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# ***Determinazione della moniliformina mediante cromatografia con ioni $\text{La}^{3+}$ in fase mobile***

***VI Congresso Nazionale  
Micotossine e Tossine Vegetali  
nella filiera agro-alimentare  
10-12 giugno 2019***

# Micotossine emergenti

Definite come: “*mycotoxins, which are neither routinely determined, nor legislatively regulated; however, the evidence of their incidence is rapidly increasing*”

(Vaclavikova et al. Food Chem. 2013, 136, 750–757)

Metaboliti da *Fusarium*



enniatine, beauvericina, **moniliformina**,  
fusaproliferina, acido fusarico,  
culmorina, butenolide

Metaboliti da *Aspergillus*



sterigmatocistina, emodina

Metaboliti da *Penicillium*



acido micofenolico

Metaboliti da *Alternaria*



alternariolo, alternariolo monometil  
etere, acido tenuazonico, tentossina

(Gruber-Dorninger et al., J. Agric. Food Chem. 2017, 65, 7052–7070)

# Moniliformina

EFSA



## SCIENTIFIC OPINION




ADOPTED: 21 November 2017

doi: 10.2903/j.efsa.2018.5082

### Risks to human and animal health related to the presence of moniliformin in food and feed

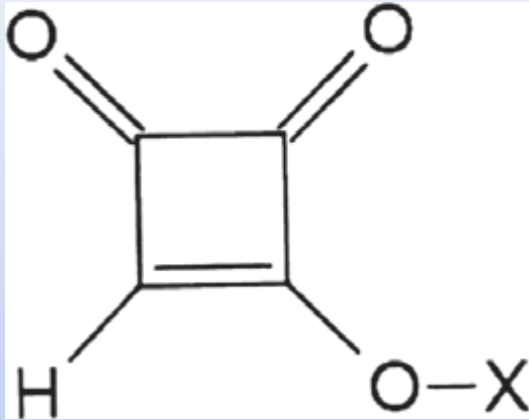
*...The limited information available on toxicity and on toxicokinetics in experimental and farm animals indicated haematotoxicity and cardiotoxicity as major adverse health effects of MON. MON causes chromosome aberrations in vitro but no in vivo genotoxicity data and no carcinogenicity data were identified...*

*...the collection of more occurrence data on MON in food and feed is recommended to enable a comprehensive human risk assessment...between 2001 and 2016 was a total of **3205, 806, and 504** analytical results of MON for food, feed and unprocessed grains... *

**4515 dati in 15 anni →300/anno**

*...the collection of more occurrence data on MON in foods and feeds with state-of-the-art validated analytical methods*

# Moniliformina



X:	Molecular weight (g/mol)
H	98
Na	120
K	136

Molecola a basso peso molecolare e altamente polare; basso pKa (< 1,7), presente come sale di Na o di K.

Principalmente prodotta da:



*Fusarium avenaceum*, *F. proliferatum*, *F. subglutinans*, *F. tricinctum* e *F. verticilloides*  
*Penicillium melanoconidium* (stoccaggio)

Tossicità:



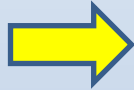
degenerazione del miocardio, debolezza muscolare e patologie respiratorie

Presente soprattutto nei cereali.

# Analisi MON

MON poco trattenuta dalle più comuni fase stazionarie per cromatografia in fase inversa.

## Determinazione analitica



RP-18 + FLD (derivatizzazione)  
*(Filek and Lindner, 1996)*

RP-18 + MS  
*(Sewram et al, 1999)*

RP-18 con formazione di coppie ioniche + UV  
*(Kandler et al, 2002; Pena et al, 2019)*

HILIC + UV o MS  
*(Sorensen et al, 2007; Scarpino et al, 2013; Barthel et al, 2018)*

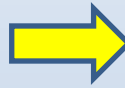
Fasi stazionarie specifiche per composti polari + MS o HRMS  
*(von Bargaen, 2012; Hallas-Moller et al, 2016; Herrera et al, 2017)*

Difficoltà: presenza co-eluzione (effetto matrice in MS), risoluzione cromatografica, 1 sola frammentazione (HRMS vs MS-MS).

Purificazione dell'estratto.

