaux A, Westwood S, Wielgosz RI. 2010. Simultaneous determination of various cardiac glycosides by liquid chromatography–hybrid mass spectrometry for the purity assessment of the therapeutic monitored drug digoxin. Journal of Chromatography A, 1217: 4535–4543

Kellmann M, Muenster H, Zomer P, Mol H. 2009. Full Scan MS in Comprehensive Qualitative and Quantitative Residue Analysis in Food and Feed Matrices: How Much Resolving Power is Required? J Am Soc Mass Spectrom 2009:1464–1476.

Khan IA. 2006. Issues related to botanicals. Life Sciences 78:2033-2038.

Kuo CH, Lee CW, Lin SC, Tsai IL, Lee SS, Tseng YJ, Kang JJ, Peng FC, Chu LW. 2010. Rapid determination of aristolochic acids I and II in herbal products and biological samples by ultra-high-pressure liquid chromatography–tandem mass spectrometry. Talanta 80:1672–1680

Lederer I, Schulzki G, Gross J, Steffe JP. 2006. Combination of TLC and HPLC-MS/MS methods. Approach to a rational quality control of Chinese star anise. J. Agric. Food Chem. 2006: 1970-1974.

Lehotay SJ, Son KA, Kwon H, Koesukwiwata U, Fud W, Mastovska K, Hoh E, Leepipatpiboon N. 2010. Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in fruits and vegetables. Journal of Chromatography A, Volume 1217:2548-2560.

Li SL, Song JZ, Qiao CF, Zhou Y, Xu HX. 2010. UPLC–PDA–TOFMS based chemical profiling approach to rapidly evaluate chemical consistency between traditional and dispensing granule decoctions of traditional medicine combinatorial formulae. Journal of Pharmaceutical and Biomedical Analysis 52:468–478.

Martena M, van der Wielen JCA, van de Laak LFJ, Konings EJM, de Groot HN, Rietjens YMCM. 2007. Enforcement of the ban on aristolochic acids in Chinese traditional herbal preparations on the Dutch market. Anal. Bioanal. Chem. 389:263:275.

McIlhenny EH, Pipkin KE, Standish LJ, Wechkin HA, Strassman R, Barker SA. 2009. Direct analysis of psychoactive tryptamine and harmala alkaloids in the Amazonian botanical medicine ayahuasca by liquid chromatography–electrospray ionization-tandem mass spectrometry. Journal of Chromatography A, 1216: 8960–8968.

Mol HGJ, Plaza-Bolaños P, Zomer P, de Rijk TC, Stolker AAM, Mulder PPJ. 2008. Toward a Generic Extraction Method for Simultaneous Determination of Pesticides, Mycotoxins, Plant Toxins, and Veterinary Drugs in Feed and Food Matrixes. Anal. Chem. 80: 9450–9459.

Nielen MWF, M.C. van Engelen MC, Zuiderent R, Ramaker R. 2007. Screening and confirmation criteria for hormone residue analysis using liquid chromatography accurate mass time-of-flight, Fourier transform ion cyclotron resonance and orbitrap mass spectrometry techniques. Analytica Chimica Acta 586:122–129.

Salgueiro L, Martins AP, Correiab H, 2010. Raw materials: the importance of quality and safety. A review. Flavour Fragr. J. 25:253–271.

Speijers G, Alink G, de Saeger S, Hardy A, Magan N, Pilegaard K, Battilani P, Riemers M. 2010. Evaluation of agronomic practices for mitigation of natural toxins. ILSI report October 2010.

Sproll C, Perz RC, Lachenmeier DW. 2006. Optimized LC/MS/MS Analysis of Morphine and Codeine in Poppy Seed and Evaluation of Their Fate during Food Processing as a Basis for Risk Analysis. J. Agric. Food Chem. 54: 5292-5298.

Staatsblad 2001. Besluit van 19 januari 2001, houdende vaststelling van het Warenwetbesluit kruidenpreparaten, Staatsblad 2001 56 [12 pp].

Sulyok M, Krska R, Schuhmacher R. 2010. Application of an LC–MS/MS based multi-mycotoxin method for the semi-quantitative determination of mycotoxins occurring in different types of food infected by moulds. Food Chemistry 119:408–416

Verpoorte R, Niessen WMA. 1994. Liquid chromatography coupled with mass spectrometry in the analysis of alkaloids. Phytochemical Analysis 5:217-232.

Wang Z, Li D, Zhou Z, Li B, Yang W. 2009. A simple method for screening and quantification of ricinine in feed with HPLC and LC-MS. J. Chromatogr. Sci. 47:585-588.

Wiedenfeld H, Edgar J. 2011. Toxicity of pyrrolizidine alkaloids to humans and ruminants. Phytochem. Rev. 10:137-151.

Yan G, Sun H, Sun W, Zhao L, Meng X, Wang X. 2010. Rapid and global detection and characterization of aconitum alkaloids in Yin Chen Si Ni Tang, a traditional Chinese medical formula, by ultra performance liquid chromatography–high resolution mass spectrometry and automated data analysis. Journal of Pharmaceutical and Biomedical Analysis 53: 421–431.

Ye M, Han J, Chen H, Zheng J, Guo D. 2007. Analysis of Phenolic Compounds in Rhubarbs Using Liquid Chromatography Coupled with Electrospray Ionization Mass Spectrometry. J Am Soc Mass Spectrom 18: 82–91.

Yue H, Pi Z, Song F, Liu Z, Cai Z, Liu S. 2009. Studies on the aconitine-type alkaloids in the roots of *Aconitum Carmichaeli Debx*. By HPLC/ESIMS/MS^n. Talanta 77:1800-1807.

Zhang H, Gong C, Lv L, Xu Y, Zhao L, Zhu Z, Chai Y, Zhang G. 2009. Rapid separation and identification of furocoumarins in Angelica dahurica by high-performance liquid chromatography with diode-array detection, time-of-flight mass spectrometry and quadrupole ion trap mass spectrometry. Rapid Commun. Mass Spectrom. 23: 2167–2175.

