# FT-MS et analyse en toxicologie alimentaire et environnementale


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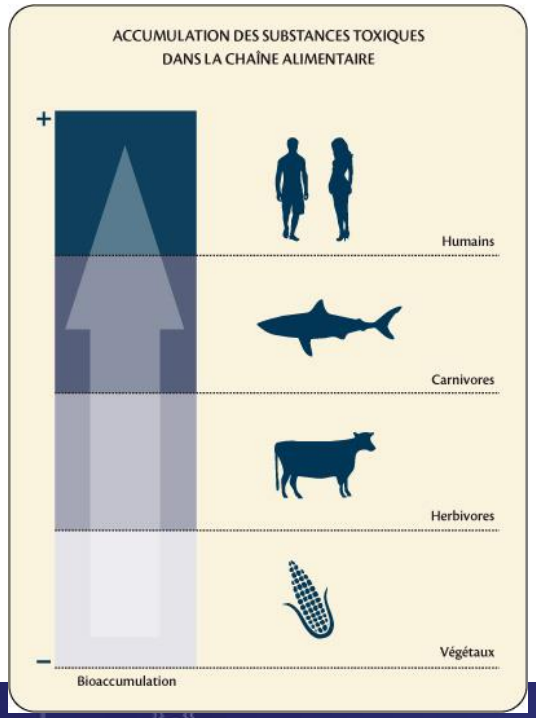
# Contaminants chimiques et alimentation

Alimentation ⇒ lien important entre l'environnement et l'homme
⇒ principale source de composés chimiques trouvés chez l'humain.

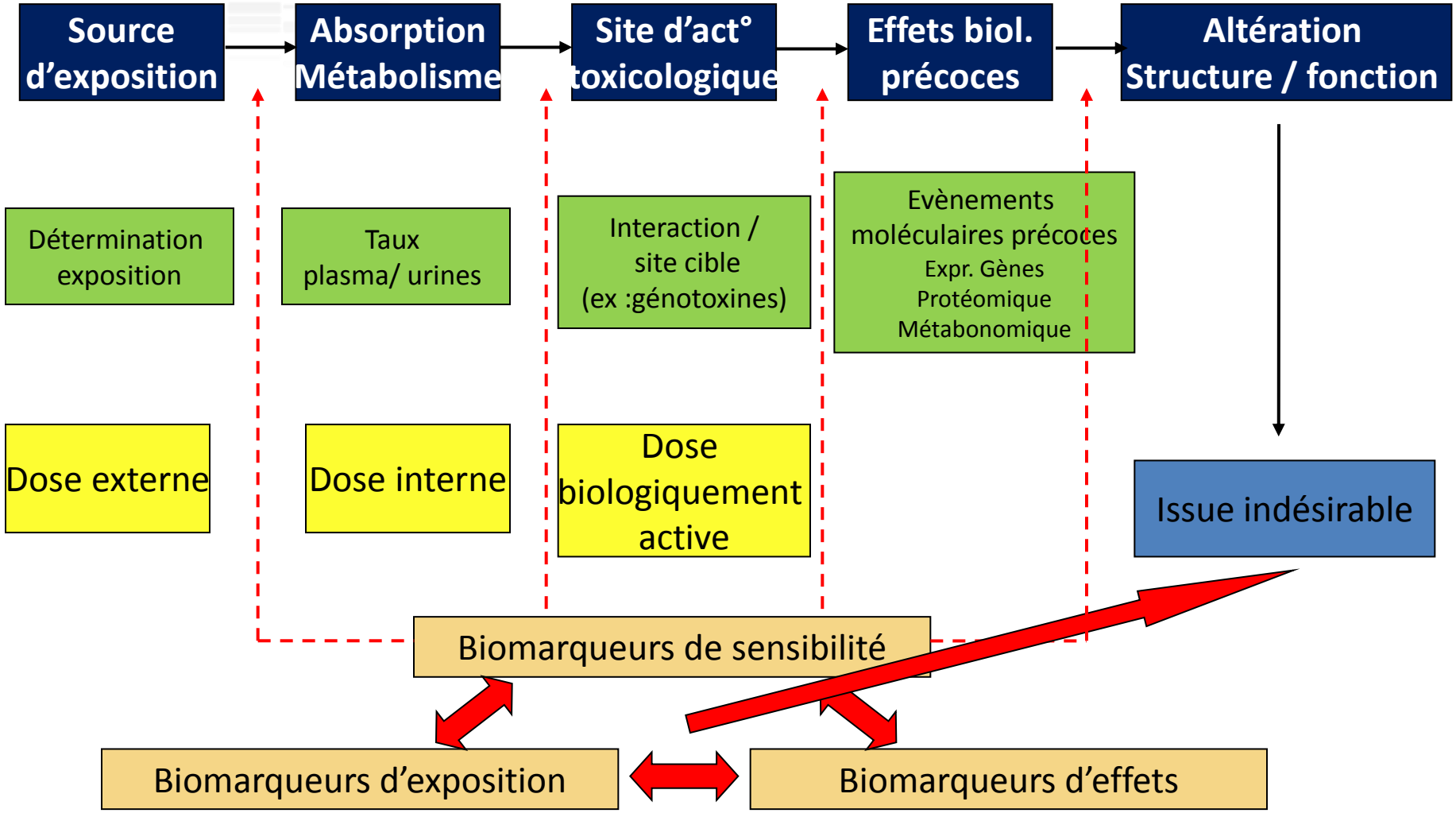
La plupart des maladies chroniques de l'homme peut être attribuée à l'exposition passée ou présente à des contaminants chimiques présents dans notre alimentation.
(source OMS : *http://www.who.int/foodsafety/chem/TDS_recipe_2005_en.pdf*).




