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LABORATOIRE DE RECHERCHE ALIMENTAIRE

AOAC Europe / ASFILAB meeting

Vitamins and Food Contaminants by UPLC/MS/MS

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LAREAL presentation

- LAREAL is a food research and analysis laboratory in food-processing :
  - Feed
  - Petfood
  - Human food
  - Health foods and nutraceuticals

- 30 compounds analysed by LC/MS/MS :
  - Food contaminants
  - Vitamins
  - Drugs

- LAREAL started with LC/MS/MS in 2004

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LC/MS at LAREAL

LAREAL develops HPLC/MS/MS with Customer agreement :

- To reduce run time :
  - *Simplification of sample preparation*
  - *Simplification of HPLC analysis (without derivatization …)*

- To improve detection limits :
  - *Sensibility of mass spectrometer*

- To secure the result :
  - *Specificity of mass spectrometer*
  - *Different confirmation levels (multiple MS/MS transitions)*

- To develop multi-analytes methods

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Quantification by MS/MS

- Advantages of MS/MS detectors :

  - *Sensibility*

  - *Selectivity*

  - *Can be coupled to high pressure LC (improve time, sensibility and selectivity)*

- Disadvantage of MS/MS -> ionization pertubation

  - *Competition between differents analysed molecules*

  - *Interference between molecule and matrix*

- To have a good accuracy, we use Internal Standards:

  - *The best choice is a stable isotope of the analyte (if available)*

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Melamine

2007 : First Melamine crisis in petfood

Development of a HPLC/UV method to detect contamination of Melamine in raw materials / petfood

oDisadvantages :

Long HPLC run time : 30 min

Low sensibility (LOD : 500 µg/kg)

Interferences in complex samples

oAdvantage :

Easy to run

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Melamine

2008 : Second Melamine crisis in infant milk and food

Development of a UPLC/MS/MS method to detect traces of Melamine in foods and raw materials

- Advantages :
  - Short run time : 10 min
  - No interferences
  - High sensibility (LOD : 10 µg/kg)
  - Specificity of mass spectrometry (2 transitions MS/MS)
  - Quantification by internal standard *(stable isotope of Melamine)*

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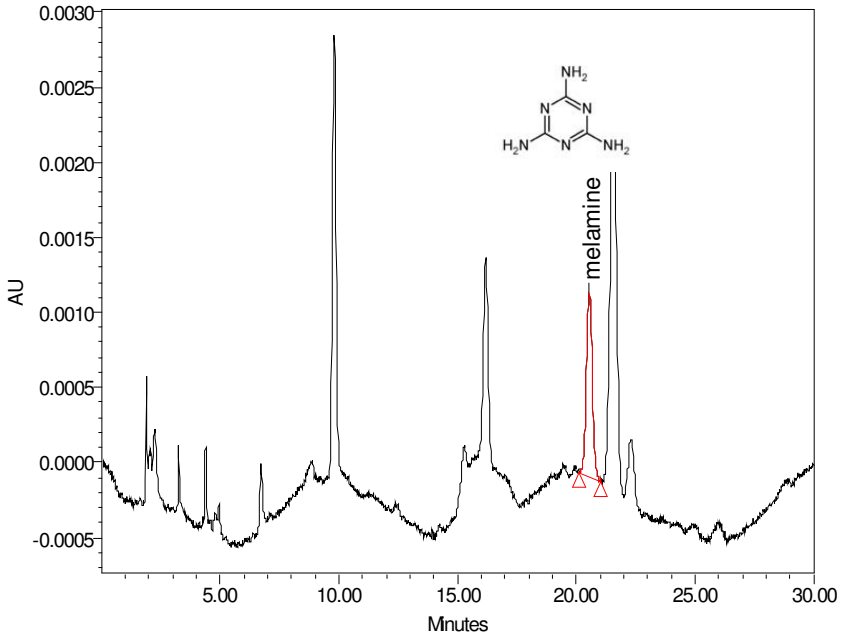
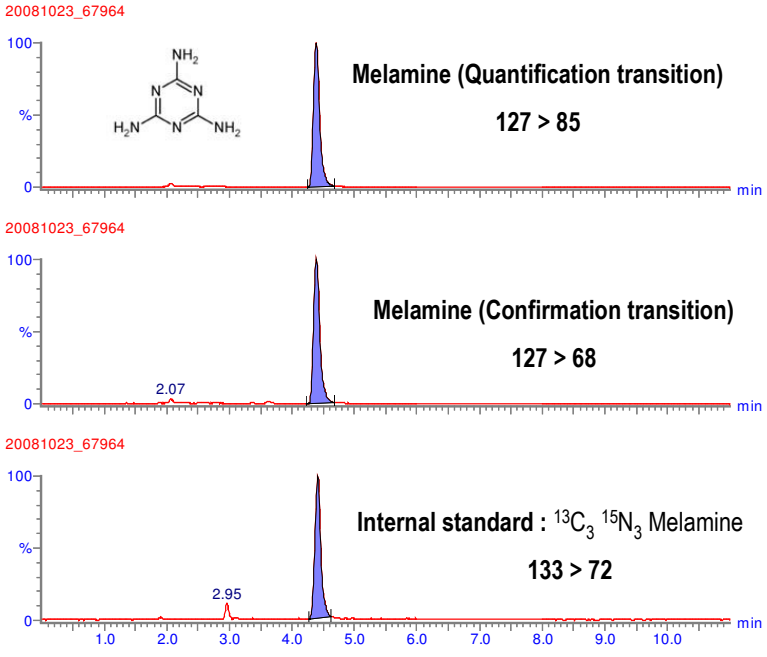
Melamine