Zhou JL, Li P, Li HJ, Jiang Y, Ren MT, Liu Y. 2008. Development and validation of a liquid chromatography/electrospray ionization time-of-flight mass spectrometry method for relative and absolute quantification of steroidal alkaloids in *Fritillaria* species. Journal of Chromatography A, 1177:126–137.

Zhou JL, Qi LW, Li P. 2009. Herbal medicine analysis by liquid chromatography/time-of-flight mass spectrometry. Journal of Chromatography A, 1216:7582–7594

Zhou Y, Li N, Choi FFK, Qiao CF, Song JZ, Li SL, Liu X, Cai ZW, Fu PP, Xu HX. 2010. A new approach for simultaneous screening and quantification of toxic pyrrolizidine alkaloids in some potential pyrrolizidine alkaloid-containing plants by using ultra performance liquid chromatography–tandem quadrupole mass spectrometry. Analytica Chimica Acta 681: 33–40.

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

								Detectability in samples			
					ESI+ relative abundance			system LOD	silage	honey	complete pig feed	food supplement
Substance	Molecular formula	RT (min)	Ion (+)	Exact mass	M+H	M+NH4	M+Na	(pg)	LOD in µg/kg (b)			
Aconitine	C34H47NO11	8.31	[M+H]	646.3222	100			2.5	10	10	10	10
Allocryptoptine	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+NH4]	475.1922		100	20	25	50	50	50	50
Anisatine	C15H20O8	6.40	[M+NH4]	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+NH4]	359.0874		100	10	2.5	50	10	10	50
Aristolochia acid II	C16H9NO6	9.34	[M+NH4]	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

									Detectability in samples			
					ESI+ relative abundance			system LOD	silage	honey	complete pig feed	food supplement
Substance	Molecular formula	RT (min)	Ion (+)	Exact mass	M+H	M+NH4	M+Na	(pg)	LOD in µg/kg (b)			
Cymarin	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

					ESI+ relative abundance			system LOD	Detectability in samples			
									silage	honey	complete pig feed	food supplement
Substance	Molecular formula	RT (min)	Ion (+)	Exact mass	M+H	M+NH4	M+Na	(pg)	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

									Detectability in samples			
					ESI+ relative abundance			system LOD	silage	honey	complete pig feed	food supplement
Substance	Molecular formula	RT (min)	Ion (+)	Exact mass	M+H	M+NH4	M+Na	(pg)	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
Seneciphylline	C18H23NO5	5.83	[M+H]	334.1649	100			2.5	10	10	10	50
Seneciphylline-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

									Detectability in samples			
					ESI+ relative abundance			system LOD	silage	honey	complete pig feed	food supplement
Substance	Molecular formula	RT (min)	Ion (+)	Exact mass	M+H	M+NH4	M+Na	(pg)	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

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

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

int = partial co-elution with interference

pos = peak at retention time of analyte

> = LOD > 200 µg/kg

na = not analysed

Table 2.

Matrix-induced ion suppression for plant toxins in crude extracts of various matrices.

		MS response in extract relative to solv. stnd			
		>50%	33-50%	20-33%	<20%
Sample	Extract (b)	% 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

(a) 'blood purifier' sample details see Supplemental Information Table S2
(b) g matrix equivalent per ml of final extract
(c) N= 98-122 substances

Figure 1.

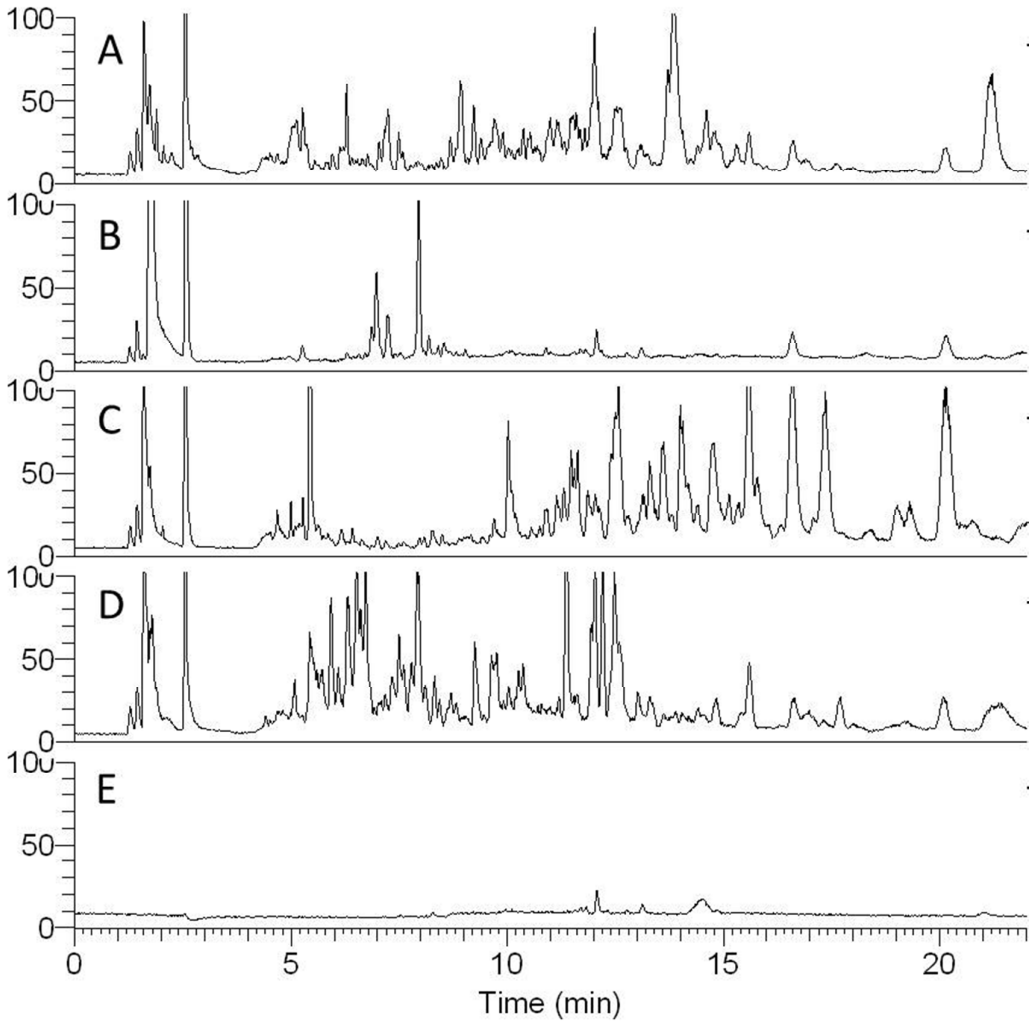

Figure 1. Total ion current chromatograms (TIC, m/z 55-1,000) obtained after LC-full scan MS analysis of crude extracts (0.125 g/ml extracts, 5 µl injection) of four matrices relevant in plant-toxin analysis. The scaling has been fixed to allow comparison of complexity. A) silage, B) honey, C) compound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

Figure 2.