**Quelques familles de contaminants organiques de l'alimentation**

Pesticides
Phtalates, Alkylphenols, Bisphenols
HAP
PCB / PCDD
Composés perfluorés
Retardateurs de flamme polybromés
Mycotoxines
Médicaments vétérinaires
Produits néoformés (HAP, AAH, acrylamide, furane…)

# Exposition aux contaminants toxiques (chronique / faibles doses)




D'après P.B. Farmer, R. Singh, *Mutat Res* 659 (2008) 68-76

# Analyse de contaminants toxiques à l'état de *traces* dans des *matrices complexes*

## Sensibilité

## Sélectivité

**La sensibilité est très souvent conditionnée par la sélectivité**

# La spectrométrie de masse pour l'analyse des résidus de contaminants organiques

**1980-1990**

GC-MS

LC-MS peu répandue dans les laboratoires d'analyse

Interfaces DLI, moving belt, thermospray, particle beam, CF-FAB

Quadripôles

*Limites de détection* *1-5 ng*

*Masse exacte / haute résolution* *non*

Sensibilité / sélectivité

**2000-2010**

LC-MS = technique de routine

ESI, APCI (APPI)

(triple) quadripôles, pièges 2D/3D, hybrides (Q-q-LIT, Q-q-ToF, Orbitrap)

*Limites de détection* *< 0.1 pg*

*Masse exacte / haute résolution* *oui*

# Plan de la présentation

Apport de la FT-MS pour la détermination ciblée de contaminants

- analyse de toxines
- comparaisons avec la méthode MRM

Intérêt de la très haute résolution pour l'identification structurale de métabolites de contaminants

- Composés polybromés
- Pesticides
- Mycotoxines

Nouvelles approches semi-ciblées / non ciblées grâce à la FT-MS

- Alcénals
- Métabolites de pesticides
- Perturbateurs endocriniens

# Analyses ciblées multi-résidus : la LC-MS/MS (MRM) comme outil de référence

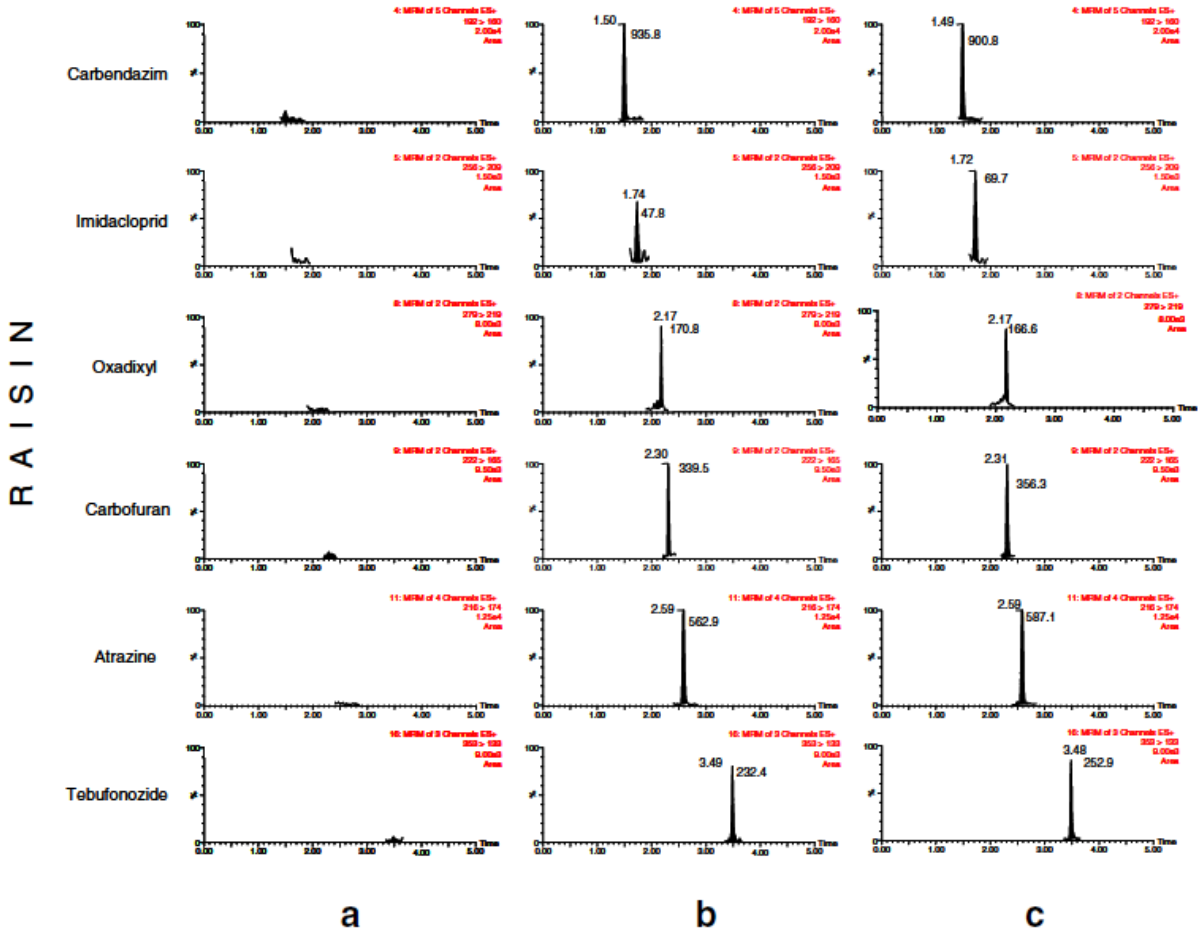



Fig. 1 UPLC–MS–MS chromatograms obtained from six representative pesticides: (a) blank sample (b) sample spiked at 0.01 mg $kg^{-1}$ (1.25 ng $mL^{-1}$ in the final extract) (c) matrix-matched standard (1.5 ng $mL^{-1}$)

O.J. Pozo et al.,
*Anal. Bioanal. Chem.* 389 (2007) 1765.