HPLC/UV chromatogram



UPLC/MS/MS chromatogram



Soya contaminated at 1.8 mg/kg

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Melamine

	HPLC/UV (mg/kg)	UPLC/MS/MS (mg/kg)
Petfood	33,8	33,1
Blood meal	9,3	9,2
Meat and bone meal	11,1	11,1
Soybean meal	3,8	3,9
Premix	1,8	2,2
Premix	9,5	9,3
Premix	21	20

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B vitamins

Microbiology : reference methods

- Time consuming
- Quantification of a microbiologic activity *(indirect quantification with possible interferences)*

Development of HPLC/MS/MS methods

- Vitamin B3, vitamin B5 and vitamin B9
- Specific method
- Good accuracy with an internal standard quantification (stable isotope vitamins)

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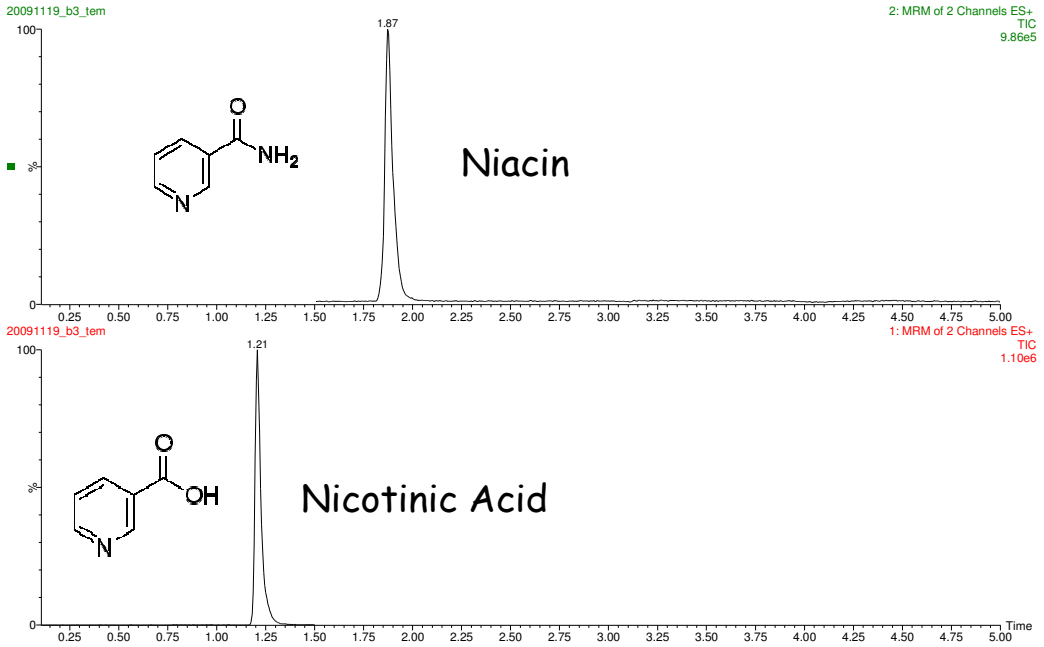
B vitamins

Vitamin B3

- 2 main forms quantified : Niacin & Nicotinic Acid
- Sample preparation : acid hydrolysis + SPE
- Quantification limit : 500 µg/kg