# Analisi MON

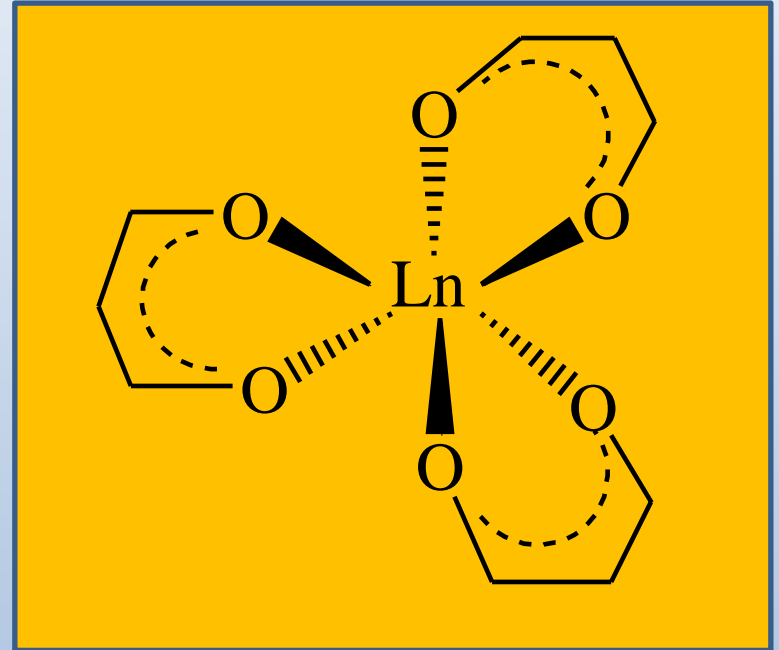
MON è un  $\alpha$ -di-chetone;  
possibilità formazione complessi  
con gli ioni dei metalli lantanidi,  
come  $\text{La}^{3+}$ ,  $\text{Tb}^{3+}$  o  $\text{Eu}^{3+}$ .