# Analyses ciblées multi-résidus : les possibilités de screening large offertes par la LC-HRMS

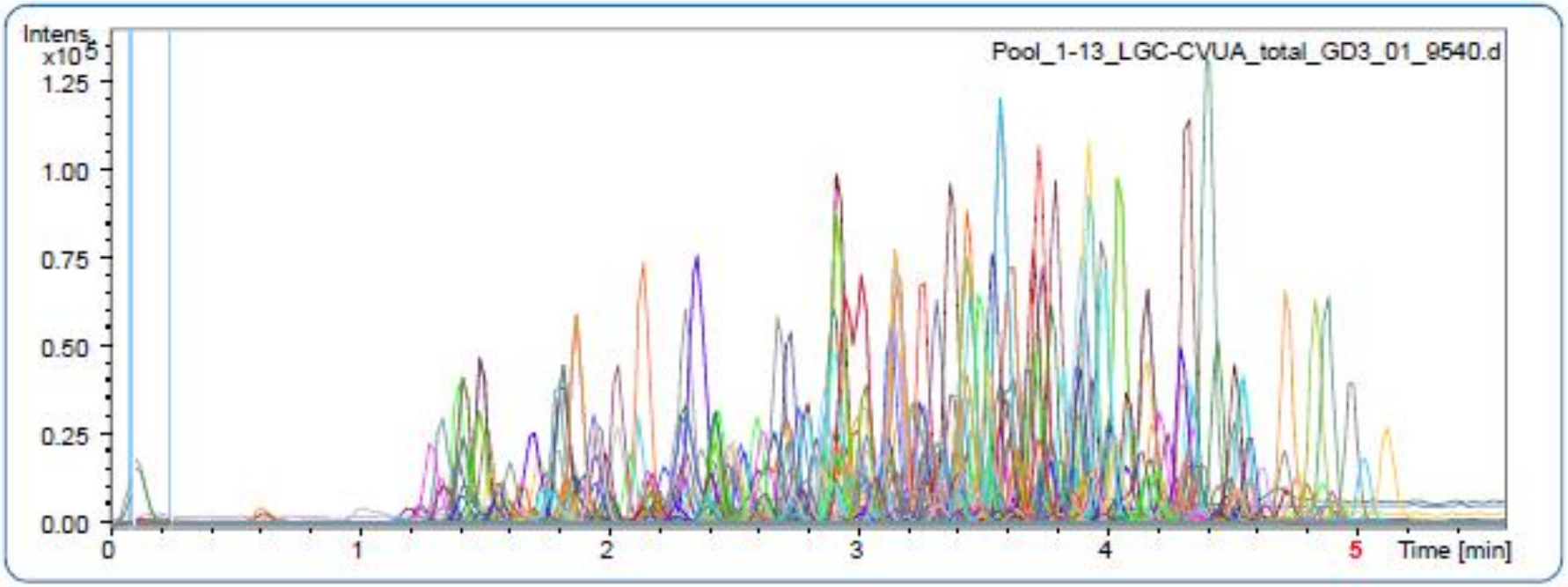



**Fig. 2:** Multi compound standard of 650 pesticides, grad. (B): overlayed compound EICs, complete pesticide elution in about 5 minutes.

L. Allen et al.
*ASMS Conference* (2009) poster WPY 605.

# Analyse de toxines sur LTQ-Orbitrap

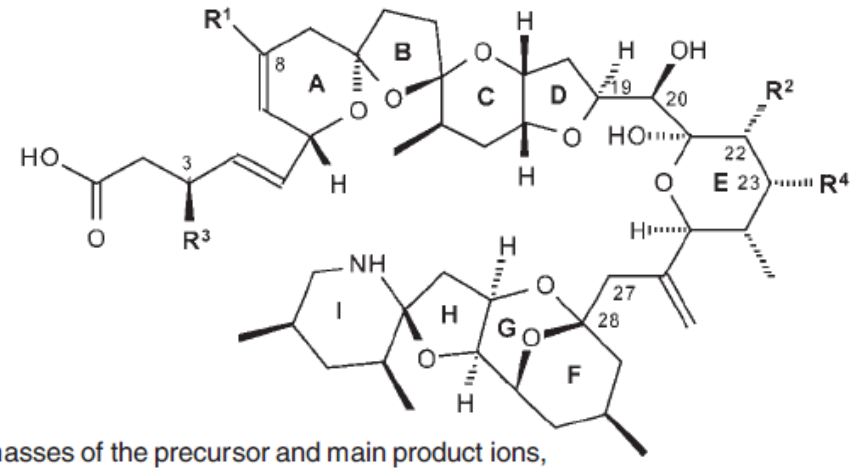


**Table 1.** Substituent designations for azaspiracids and the theoretical and found masses of the precursor and main product ions, determined using data-dependent scans with HCD

| | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $[M+H]^+$ theoretical $m/z$ | $[M+H]^+$ found $m/z$ **Error (ppm)** | $[M+H\text{-}H_2O]^+$ theoretical $m/z$ | $[M+H\text{-}H_2O]^+$ (Found) **Error (ppm)** | $[M+H\text{-}H_2O\text{-A ring}]^+$ theoretical $m/z$ | $[M+H\text{-}H_2O\text{-A ring}]^+$ found $m/z$ **Error (ppm)** |
|---|---|---|---|---|---|---|---|---|---|---|
| **AZA1** | H | $CH_3$ | H | H | 842.50545 | 842.50564 **0.22** | 824.49489 | 824.49492 **0.04** | 672.41116 | 672.41091 **-0.37** |
| **AZA2** | $CH_3$ | $CH_3$ | H | H | 856.52110 | 856.52160 **0.58** | 838.51054 | 838.51078 **0.29** | 672.41116 | 672.41092 **-0.35** |
| **AZA3** | H | H | H | H | 828.48980 | 828.48953 **-0.33** | 810.47924 | 810.47855 **-0.85** | 658.39551 | 658.39464 **-1.32** |
| **AZA4** | H | H | OH | H | 844.48472 | 844.48491 **0.23** | 826.47415 | 826.47423 **0.09** | 658.39551 | 658.39516 **-0.53** |
| **AZA5** | H | H | H | OH | 844.48472 | 844.48523 **0.61** | 826.47415 | 826.47432 **0.20** | 674.39042 | 674.39021 **-0.31** |
| **AZA6** | $CH_3$ | H | H | H | 842.50545 | 842.50602 **0.67** | 824.49489 | 824.49498 **0.11** | 658.39551 | 658.39527 **-0.36** |

Z. Skrabakova et al.
*Rapid Commun. Mass Spectrom.* 24 (2010) 2966.

# Analyse de toxines sur LTQ-Orbitrap

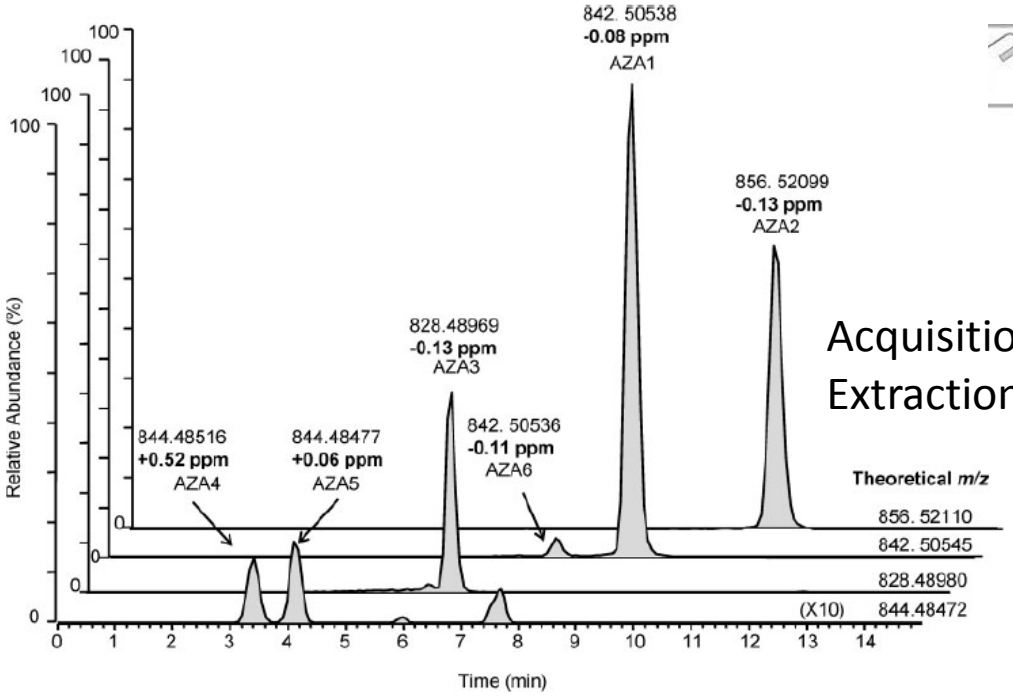
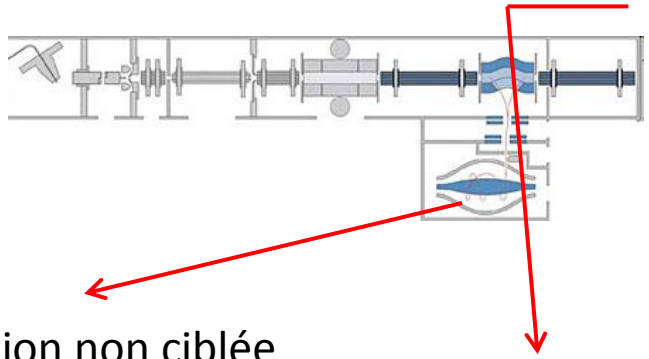
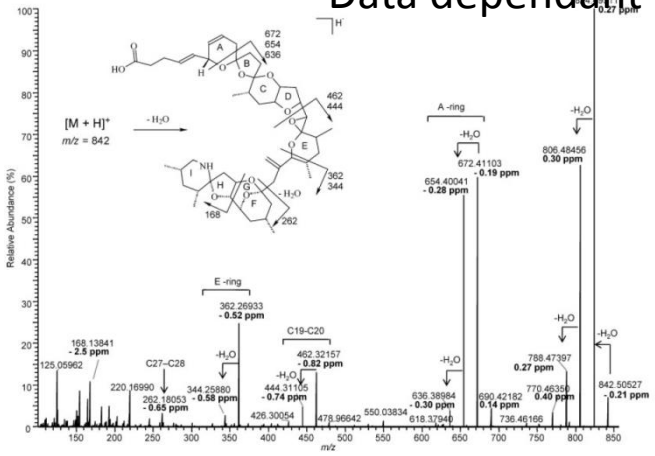



**Figure 4.** LC/MS ion chromatograms of azaspiracids in an extract of mussel (*M. edulis*) tissues, obtained using full-scan FTMS at 100 000 resolution. The found masses and error values are shown above each peak. The chromatograms were generated post-acquisition using four selected theoretical mass values with a mass tolerance window of ±2 mDa.



