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Pharma

Analysis of pyrrolizidine alkaloids in various plants

Analysis of pyrrolizidine alkaloids in various plants by means of LC-MS/MS

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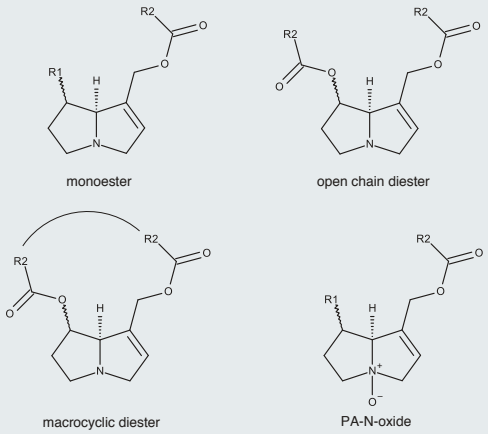
Pyrrolizidine alkaloids are derivates of alkaline pyrrolizidine. Numerous flowering plants contain pyrrolizidine alkaloids. Until now more than 600 different pyrrolizidine alkaloids have been identified in more than 6000 types of plants; particularly often they have been evidenced in composite flowers and boraginaceae.

In human and animal organism the liver partially breaks pyrrolizidine alkaloids down to strongly hepatotoxic compounds, whereas the macrocyclic diesters exhibit the highest toxicity. In higher dosages fatal liver function disorders might occur.

In Central Europe humans are only rarely harmed, but horses are often poisoned with pyrrolizidine alkaloids from ragwort. Ragwort is a very frugal plant, which often populates extensively farmed meadows. Pyrrolizidine alkaloids are also contained in numerous plants, which are used for teas or medicinal purposes. Typical indigenous representatives are for example Comfrey, Coltsfoot, Butterbur and Borage. →

Basic structures of pyrrolizidine alkaloids



Indigenous medicinal plants, which contain pyrrolizidine


Comfrey


Coltsfoot


Butterbur


Borage

Plant	Indication	Pyrrolizidine alkaloids
Comfrey (Symphytum officinale)	Bruises, strains, sprains	Lycopsamine, intermedine and their acetates
Coltsfoot (Tussilago farfara)	Cough, loosening mucus	Senkirkine, senecionine
Butterbur (Petasites hybridus)	Migraine, cramps, allergies	Senecionine, integerrimine
Borage (Borago officinalis)	Fever, diarrhoea, inflammations	Amabiline, intermedine, lycopsamine and supinine

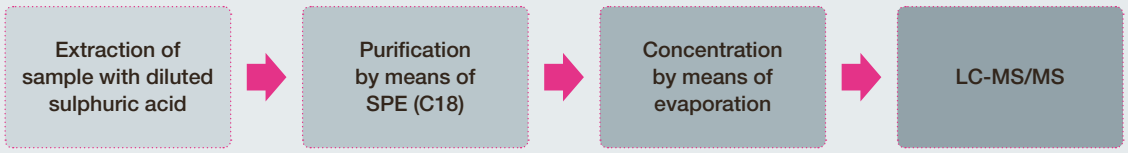
Method

For the analysis of pyrrolizidine alkaloids numerous GC methods have been developed in the past. They are basing to a large extent on the works of Wiedenfeld from the 1980s [1]. After extraction and reduction of non-volatile N-oxides to free amines and one or more steps for purification the determination takes place by means of GC-MS or GC-NPD.