Aggiunta di un sale di Lantanide nella fase  
mobile



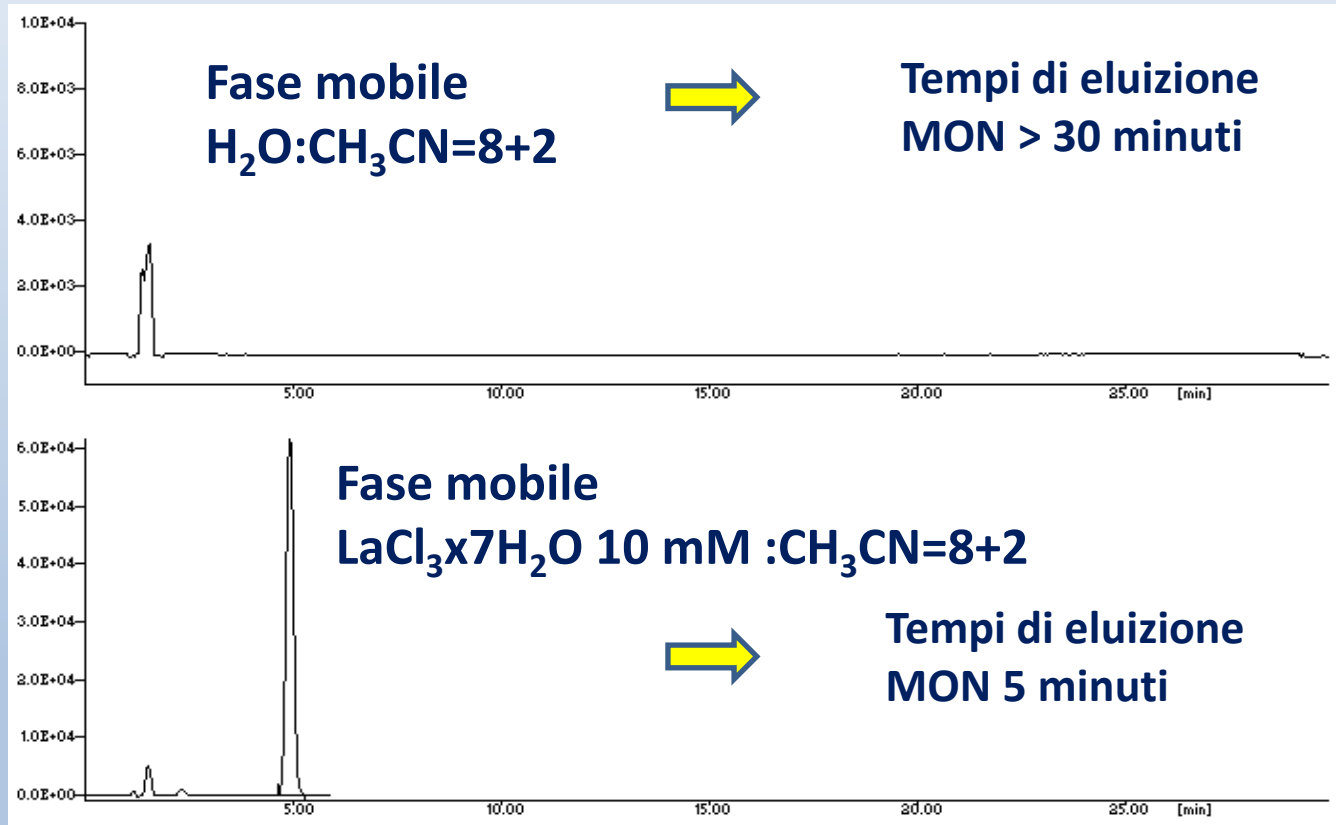
**migliore separazione cromatografica**



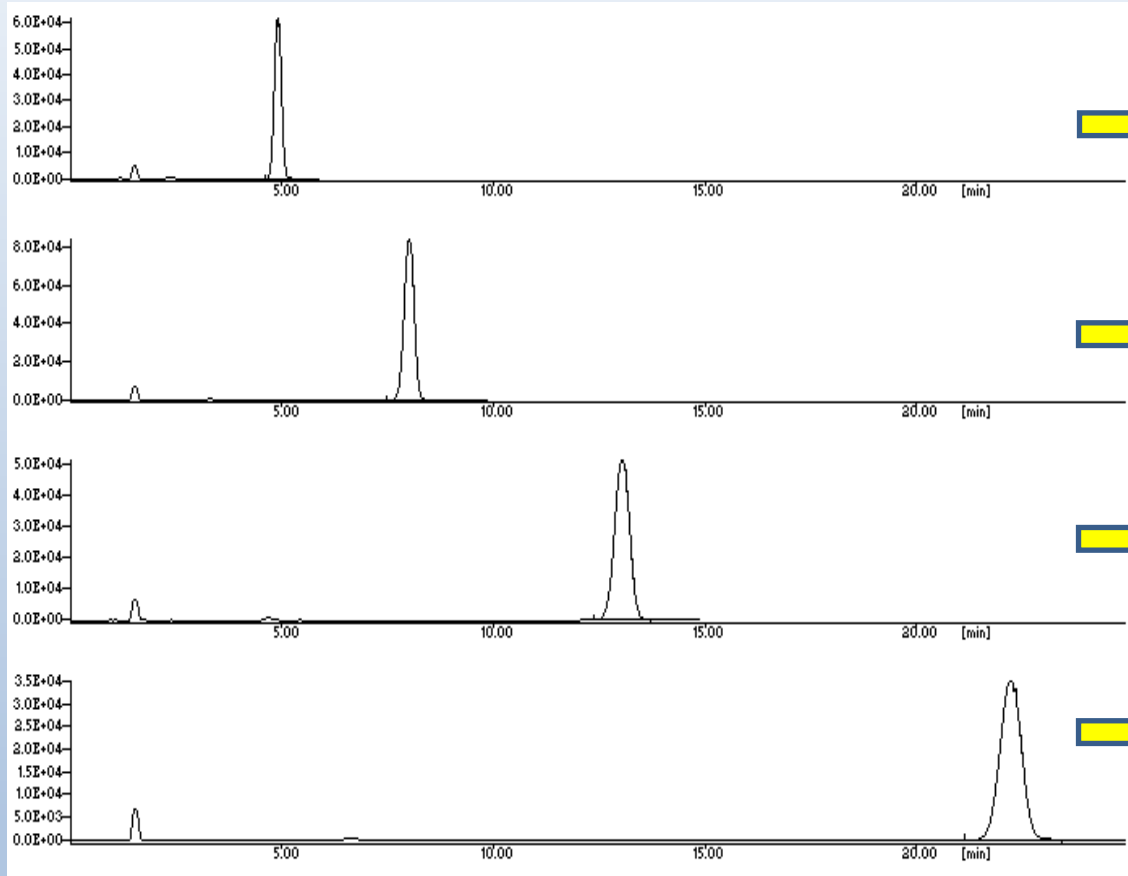
K.Binnemans, Rare Earth  $\beta$ -diketonates, K.A. Gschneidner Jr., J.C.G. Bünzli, V.K., Perchasky (Eds.), Handbook of the Physics and Chemistry of Rare Earths, 25, Elsevier, Amsterdam, 2005.

