An apple per da

Mycotoxins are a world-wide probl

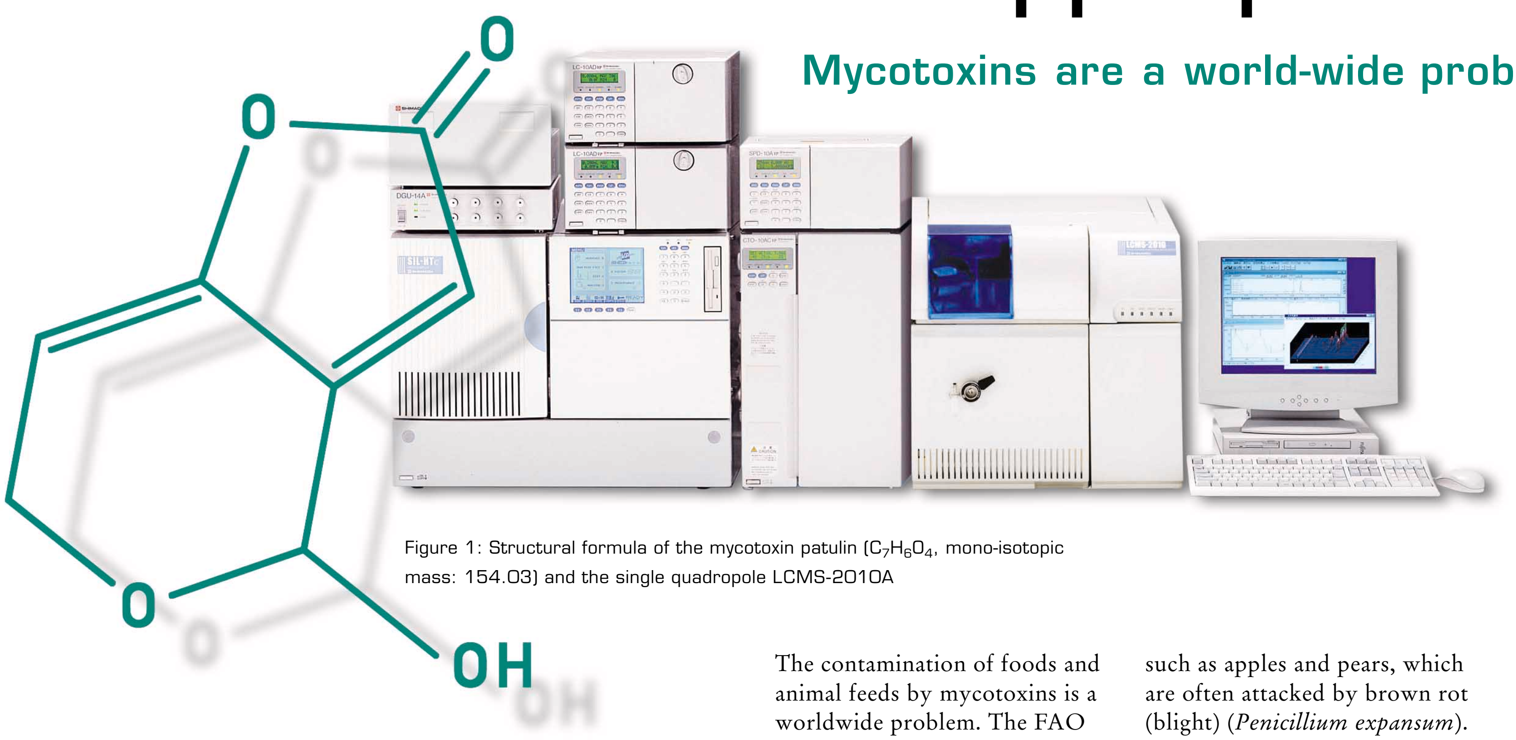

Figure 1: Structural formula of the mycotoxin patulin ($C_7H_6O_4$, mono-isotopic mass: 154.03) and the single quadropole LCMS-2010A

Fungal toxins have posed a human health hazard since the early days of food crop cultivation. Already in the Bible, there are reports of a disease that can occur after digestion of ergot. In the Middle Ages hundreds of thousands of people died due to ergot poisoning.

In search of the cause for particular diseases that rule out known triggers such as microorganisms, plant toxins or pesticide residues, mycotoxins were discovered. Over time it was established that foods infected with fungi are responsible for several diseases in livestock. That the same compounds could also be present in human foods and be the possible causes of disease was only discovered when analytical research methods became more sensitive and a large number of foods and food components could be analysed.

The contamination of foods and animal feeds by mycotoxins is a worldwide problem. The FAO (UN *Food and Agriculture Organisation*) estimates that up to 25 % of the world food production is affected by mycotoxins. In approximately 20 % of the cereal crops in the EU, mycotoxins can be detected. To date there are hardly any consolidated findings about the effects of small amounts – especially during lifelong uptake.

The Mycotoxin Patulin

Patulin is formed by various *Penicillium-*, *Aspergillus-* and *Byssochlamis-species*. *Penicillium-expansum* is the main cause of rot in apples and many other types of fruits and vegetables. Main sources are therefore all types of fruit, especially pomaceous fruit such as apples and pears, which are often attacked by brown rot (blight) (*Penicillium expansum*).

Mycotoxins can also attack peaches, apricots and cherries. Citrus fruits and plums are less problematic. In approximately 40 % of brown rot in apples, patulin could be detected. The infected areas in apples can contain more than 80 mg/kg patulin. This means that only a small number of mouldy apples can cause patulin contaminations in large volumes of apple juice to patulin concentrations of 50 µg/kg or even higher.