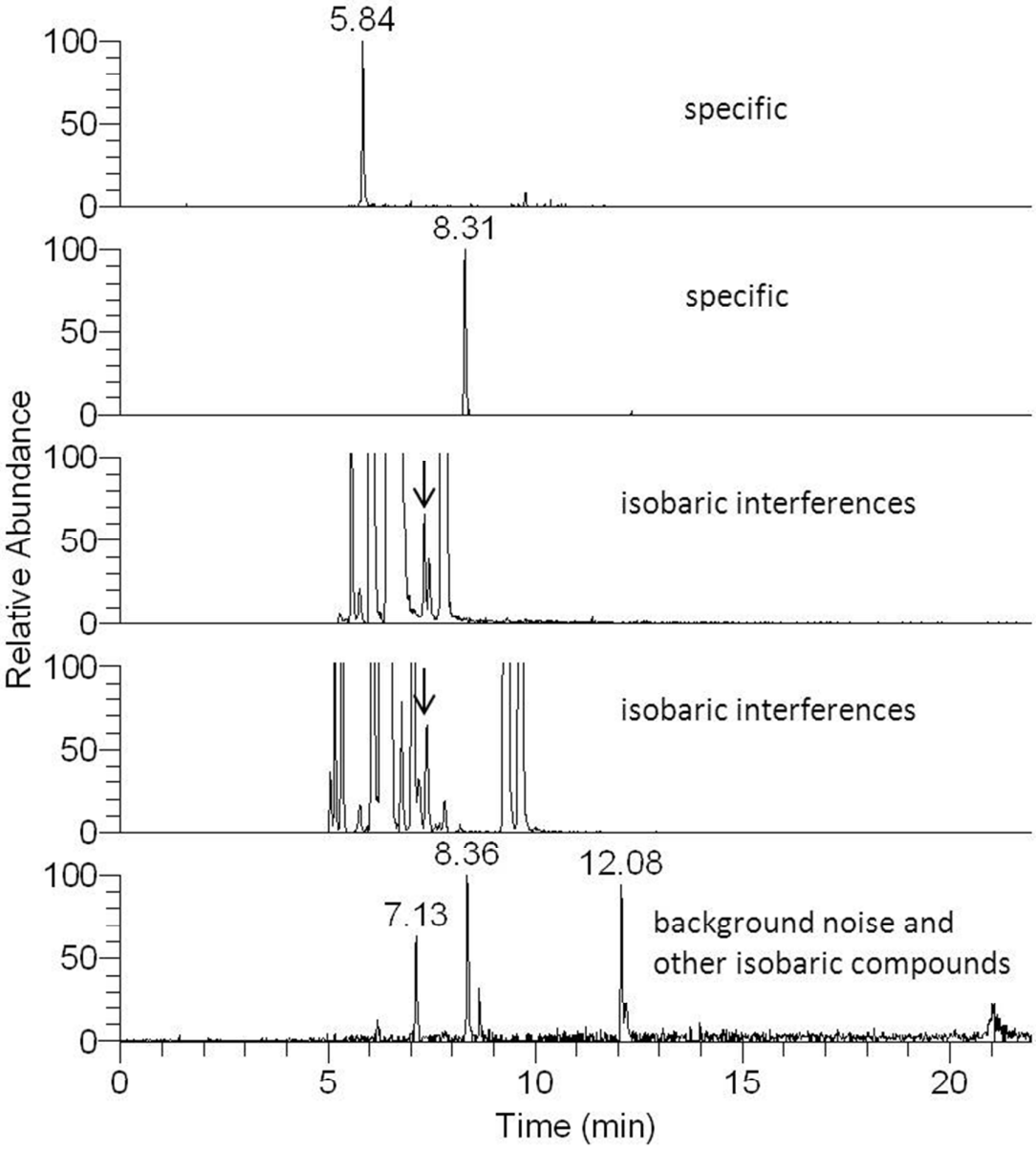

Figure 2. Example of extracted ion chromatograms of plant toxins (exact mass ±5ppm) in spiked crude extracts of various matrices. A) atropine (5.84 min, m/z 290.1751) in silage at the level of 0.05 mg/kg; B) aconitine (8.31 min, m/z 646.3222) in a herbal food supplement, 0.05 mg/kg; C) chelidonine (7.32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

736

737

738

739

Figure 3a.