# Analisi MON

Fase stazionaria colonna RP-ammino → alta polarità.



# Analisi MON



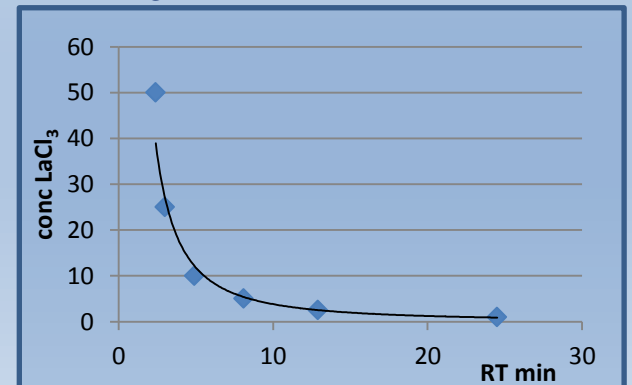
→  $\text{LaCl}_3 \cdot 7\text{H}_2\text{O}$  10 mM

→  $\text{LaCl}_3 \cdot 7\text{H}_2\text{O}$  5 mM

→  $\text{LaCl}_3 \cdot 7\text{H}_2\text{O}$  2,5 mM

→  $\text{LaCl}_3 \cdot 7\text{H}_2\text{O}$  1 mM

Stessi risultati con  $\text{Tb}^{3+}$  e  $\text{Eu}^{3+}$ .