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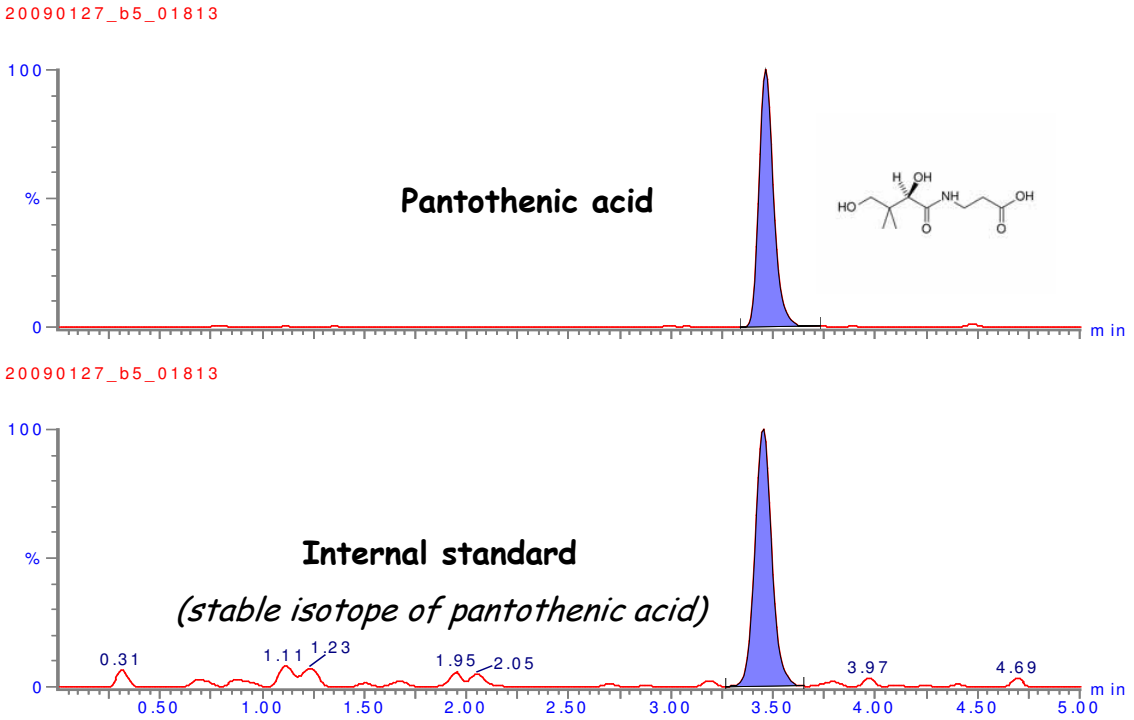
Vitamin B5

- Quantification of Pantothenic Acid
- Sample extraction : enzymatic hydrolysis + SPE
- Quantification limit : 100 µg/kg



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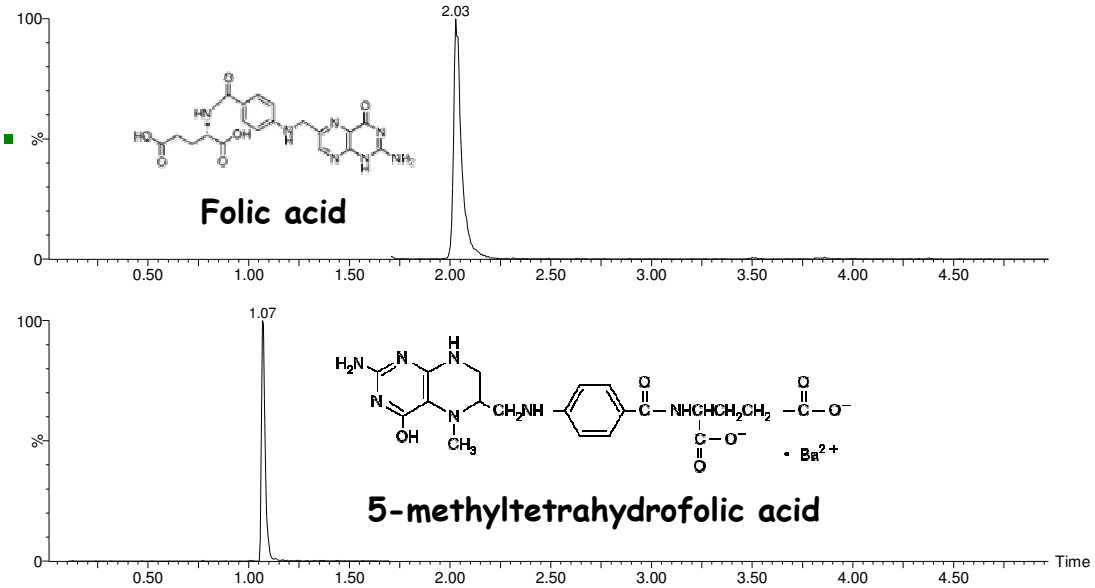
Vitamin B9