Acquisition non ciblée
Extraction signaux HR

Confirmation
MS/MS (HCD)
Data dependant





Z. Skrabakova et al.
*Rapid Commun. Mass Spectrom.* 24 (2010) 2966.

# SRM *vs.* HRMS

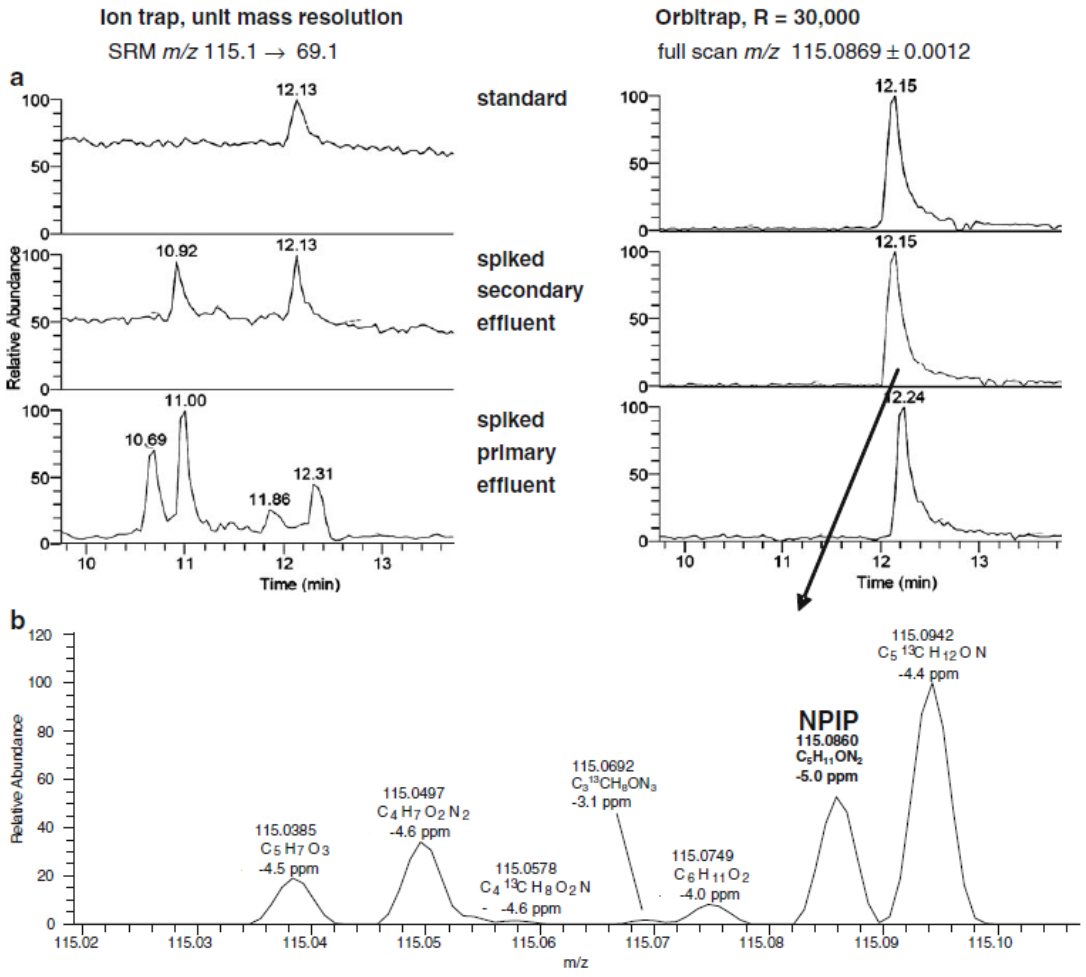
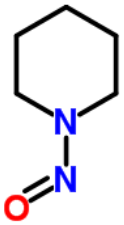



Fig. 2 a LC-MS chromatograms of *N*-nitrosopiperidine (NPIP) obtained from SRM at unit mass resolution (ion trap) and HRMS full scan at $R$=30,000 using an LTQ Orbitrap. A calibration standard of 10 ng/mL in water/methanol 95:5, and extracts of a secondary and a primary effluent sample from a sewage treatment plant both spiked at 20 ng/L are shown. All peaks correspond to about 200 pg of compound on column. b Section of the high resolution full-scan mass spectrum from the secondary effluent sample extract showing NPIP and interfering compounds of the same nominal mass with molecular formula assignments and mass accuracy. Further analytical details are given in [12]

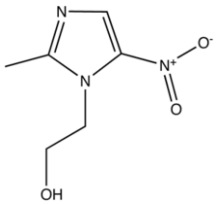


N-nitrosopiperidine
Détectée dans certains aliments fumés (poisson,bacon,…) et autres aliments conservés avec du nitrite de sodium