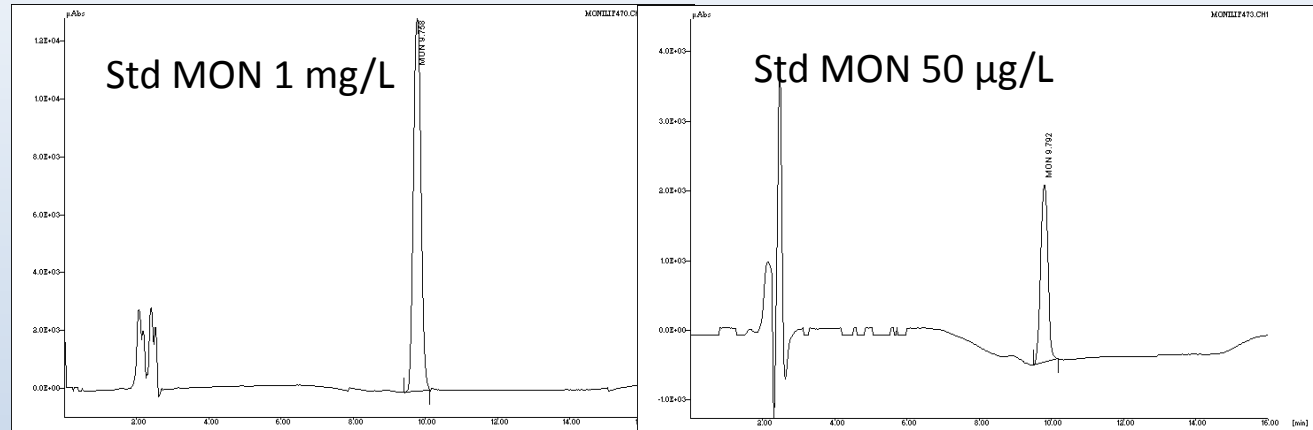


# Analisi MON

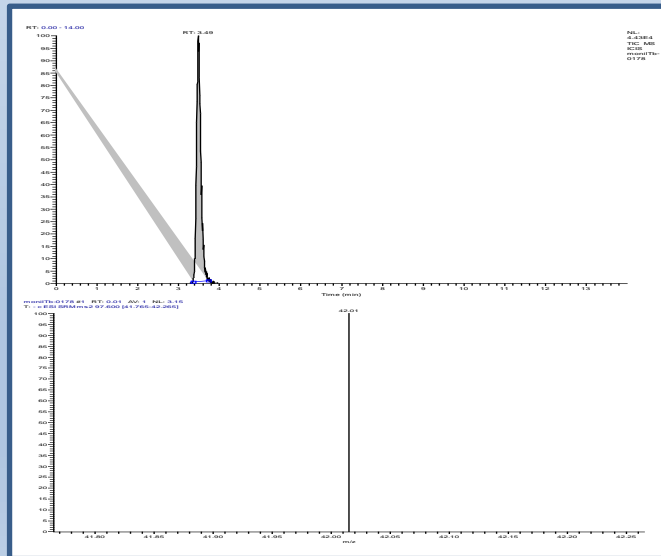
Gradiente

$\text{LaCl}_3 \cdot 7\text{H}_2\text{O}$  2,5 mM :  $\text{CH}_3\text{CN}$

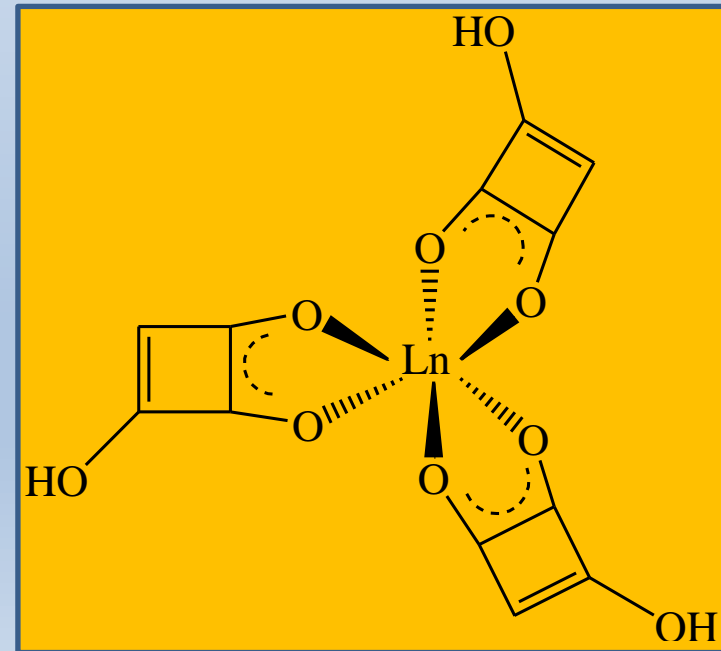
UV 260 nm



LC-MS/MS



Possibile struttura complesso  $[\text{Ln}(\text{MON})_3]$



# Analisi MON

## Purificazione estratto campione (no colonne immuno-affinità)

SPE C18 (trattiene impurità) (*Kandler et al, 2002*)

SPE SAX + Evaporazione N<sub>2</sub> (*Sorensen et al, 2007; von Bargen et al, 2012*)

Polimeri molecolari (MISPE) (*Appell et al, 2007*)

Mycosep 240 (*Scarpino et al, 2013*)

Evaporazione N<sub>2</sub> ❌

Estrazione H<sub>2</sub>O (*Herrera et al, 2017*)

o H<sub>2</sub>O:CH<sub>3</sub>CN 1+1

No purificazione

Evaporazione N<sub>2</sub> ❌

Quechers ❌

Estrazione H<sub>2</sub>O:CH<sub>3</sub>CN 1+1 (*Barthel et al, 2018*)

No purificazione

Extraction	Clean-up	Evaporation	<sup>a</sup> Recovery (%)
<sup>a</sup> ACN/water (84:16, v/v)	No	No	69%
<sup>a</sup> ACN/water (84:16, v/v)	SAX	Dryness	0%
<sup>a</sup> ACN/water (84:16, v/v)	MycoSep@ 240 MON	No	55%
<sup>a</sup> ACN/water (84:16, v/v)	MycoSep@ 240 MON	0.5 mL	20%
<sup>a</sup> ACN/water (84:16, v/v)	MycoSep@ 240 MON	Dryness	25%
<sup>a</sup> ACN/water (84:16, v/v) + 1% acetic acid	MycoSep@ 240 MON	0.5 mL	30%
<sup>a</sup> ACN/water (84:16, v/v) + 1% formic acid	MycoSep@ 240 MON	0.5 mL	5%
<sup>a</sup> QuEChERS	MycoSep@ 240 MON	0.5 mL	27%
<sup>b</sup> ACN/water (50:50, v/v)	No	No	85%
<sup>b</sup> 100% water	No	No	102%