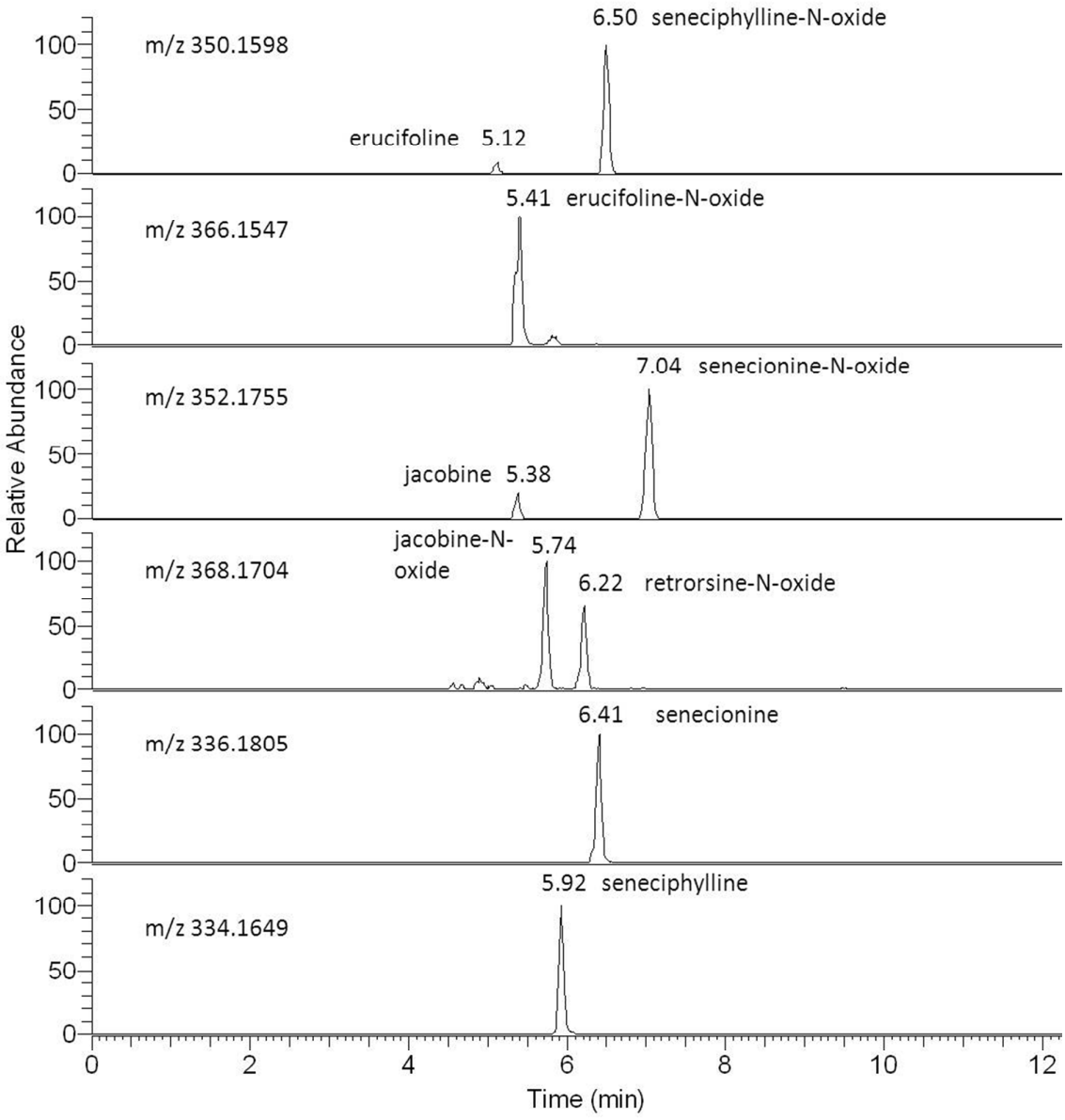

Figure 3a. XICs of a honey sample contaminated with pyrrolizidine alkaloids. Estimated levels range from 0.05 to 0.6 mg/kg (see Supplemental Information Table S4).

Figure 3b.

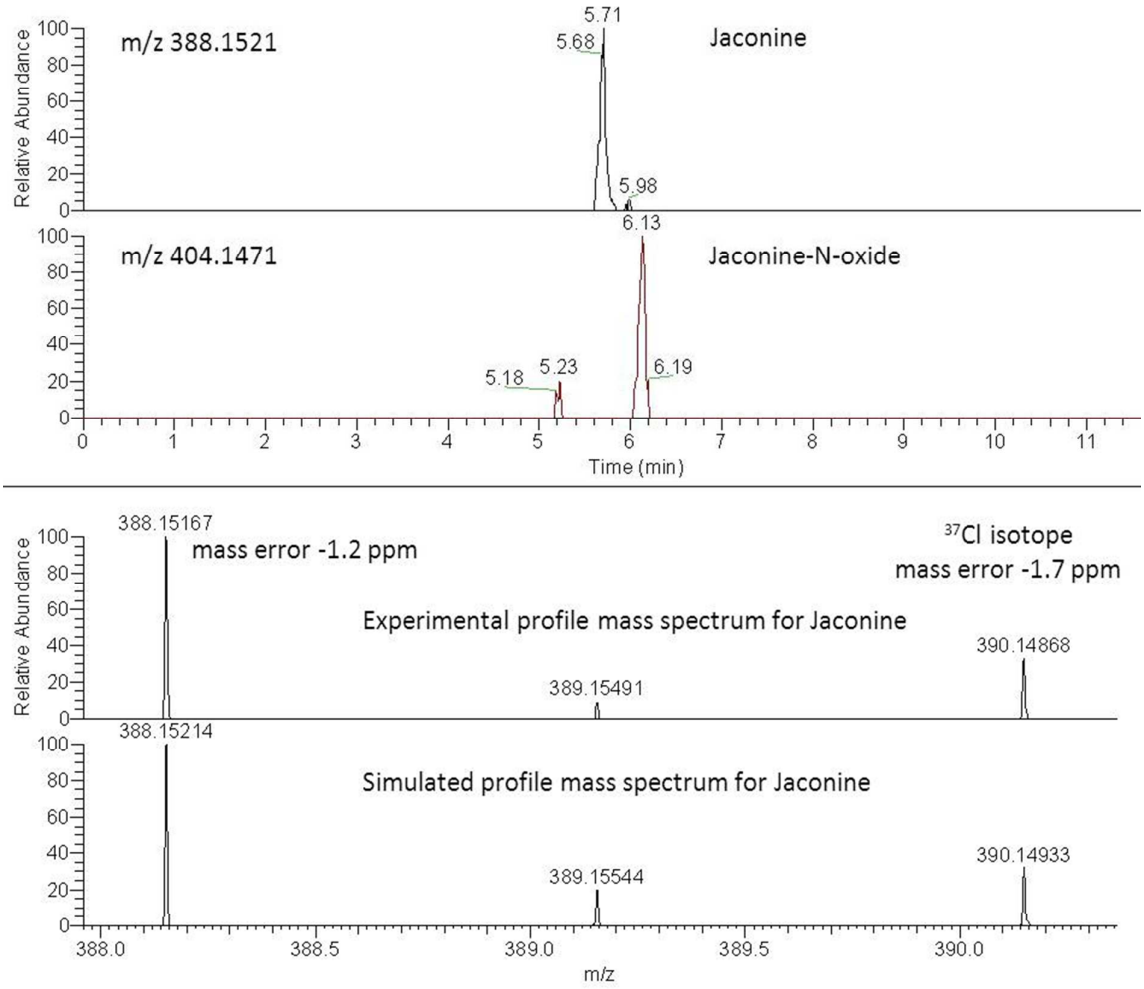

Figure 3b. Upper two traces: XIC for jaconine ($C_{18}H_{26}ClO_6$ as $[M+H]^+$)and its N-oxide, present at low levels (< 0.05 mg/kg) in honey. Lower two traces: experimental and theoretical mass spectrum of jaconine

Figure 4.