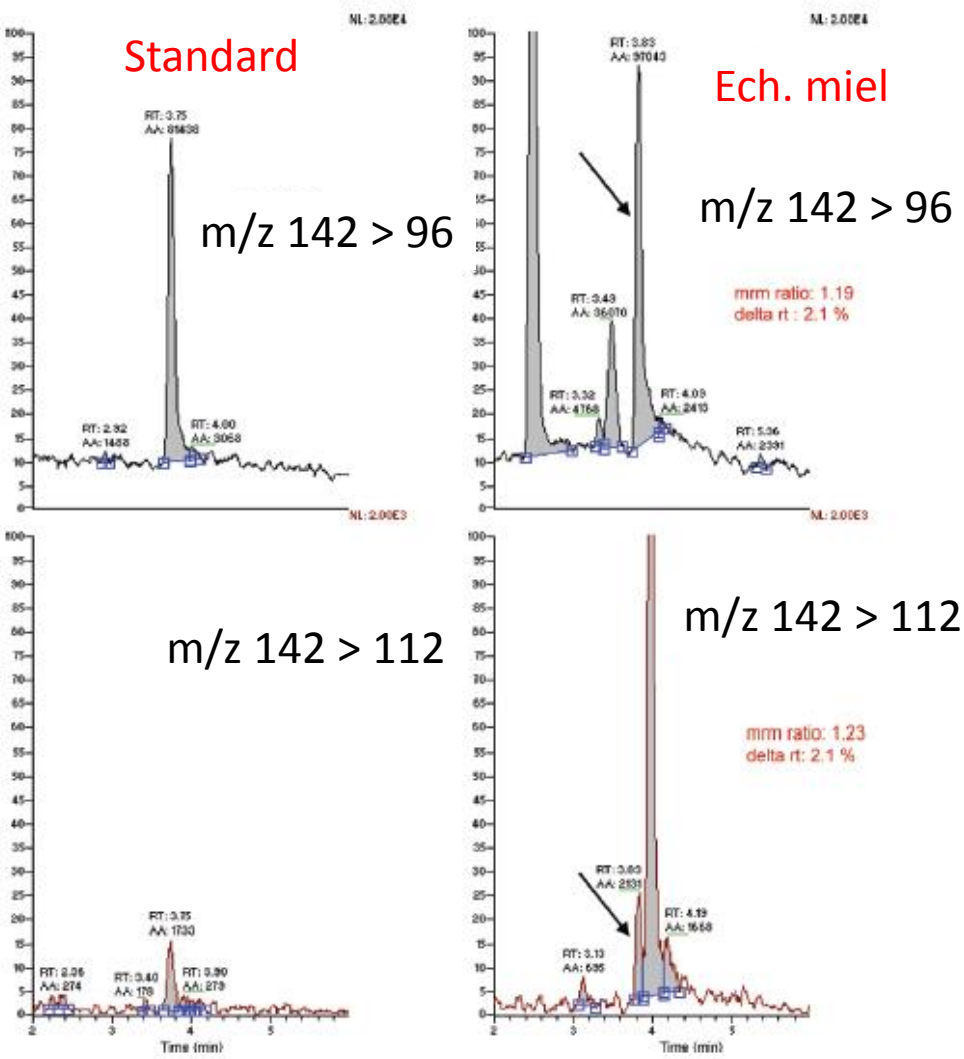
M. Krauss, H. Singer, J. Hollender.
*Anal. Bioanal. Chem.*, 397 (2010) 943-951.

# MRM *vs.* HRMS

**MRM**
**(faux positif)**




Nitroimidazole (miel)