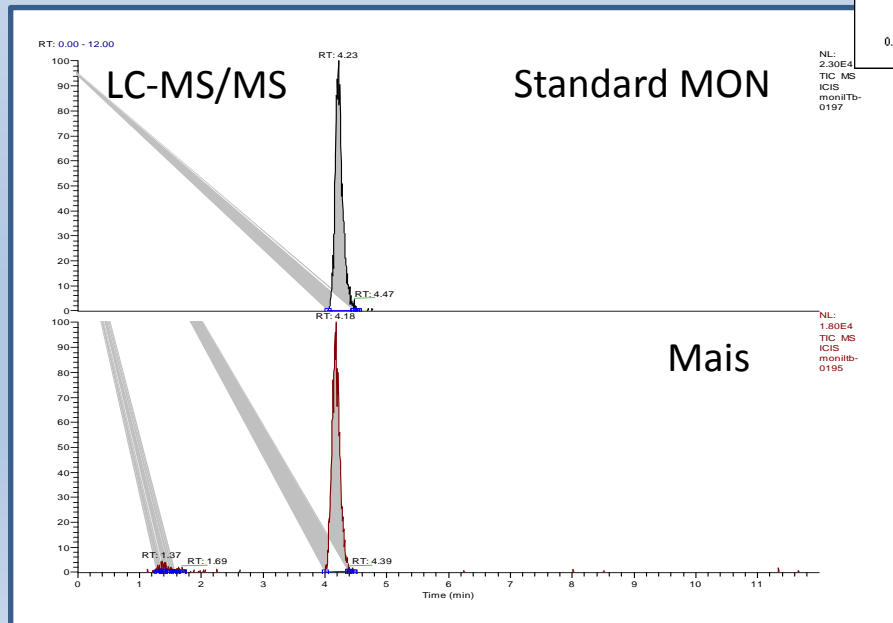
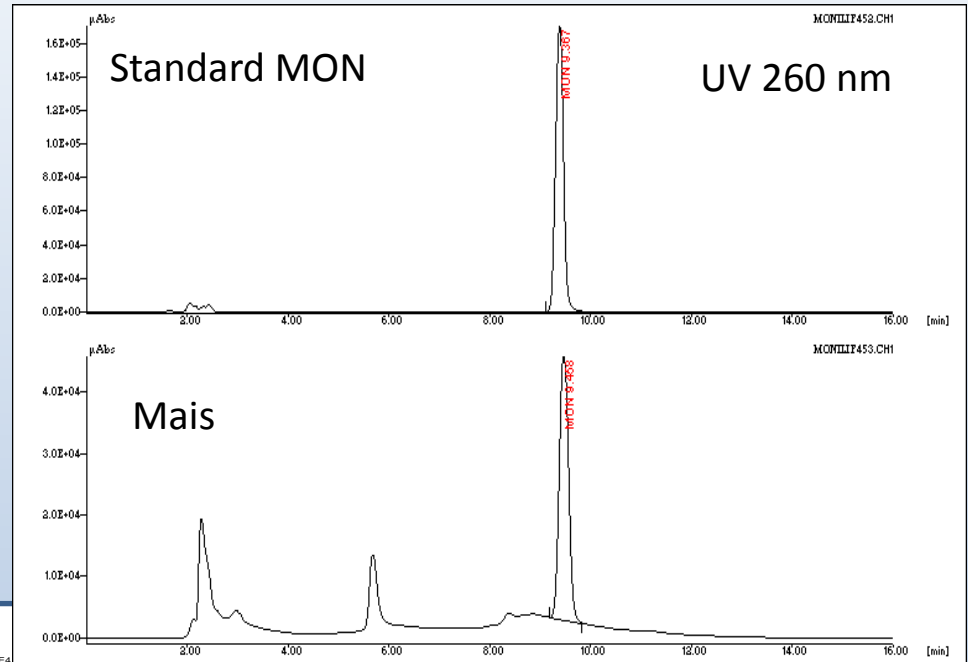
# Analisi MON