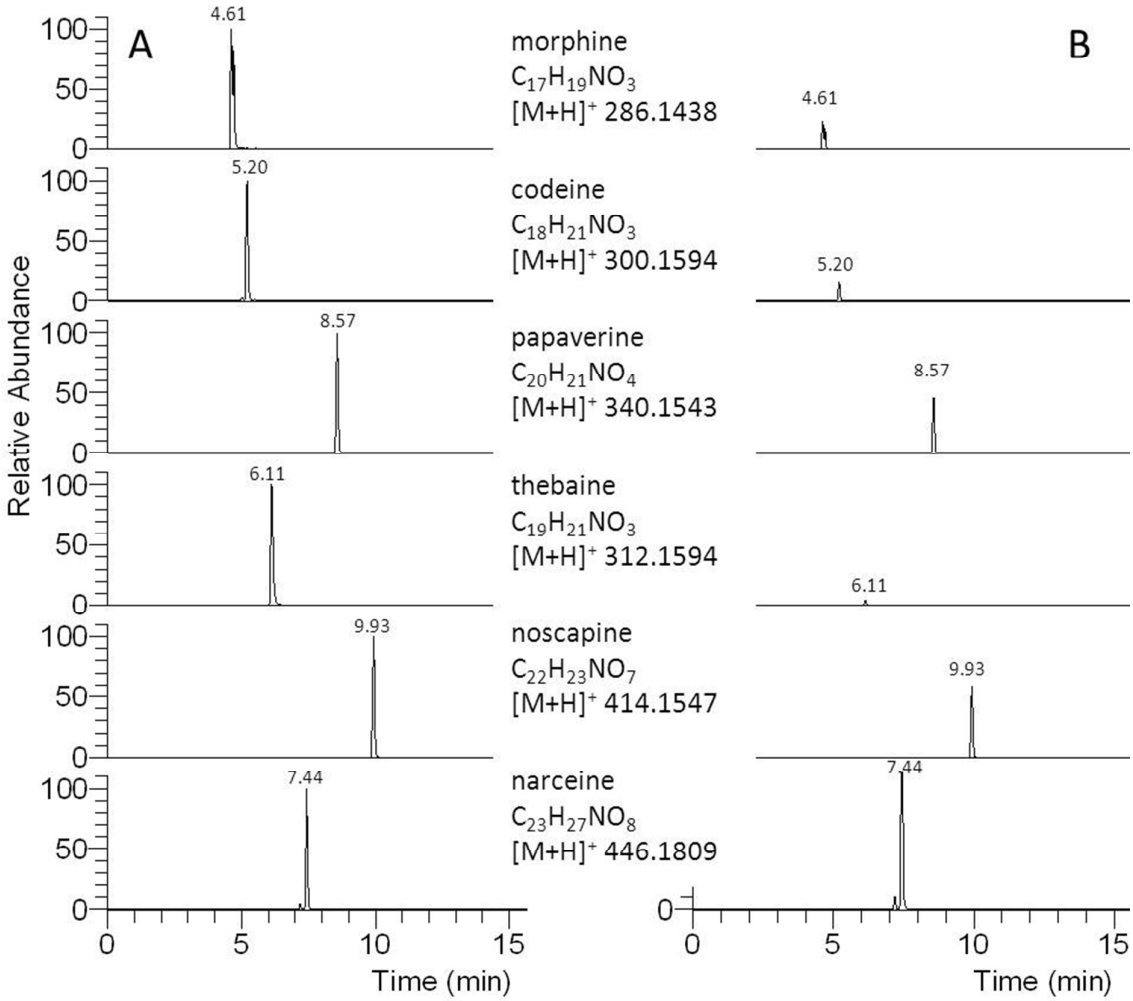

Figure 4. XICs of opium alkaloids after analysis of poppy seeds purchased as food ingredient. A) white poppy seeds, B) blue poppy seeds. Extracts were 10-fold diluted before analysis. For each alkaloid, the Y-axis has been fixed to allow direct comparison of the levels in the two samples.

Figure5.