- 2 main forms quantified : Folic acid & 5-methyltetrahydrofolic acid
- Sample preparation : enzymatic extraction + SPE
- Quantification limit : 20 µg/kg



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Vitamin D_3 (Cholecalciferol)

Reference method in Foodstuffs : NF EN 12821 (HPLC/UV)

- Long and complex sample preparation

Development of a HPLC/MS/MS method

- Simplification of sample preparation
- Quantification by internal standard (stable isotope of vitamin D3)
- Good accuracy and best reproducibility

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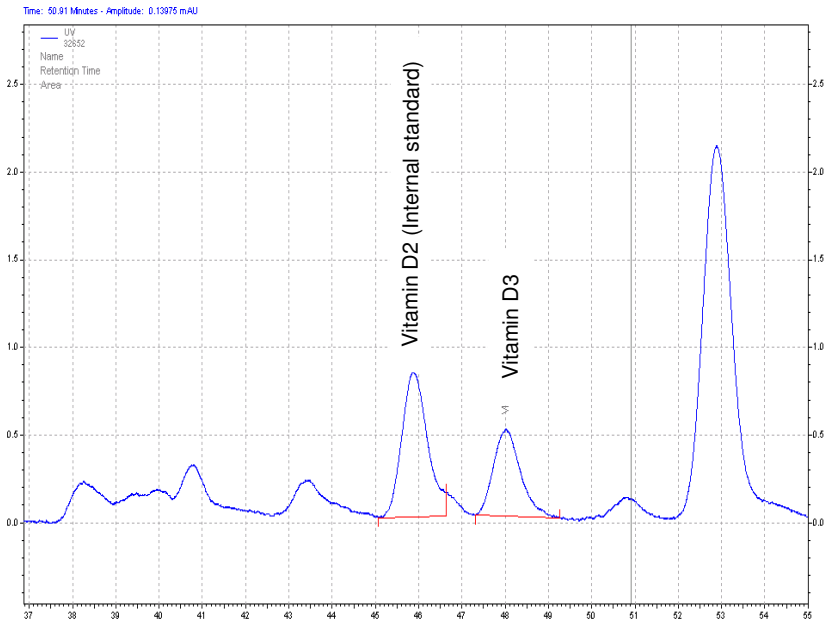
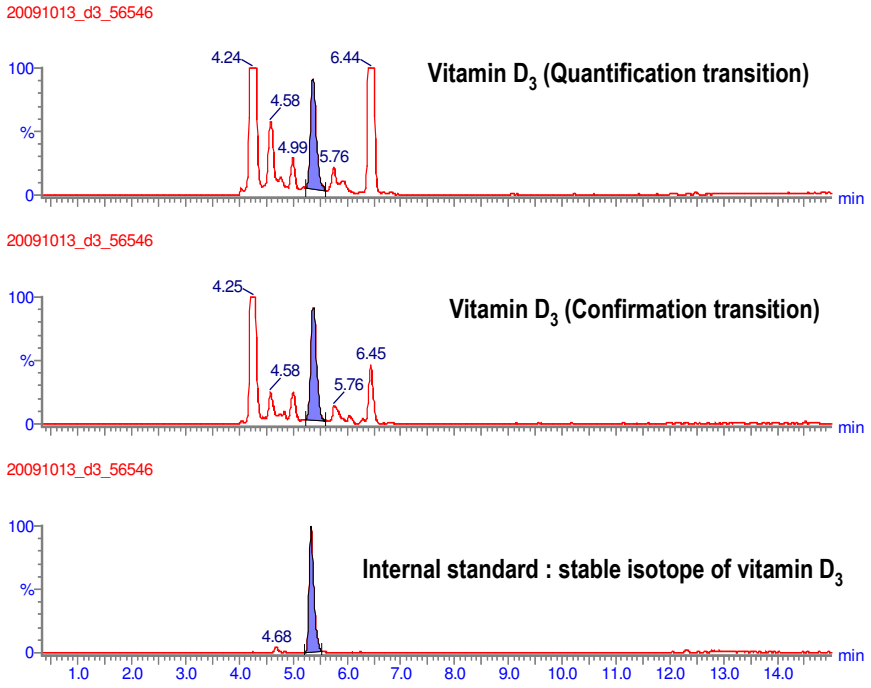
Vitamin D_3 (Cholecalciferol)