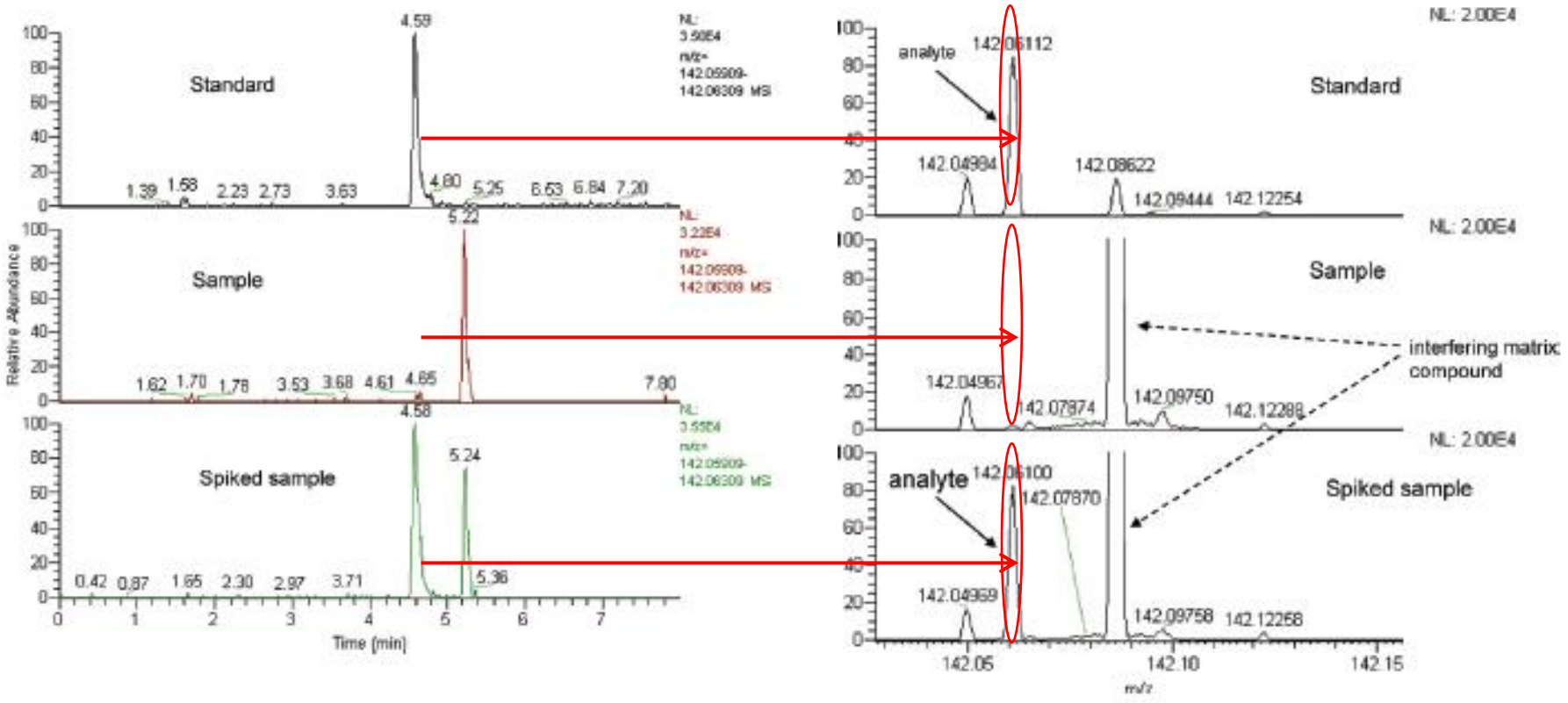




Kaufmann A. et al.,
Anal. Chim. Acta (2010) 673, 60

# MRM *vs.* HRMS

142,06109 ± 2 mDa




Kaufmann A. et al.,
*Anal. Chim. Acta* (2010) 673, 60

# MRM *vs.* HRMS

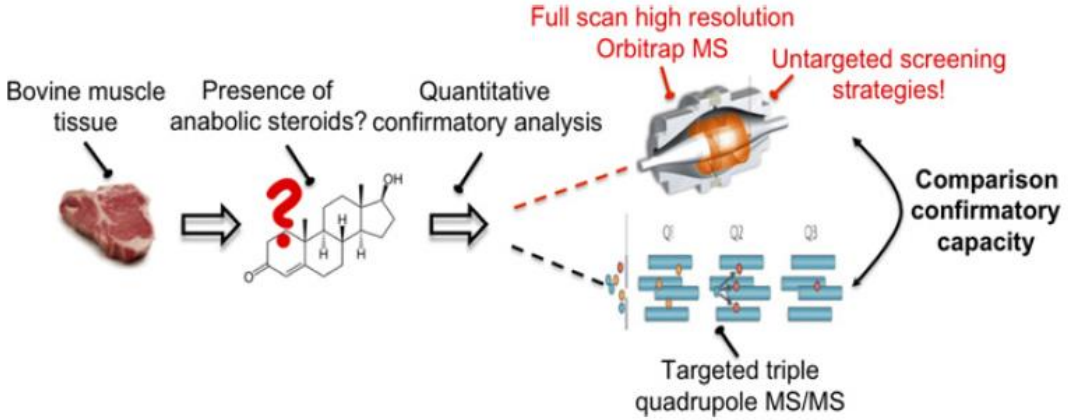



**Table 1**
Mean corrected recovery (at the RC or AL) and precision of the MS/MS and HRMS methods, developed for the analysis of 34 anabolic steroids in meat.

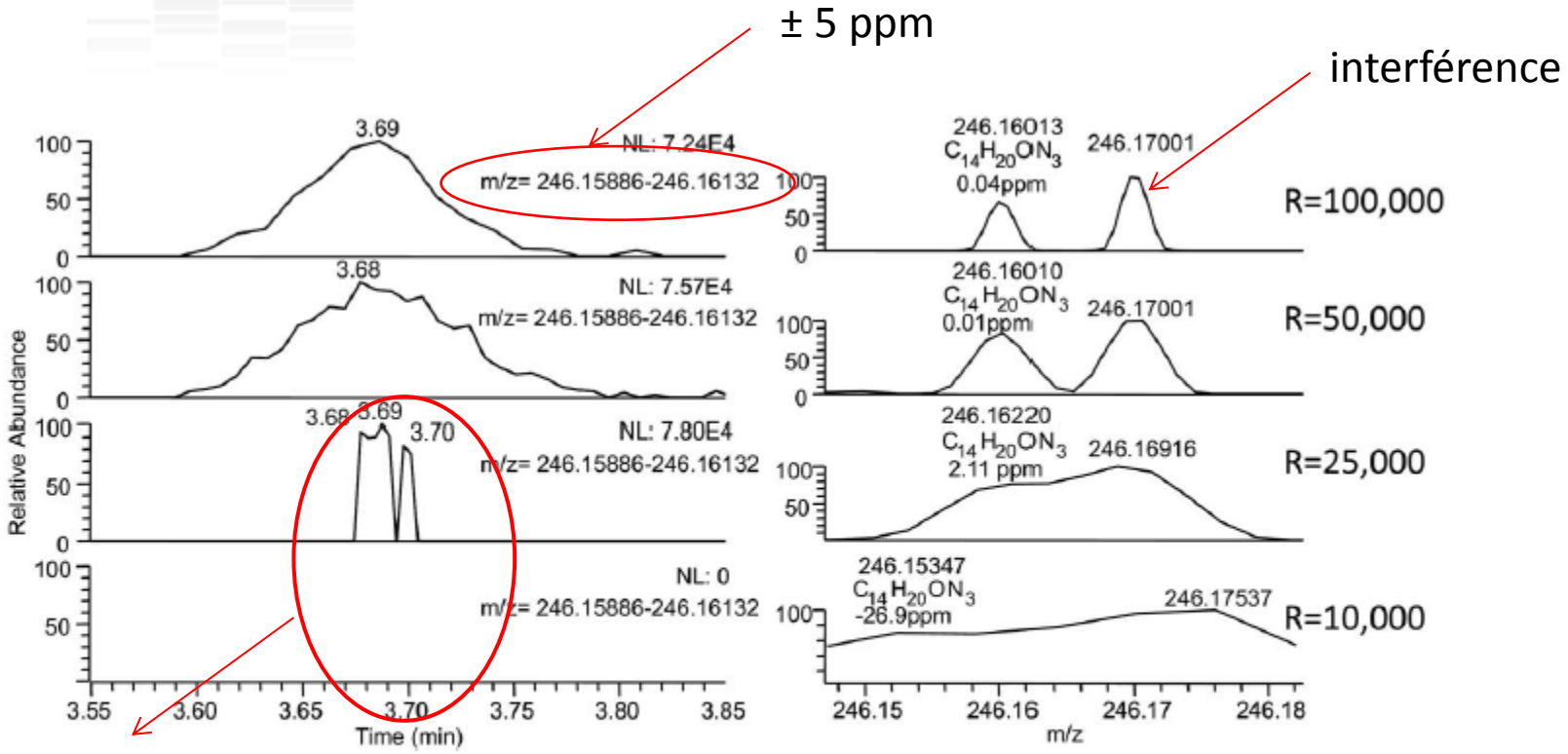
| Analyte | Mass Spectrom. Technique | RC/AL ($\mu g\,kg^{-1}$) | Recovery[a] Mean ± SD (%) | Repeatability[a] RSD (%) | Within-lab. Reprod[b] RSD (%) |
|---|---|---|---|---|---|
| 17α-estradiol | MS/MS | 1 | 93.4 ± 7.9 | 7.3 | 8.2 |
| | Orbitrap | 1 | 104.1 ± 21.8 | 26.8 | 27.5 |
| 17β-estradiol | MS/MS | 1 | 101.4 ± 10.8 | 10.2 | 9.9 |
| | Orbitrap | 1 | 81.8 ± 25.1 | 28.6 | 28.5 |
| dienestrol | MS/MS | 1 | 97.4 ± 14.3 | 17.0 | 18.5 |
| | Orbitrap | 1 | 98.7 ± 12.3 | 19.7 | 18.9 |
| diethylstilbestrol | MS/MS | 1 | 85.4 ± 16.3 | 19.0 | 17.5 |
| | Orbitrap | 1 | 153.6 ± 71.9 | 39.3 | 37.9 |
| 17β-ethinylestradiol | MS/MS | 1 | 93.5 ± 12.5 | 13.0 | 12.1 |
| | Orbitrap | 1 | 102.6 ± 13.5 | 19.7 | 18.9 |
| α-zearalanol | MS/MS | 5 | 81.4 ± 11.3 | 18.3 | 16.8 |
| | Orbitrap | 5 | 116.4 ± 45.4 | 27.9 | 29.3 |
| β-zearalanol | MS/MS | 1 | 81.6 ± 11.2 | 18.5 | 17.1 |
| | Orbitrap | 1 | 154.0 ± 80.1 | 26.7 | 47.54 |
| estrone | MS/MS | 1 | 110.0 ± 14.3 | 8.4 | 9.6 |
| | Orbitrap | 1 | 101.1 ± 16.6 | 18.6 | 18.5 |
| estriol | MS/MS | 1 | 119.2 ± 13.9 | 11.7 | |
| | Orbitrap | 1 | n.d. | n.d. | |
| hexoestrol | MS/MS | 1 | 99.5 ± 7.6 | 7.7 | |
| | Orbitrap | 1 | n.d. | n.d. | |
| 17α-testosterone | MS/MS | 1 | 93.4 ± 9.3 | 6.9 | |
| | Orbitrap | 1 | 94.9 ± 9.8 | 15.4 | 12.6 |