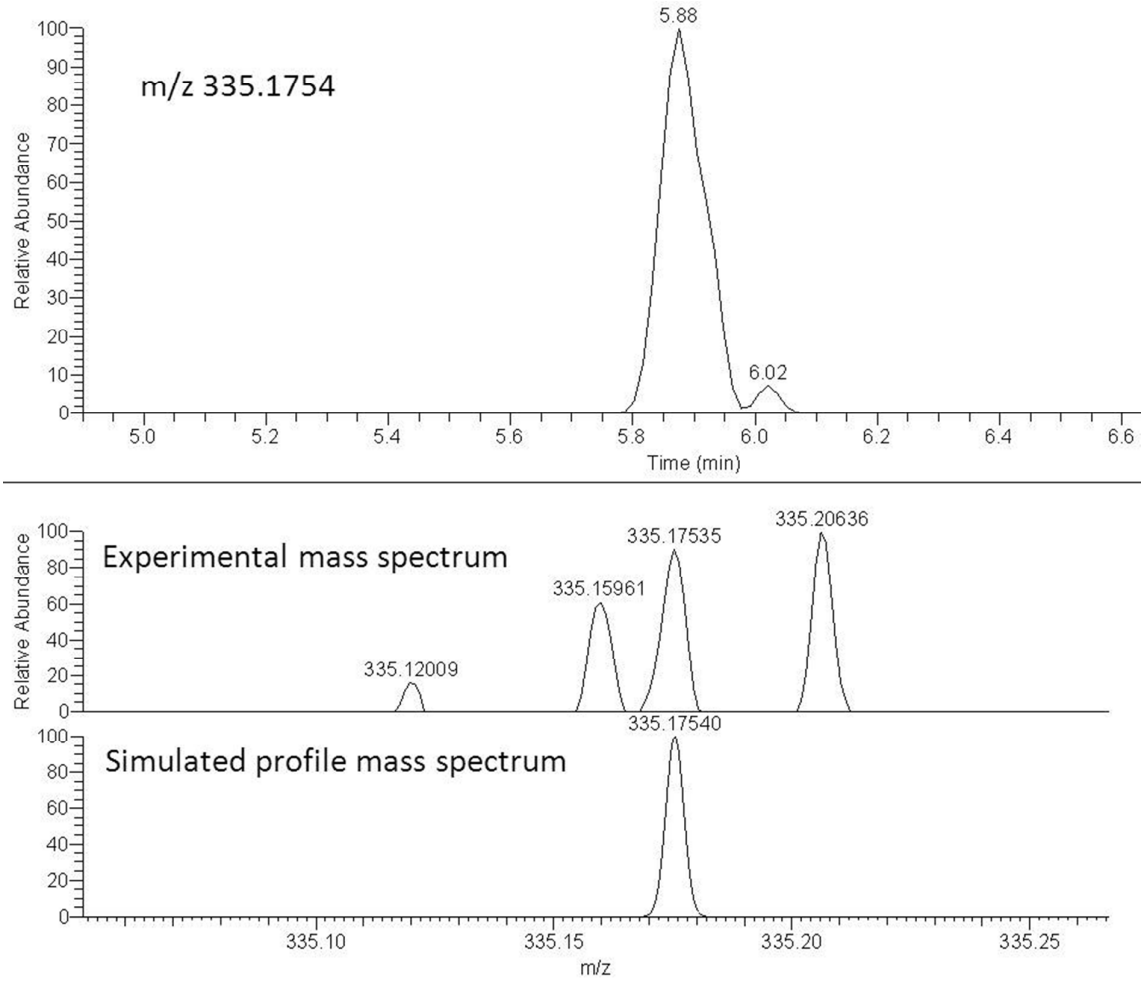

Figure 5. Top: XIC of strychnine (0.02 mg/kg) in a food supplement ('testosterone booster'). Bottom: experimental and theoretical mass spectrum.

Figure 6.

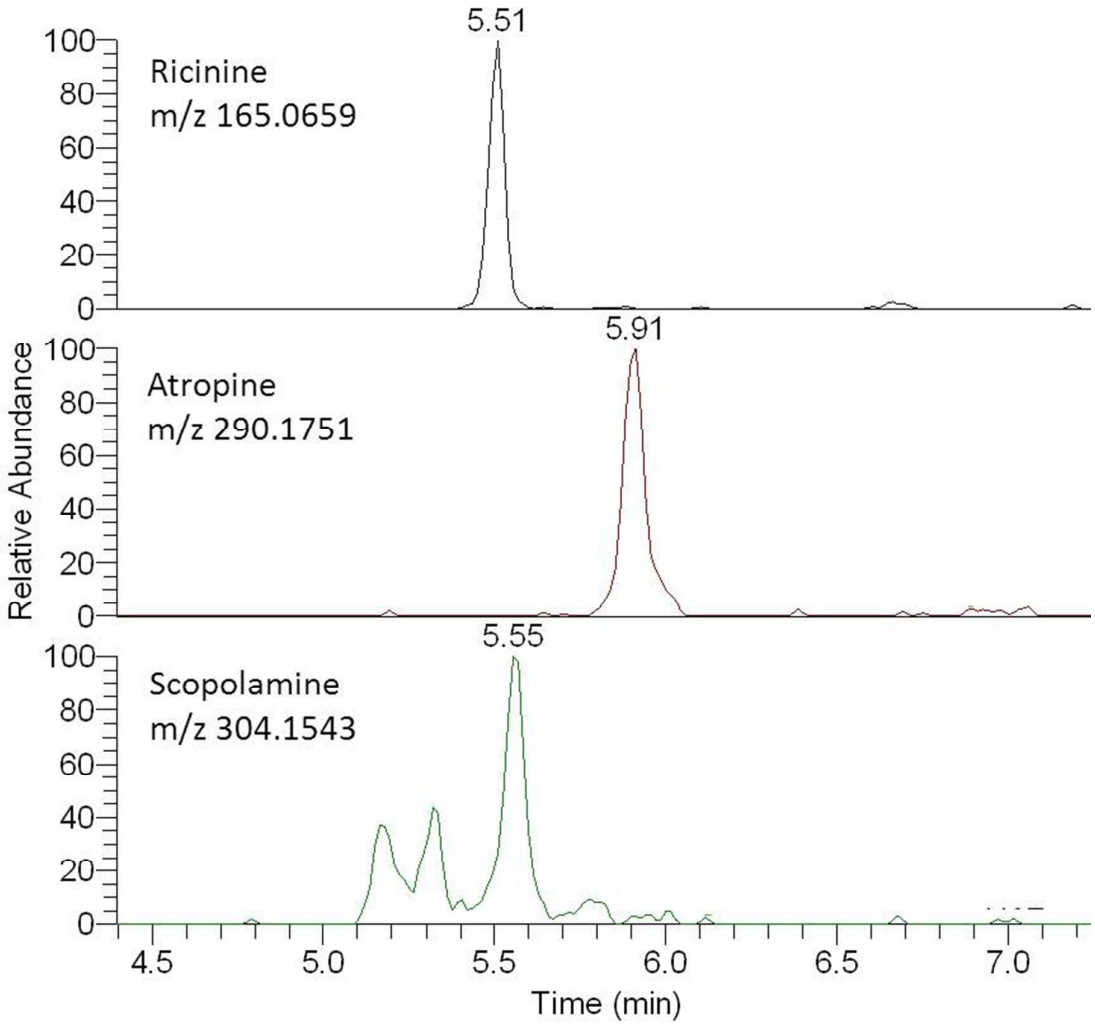

Figure 6. Detection of ricinine (alkaloid marker for ricin), atropine and scopolamine which were spiked to a silage sample at 0.05 mg/kg.