HPLC/UV chromatogram



UPLC/MS/MS chromatogram



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Petfood sample at 100 IU/100g (2.5 µg/100 g)

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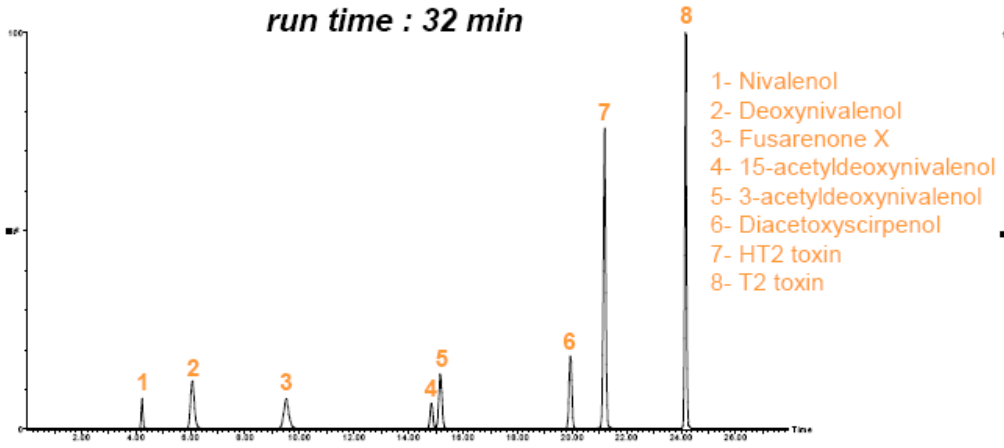
Comparison HPLC / UPLC

Comparison Trichothecenes (Mycotoxins) analysis by HPLC/MS/MS and UPLC/MS/MS

- Analysis time : UPLC is 4 times faster than HPLC

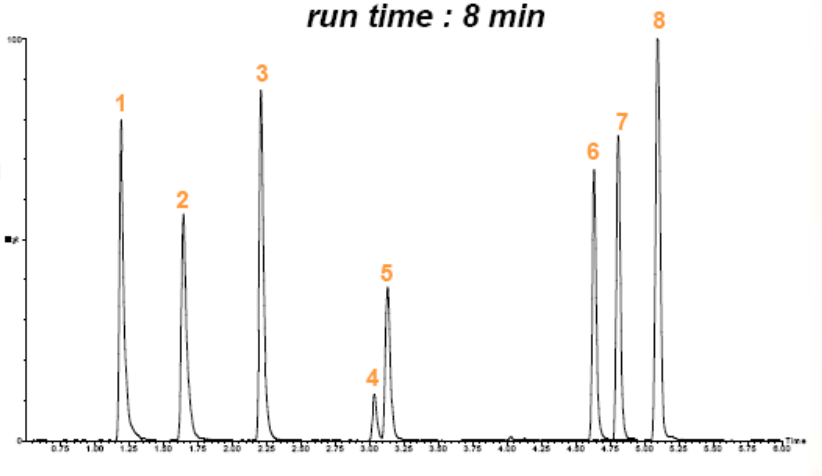
HPLC

run time : 32 min



UPLC

run time : 8 min



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Comparison HPLC / UPLC

Comparison Trichothecenes (Mycotoxins) analysis by HPLC/MS/MS and UPLC/MS/MS

- Chromatographic separation : no change

- Quantification : no significant difference

  Intra-lab reproducibility of Deoxynivalenol, in Quality Control sample :

  HPLC : RSD = 8.8 %

  UPLC : RSD = 9.0 %

- Productivity : better with UPLC

  HPLC : 1.8 sample / hour

  UPLC : 7.5 samples / hour

- Sensibility : better with UPLC

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LC/MS/MS LAREAL applications

Food contaminants	Melamine
	Cyanuric acid
	Acrylamide
	Bisphenol A
	Trichothecenes profile
	Zearalenone
	Ochratoxin A
	Wortmannin
Vitamins	Vitamin B3
	Vitamin B5
	Vitamin B9
	Vitamin D3
Others	Monensin
	Narasin
	Salinomycin
	Maduramicin
	Lasalocid
	Sulfadiazine
	Sulfadimethoxine
	Ginkgolides *(Ginkgo Biloba)*

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Thank you for your attention

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