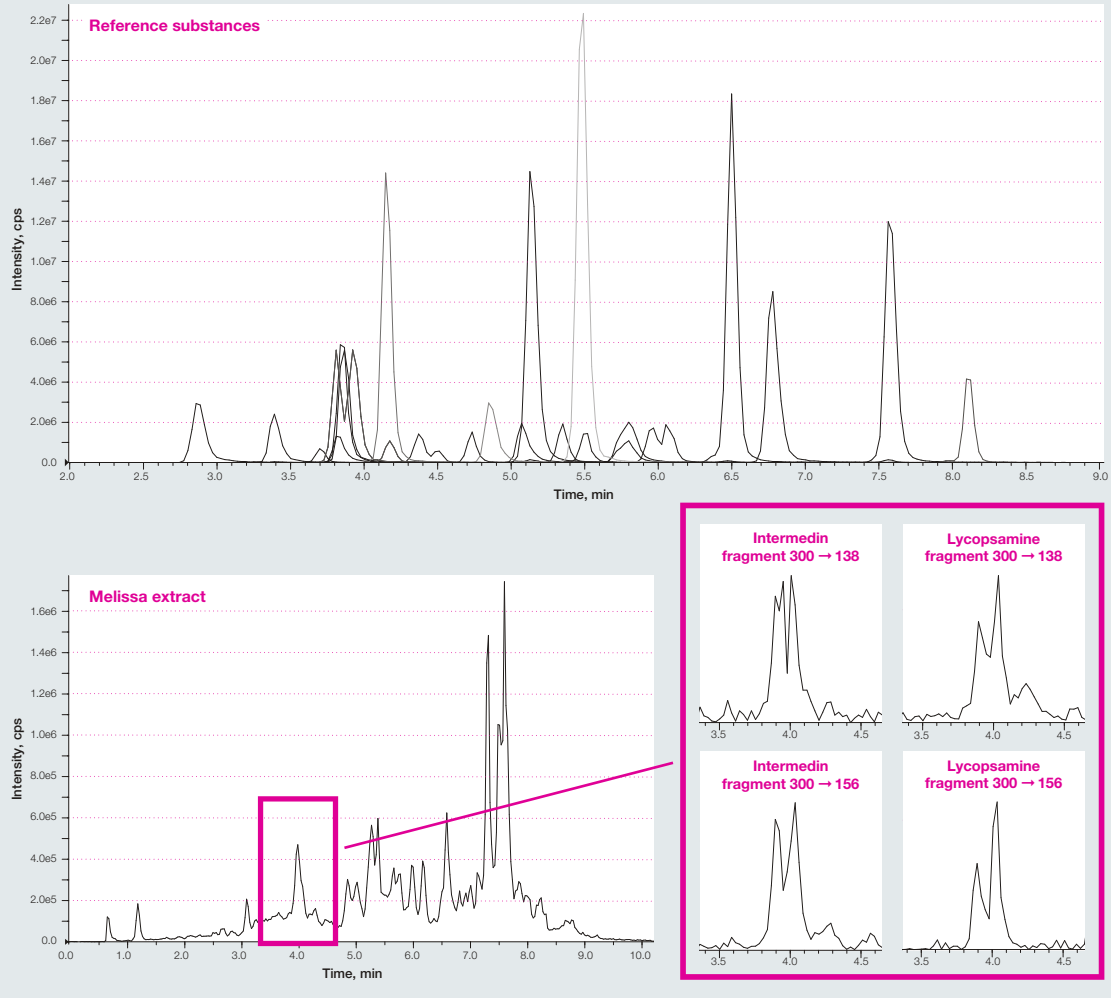
All these methods have in common that the reduction step is often hard to reproduce and the GC determination of the strong alkaline analytes is quite sensitive. By means of HPLC-MS a huge part of these problems can be circumvented. In this way it is now possible to directly determine the N-oxides, what makes it much easier. The Federal Institute for Risk Assessment published in 2014 a method, which allows the reliable determination of pyrrolizidine alkaloids and their N-oxides in plant material [2].

After extraction of the crushed plant material the extract is purified by solid phase purification and analysed after accumulation with HPLC-MS/MS. In this way numerous pyrrolizidine alkaloids can be determined. →

Analysis scheme of the BFR method



Chromatogram of a reference solution, which contains 28 pyrrolizidine alkaloids



Results

With this method pyrrolizidine alkaloids can be determined in many plant materials (teas, drugs, fresh plants, CO_2 extracts etc.). The determination limits lie – depending on the sample and type of pyrrolizidine alkaloid – in the range from 1 to 20 µg/kg. However, the method requires the availability of reference substances. This fact is currently the limiting factor, as only about 30 pyrrolizidine alkaloids are commercially available as pure substances.
An HPLC chromatogram of 28 reference compounds is shown in the upper part of the chromatogram (see previous page), whereby Monocrotalin with a retention time of 2.9 minutes eluted as first compound and Lasiocarpin-N-oxide with 8.1 minutes as the last compound [2]. As current example the HPLC chromatogram of a melissa extract is depictured (lower part of the chromatogram), in which the two compounds Intermedin (0.004 µg/kg) and Lycopsamine (0.004 µg/kg) can be evidenced. The isomer compounds Intermedin and Lycopsamine have both been clearly identified by means of the two characteristic fragments m/z = 138 and 156. The respective signals are reflected in the chromatogram. Basing on the achieved results the presence of comfrey can be assumed.

Discussion & conclusion:

For some time there are general recommendations for the maximum exposition of pyrrolizidine alkaloids from various sides. Against this background numerous studies have been conducted in the last years. In these pyrrolizidine alkaloids have been detected in numerous preparations, which have been produced with plants, which biosynthesise no pyrrolizidine alkaloids. It is assumed that impurifications with weeds such as plant species of the genus Senecio (Ragwort) are responsible for this. As already small amounts of weed can lead to toxic relevant concentrations, the German Federal Institute decided that for herbal drug products the limits of 1 µg pyrrolizidine alkaloids related to the maximum daily dose must not be exceeded [3].

Assuming that per day a maximum of 10 g of the herbal drug is consumed, the required determination limit for the sum of pyrrolizidine alkaloids is 100 µg/kg. Depending on the number of pyrrolizidine alkaloids contained in a sample the determination limits must be set respectively lower. In most cases there are no more than five relevant pyrrolizidine alkaloids contained in a plant in relevant amounts. Thus the method described here is even sensitive enough in the worst case, to test an herbal drug product on pyrrolizidine alkaloids. □

About the author

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1) Wiedenfeld et al., Planta Medica, 1981, Vol. 41

2) Federal Institute for Risk Assessment PA-Tee-2.0/2014

3) Federal Institute for Medicinal Products, announcement from 01st March 2016

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