SUPPLEMENTAL INFORMATION to the paper entitled:

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

Hans G.J. Mol*), Ruud C.J. van Dam, Paul Zomer, Patrick P.J. Mulder

RIKILT Institute of Food Safety, Wageningen University and Research Centre, Akkermaalsbos 2, 6708 WB, Wageningen, Netherlands

* To whom correspondence should be addressed. E-mail: hans.mol@wur.nl

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

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-vinyloxazolidine-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*
- Datura sp.	all feeding stuffs	1000 mg/kg*
- Seeds and husks from Ricinus communis L., Croton tiglium L. and Abrus precatorius L. as well as their processed derivatives (20), separately or in combination		10 mg/kg*
- Crotalaria spp.	all feeding stuffs	100 mg/kg*
- Unhusked beech mast — Fagus silvatica L. - Purghera — Jatropha curcas L. - Indian mustard — Brassica juncea (L.) Czern. And Coss. ssp. Intergrifolia (West.) Thell. - Sareptian mustard — Brassica juncea (L.) Czern. And Coss. ssp. juncea - Chinese mustard — Brassica juncea (L.) Czern. And Coss. ssp. Juncea var. lutea Batalin - Black mustard — Brassica nigra (L.) Koch - Ethiopian mustard — Brassica carinata A. Braun	all feeding stuffs	Seeds and fruit of the plant species listed opposite as well as their processed derivates 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)		
Aristolochia spp. and preparations thereof	foodstuffs of animal origin	prohibited substance (MRL cannot be established)
Isoeugenol	fin fish	6 mg/kg

Table S2. Sample details

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

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	C21H22O9	8.49/8.66	[M-H]	417.1191
Amygdalin	C20H27NO11	5.87	[M-H]	456.1511
Anisatine	C15H20O8	6.40	[M-H]	327.1085
Curcumin	C21H20O6	10.58	[M-H]	367.1187
Digitoxin	C41H64O13	11.34	[M+HCOOH-H]	809.4329
Digoxin	C41H64O14	9.57	[M+HCOOH-H]	825.4278
Evodiamine	C19H17N3O	10.68	[M-H]	302.1299
Forskolin	C22H34O7	10.66	[M-H]	409.2232
Grayanotoxin III	C20H34O6	6.95	[M-H]	369.2283
Lapachol	C15H14O3	10.35	[M-H]	241.0870
Physcion	C16H12O5	14.33	[M-H]	283.0612
Picrotin	C15H18O7	6.69	[M-H]	309.0980
Picrotoxinin	C15H16O6	7.26	[M-H]	291.0874
Quercetin	C15H10O7	8.71	[M-H]	301.0354
Quercitrin	C21H20O11	7.86	[M-H]	447.0933
Rutin	C27H30O16	7.35	[M-H]	609.1461
Scopoletin	C10H8O4	7.00	[M-H]	191.0350
Substances for which response was inconsistent and/or issues related to multi-analyte standard or stability				
Chrysophanic acid	C15H10O4	13.16	[M-H]	253.0506
Glycyrrhizic acid	C42H62O16	10.06	[M-H]	821.3965
Methyllycaconitine	C37H50N2O10	7.07	[M+H]	683.3538
Oenin (cyclamin)	C23H25O12	6.00	[M+]	493.1341
Vincristine	C46H56N4O10	10.42	[M+H]	825.4069
Substances for which no response was obtained under the applied generic conditions				
Coumaric acid p-	C9H8O3		no response	
Epigallocatechin gallate	C22H18O11		no response	
Eucalyptol	C10H18O		no response	
Gossypol	C30H30O8		no response	
Hydroxycitric acid (HCA)	C6H8O8		no response	
Limonene	C10H16		no response	
Menthofuran	C10H14O		no response	
Methyl eugenol	C11H14O2		no response	
Methyl salicylate	C8H8O3		no response	
Myristicin	C11H12O3		no response	
Safrole	C10H10O2		no response	
Sarsasapogenin	C27H44O3		no response	
Sennoside B	C42H38O20		no response	
Strophanthin K-	C36H54O14		no response	
Terpineol	C10H18O		no response	
Thujone	C10H16O		no response	
Thymol	C10H14O		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				
Allocryptopine							+	+			+									
Aloe-emodin/emodin										+		+	+		+	+				
Aloin												+	+							
Amentoflavone						+			+		+									
Amygdalin						+?									+					
Anisatine						+														
Arbutin																				+ ?
Aristolochic acid I															0.3					
Berberine							0.03					1			0.5					
Chelidonine											+									
Curcumin						+								+						
Digitoxigenin																				+
Digitoxin																				180
Digoxin																				1700
Ephedrine																			X	
Erucifoline	0.05																			
Erucifoline-N-oxide	0.18																			
Gerannyloxypsoralen 5-															0.5					0.04
Granyanotoxin III			30																	
Harmaline															0.06					
Harmine															+					
Hydrastine beta												X								
Imperatorin															9					0.4
Jacobine	0.16																			

	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
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
Prenylnaringenin 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	+				+	+			+		+			+	+	+	+		+	+

	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			
Seneciphylline	0.11																			0.02
Seneciphylline-N-oxide	0.31																			
Senkirkine																	60			
Solanine alpha									+											
Strychnine														0.02						
Synephrine														+	+	+				
Tetrahydrocannabinol					+															
Trigonelline					+	+	+	+	+		+			+++	+				+	
Tropine																X			0.03	
Tryptamine					+	+	+	+	+		+					+			+	+
Umbelliferone											+				+	+				

+ : peak detected; retention time < ±0.1 min, accurate mass < ±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.