## Purificazione estratto

Estrazione  $\text{H}_2\text{O}:\text{CH}_3\text{CN}$  1+1

Purificazione su SPE RP-Ammino

Eluizione sol.  $\text{LaCl}_3 \cdot 7\text{H}_2\text{O}$  12,5 mM

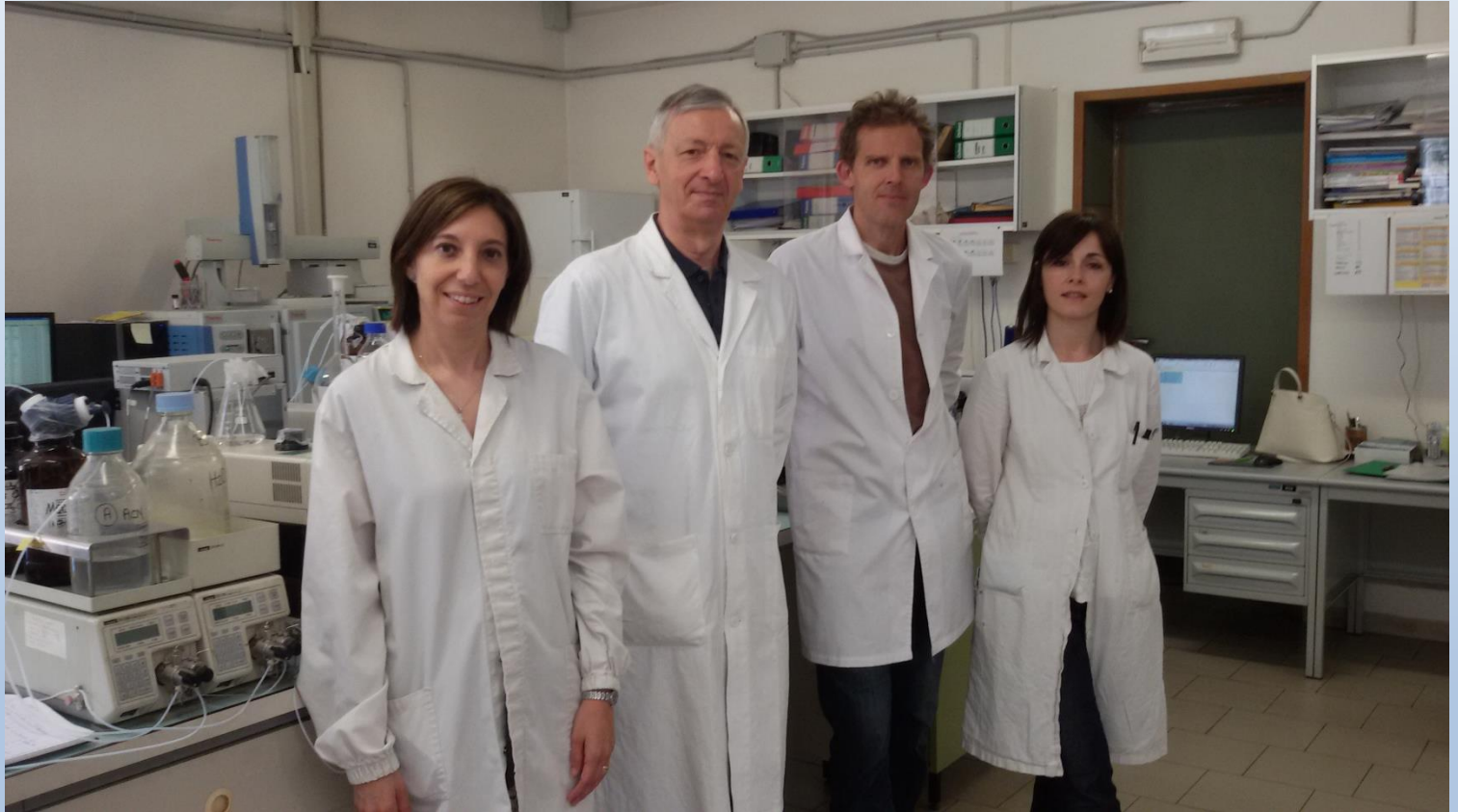


Estrazione  $\text{H}_2\text{O}:\text{CH}_3\text{CN}$  1+1

No Purificazione

Gradiente  $\text{CH}_3\text{OH}:\text{LaCl}_3 \cdot 7\text{H}_2\text{O}$  1,25 mM  
ESI – 97 → 42.

*Grazie per l'attenzione*



*VI Congresso Nazionale Micotossine e Tossine Vegetali  
nella filiera agro-alimentare 10-12 giugno 2019*