UNIVERSITEIT GENT

FACULTEIT FARMACEUTISCHE WETENSCHAPPEN

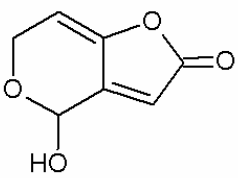
New insights into electrospray ionization of patulin

S. Malysheva [1]*, J. Diana Di Mavungu [1], J. Boonen [2], B. De Spiegeleer [2], I. Yu. Goryacheva [3], C. Van Peteghem [1] and S. De Saeger [1]

[1] *Laboratory of Food Analysis, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium*
[2] *Drug quality and registration (DruQuaR) group, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium*
[3] *Saratov State University, Chemistry Institute, Department of Common and Inorganic Chemistry, Saratov, Russia*

Introduction

Patulin is a mycotoxin produced by several fungal species, mainly by *Penicillium* spp. and *Aspergillus* spp. Since patulin-producing fungi are widely spread, this toxin has been detected in food (fruit- and vegetable-based products, cereal products, cheese), feed and even in mouldy water-damaged dwellings. Co-occurrence of patulin with other mycotoxins has also been reported.



Structure of patulin
(MW=154 g/mol)

Patulin is commonly analyzed by liquid chromatography (LC) with UV-detection. LC coupled to mass spectrometry (MS) is considered as a more specific tool for mycotoxin detection and confirmation. However, the implementation of this technique for the determination of patulin, especially in the context of a multi-mycotoxin analysis, is limited due to ionization problems.

The aim of the current study was the optimisation of the electrospray ionization of patulin in positive mode (ESI+) with the view of a concomitant LC-MS/MS analysis with other mycotoxins.

Experimental

A Micromass Quattro LC triple quadrupole (Waters, Milford, MA, USA) operated in positive and negative ionization mode equipped with ESI source was used for the preliminary experiments. The data were acquired using MassLynx™ software (Micromass, Manchester, UK).
For the investigation of patulin fragmentation pattern and for fragment assignment an Ion Trap Thermo Finnigan MS (Thermo Electron, San José, USA) and an Orbitrap Exactive™ (Thermo Electron, San José, USA) were utilized. For both instruments, an Xcalibur software was used for data acquisition.

Different organic modifiers, mobile phase additives and pH were investigated:

1. H_2O/0.2 M NH_4HCO_3 (pH 10)/MeOH (29/5/66)
2. H_2O/0.2 M NH_4HCO_3 (pH 10)/ACN (29/5/66)
3. H_2O/MeOH (30/70) containing 5 mM CH_3COONH_4 (pH 6.8)
4. H_2O/ACN (30/70) containing 5 mM CH_3COONH_4 (pH 6.8)
5. H_2O/MeOH/CH_3COOH (30/69/1) containing 5 mM CH_3COONH_4 (pH 4.8)
6. H_2O/ACN/CH_3COOH (30/69/1) containing 5 mM CH_3COONH_4 (pH 4.8)
7. H_2O/MeOH (30/70) containing 0.1 % HCOOH (pH 3)
8. H_2O/ACN (30/70) containing 0.1 % HCOOH (pH 3)

Tuning solution of patulin was prepared in the mobile phase at concentration 10 ng/µL. The solution of patulin coming from a syringe pump at a flow rate of 10 µL/min was mixed with the mobile phase (0.15 mL/min) through a T-shaped connector.
An experimental design (Modde 5.0) was applied for the optimisation of MS conditions for patulin signal in the most suitable mobile phase.

Results

Preliminary experiments demonstrated that a very low patulin signal was observed in ESI- in all tested mobile phases. However, under alkaline conditions and using methanol as organic modifier, a high ESI+ signal was obtained, corresponding to a patulin adduct with a *m/z* 187. Its product ion spectra were overall more informative and intense than those obtained with the protonated molecule (Fig. 1). Besides, the ion at *m/z* 187, an ion at *m/z* 169 was observed in the full MS spectrum, most due to a loss of water. This methanol adduct of patulin was further examined and confirmed by MS^n experiments (Fig. 1 and 2).

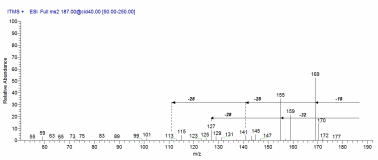


Fig. 1. MS^2 spectrum of patulin (precursor ion at *m/z* 187)

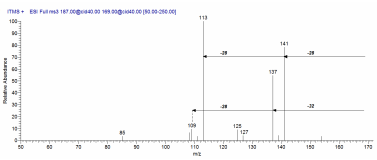


Fig. 2. MS^3 spectrum of patulin (precursor ion at *m/z* 187).

Combining the MS^n data and accurate mass measurement the assignment of fragments was achieved and a fragmentation pattern was proposed (Fig. 3).

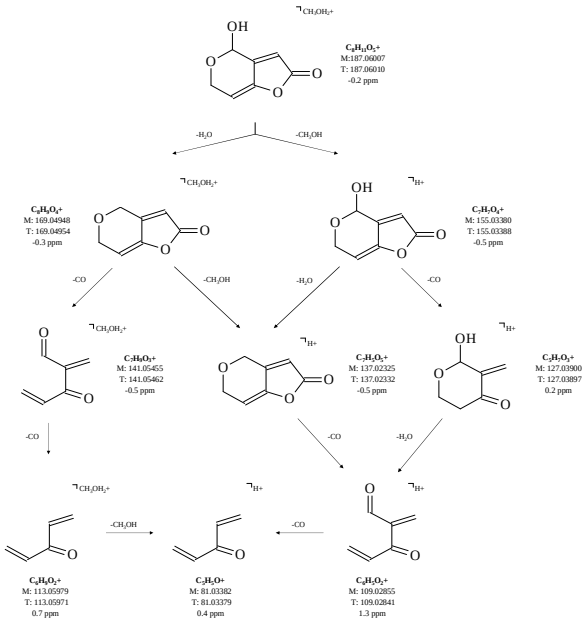


Fig. 3. Proposed fragmentation mechanism of patulin (precursor ion at m/z 187).
T – theoretical mass; M – measured mass.

Conclusions

This study for the first time suggests the use of a methanol-adduct formed under alkaline conditions for determination of patulin by means of LC-MS/MS operating in ESI+ mode. Owing to these data, patulin can be easily included in a multi-mycotoxin analysis.

Acknowledgments

This research was financially supported by FWO project G.0034.07N and BOF project, Ghent University, 24J01J08409.

* Corresponding author. Address: Harelbekestraat 72, B-9000, Ghent, Belgium.
E-mail: Svetlana.Malysheva@UGent.be