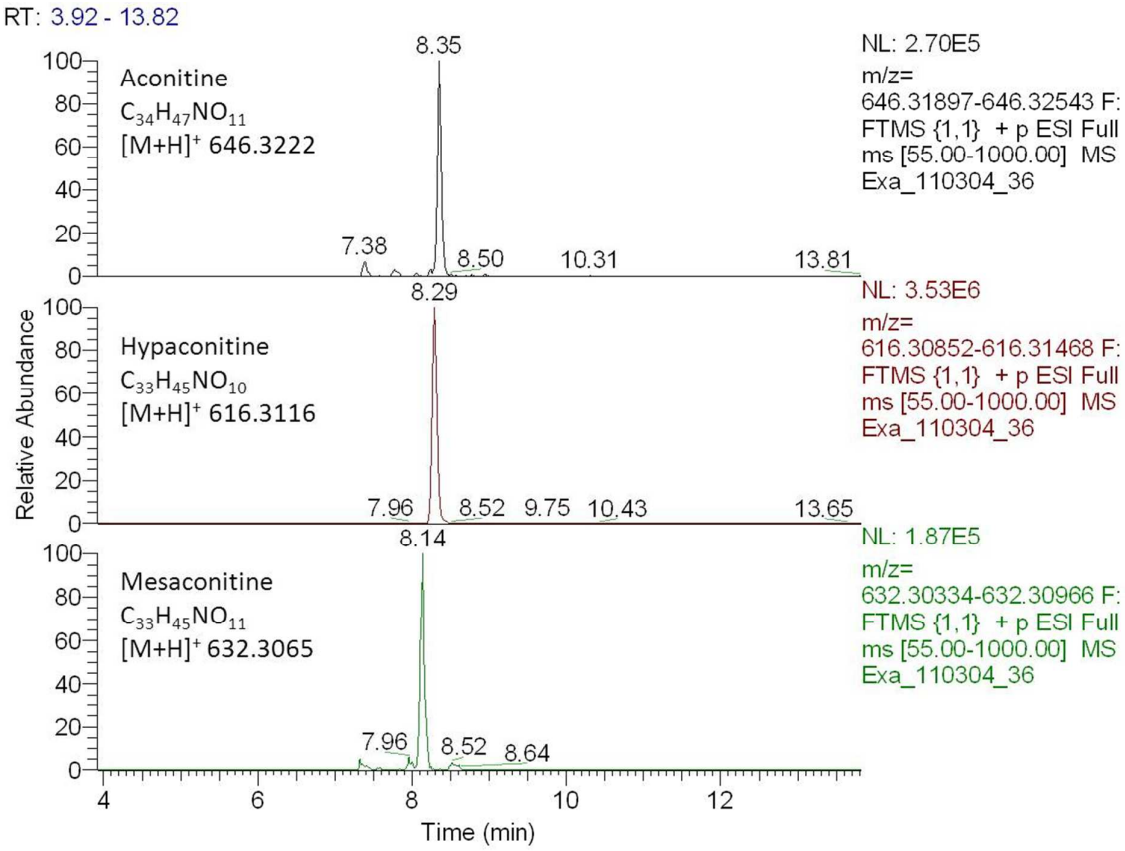

Figure S1. XICs of aconitine-alkaloids in the TCM Chuan Wu (*Aconitum carmichaeli*). Estimated level of aconitine is 0.1 mg/kg.

Figure S2.

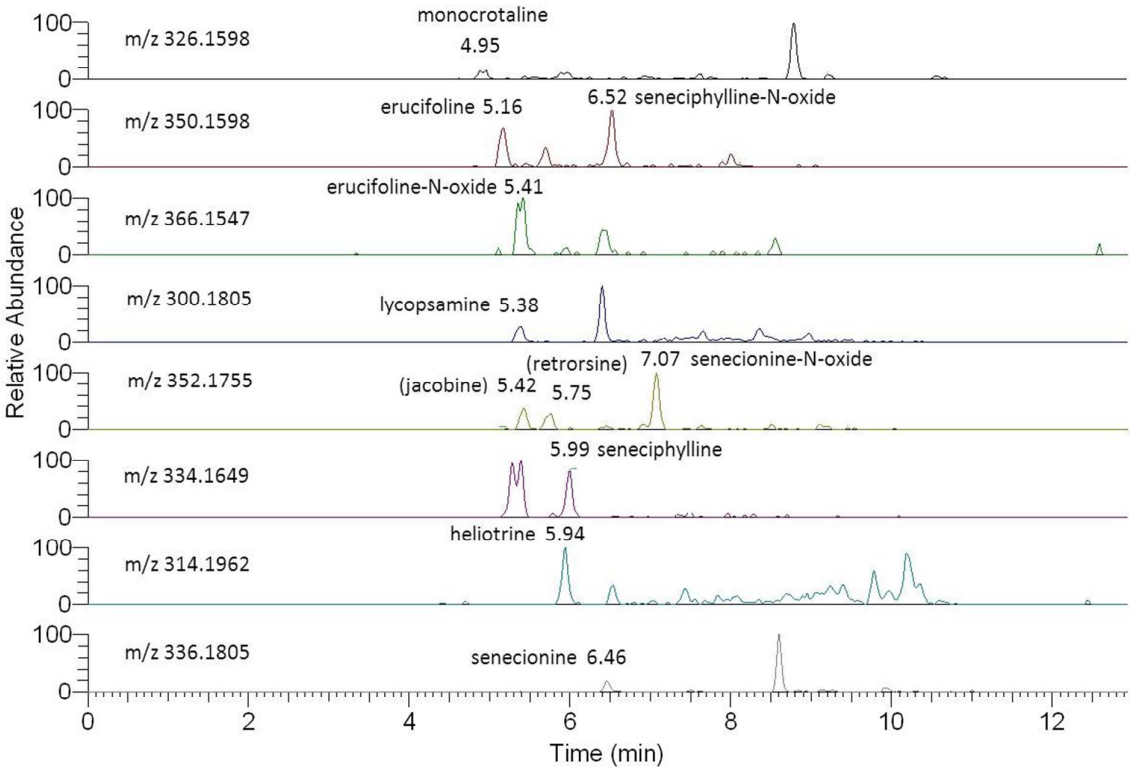

Figure S2. XICs of pyrrolizidine alkaloids spiked to a silage samples at 0.05 mg/kg.