EU Commission limits the patulin mycotoxin in apple products

Since November 2003, the following maximum concentrations

Concentration (ppb)	1	2	3	Mean value (1-3)	%RSD
5	1293	1266	1216	1258	3.10
10	2773	2815	3027	2872	4.74
25	6560	6271	7081	5402	7.60
50	14006	14425	14441	14291	1.73
100	28602	29175	28655	28811	1.10
250	69991	75810	74773	73525	4.22
500	165437	161378	156406	161074	2.81
1000	291363	280538	278373	283425	2.46

Table 1: Calibration from 5 ppb up to 1000 ppb

y keeps the doctor away?!

em – Highly sensitive determination of the fungal toxin patulin

of patulin in apple products have been established in the European Union.

The mycotoxin patulin is a five-ring unsaturated lactone [4-Hydroxy-4H-furo(3.2-c) pyran-2(6H)-on ($C_7H_6O_4$)].

More selective, faster detection using LC/MS

The analysis of patulin in foods is commonly carried out according to the DIN ISO 8128-1 method using HPLC/PDA. There is, however, a growing trend to use the hyphenated technique HPLC-MS for identification and quantification. In order to attain a higher sensitivity and selectivity for the determination of patulin at the trace level, an HPLC method using mass spectrometric detection was developed as described below. This method uses Shimadzu's single quadrupole LCMS-2010A for mass spectrometric detection.

The chromatographic separation was carried out on a 2 mm reversed phase column in less than 15 minutes. The toxin is protonated in the mass spectrometer via APCI (atmospheric pressure chemical ionisation) (detected at $[M^{+}H^{+}]^{+}$ = 155 m/z). Using this analysis method it is possible to detect patulin safely down to the sub-ppb range. Figures 2 and 3 show the chromatogram in single ion monitoring (SIM) mode (at m/z = 155) and a calibration from 5 to 1000 ppb with excellent linearity.

Not only patulin can be detected with excellent sensitivity using LC/MS. Other highly sensitive routine LC/MS methods exist for mycotoxins such as aflatoxins, ochratoxin and fusaric toxins such as deoxynivalenol. Due to the high selectivity of MS detection, the simultaneous determination of various toxins in a single analysis is also possible.

The use of such highly sensitive detection methods for mycotoxins in foods enables accurate monitoring of the mycotoxin concentration and forms the basis for further research into health hazards that can arise from mycotoxin contamination in foods.

We will gladly send you further information. Please note the appropriate number on your reader reply card.
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Product	Maximum concentration (µg/kg)
Fruit juices, especially apple juice, and fruit juice additives in other beverages, fruit nectars and fruit concentrates, spirits, apple cider and other beverages derived from apples or apple juice containing fermented beverages	50
Solid apple products intended for direct consumption, including apple compote and apple sauce	25
Apple juice and solid apple products, including apple compote and apple puree for nursing and infants, which are marked and sold for this purpose. Other supplements for nursing and infants.	10

Table 2: Maximum concentration for Patulin

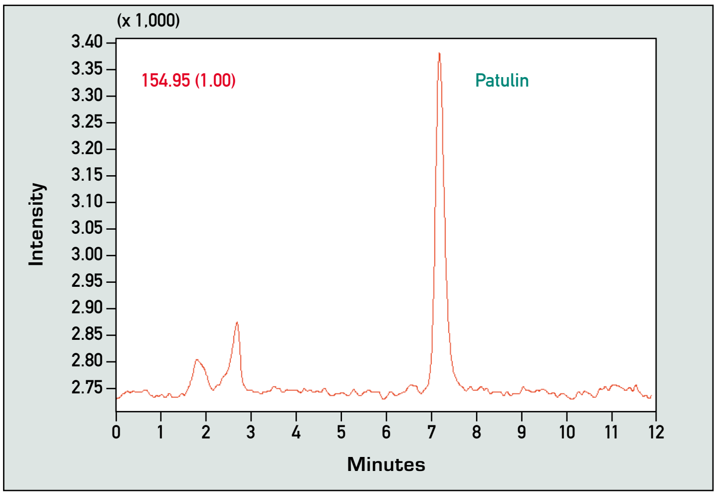

Figure 2: Detection of patulin using APCI in SIM mode

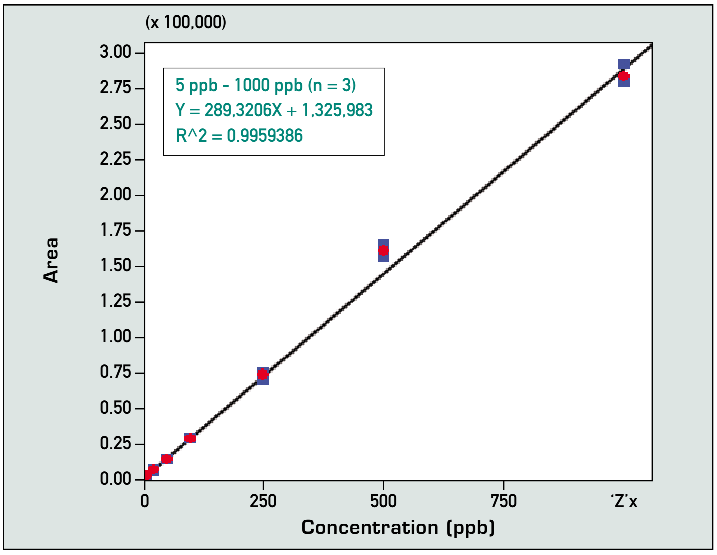

Figure 3: Calibration from 5 ppb up to 1000 ppb