Sensibilité plus faible
→ Valeurs élevées

Etc…

Vanhaecke L. et al.,
*Anal. Chim. Acta* (2013) 767, 118

# Utilité de la très haute résolution pour la sélectivité de détection




Risque de faux négatif à R < 25000

**Figure 3.** Effect of resolving power on assigned mass accuracy of an analyte in matrix. Isopyrin ($C_{14}H_{19}N_3O$, RT = 3.69 min; MH+ = 246.16009, 25 ng/g in animal feed. Left-hand side: XICs with ±5 ppm mass extraction window. Right-hand side: mass profiles of analyte and matrix interference at RT = 3.69 min.


Kellmann M. et al.,
*J. Am. Soc. Mass Spectrom.* (2009) 20, 1464

# Sélectivité de détection : importance de la précision de la mesure

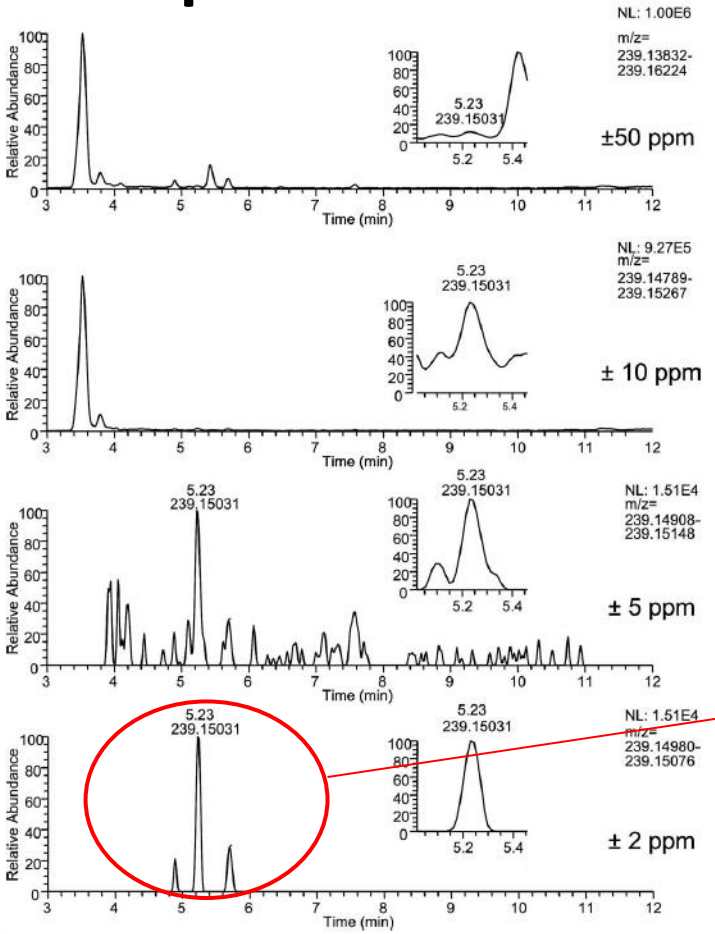



**Figure 1.** Effect of mass extraction window on selectivity. Extracted ion chromatograms for the pesticide pirimicarb (MH+, $C_{11}H_{19}N_4O_2$, $m/z_{theo.}$ = 239.15028, retention time = 5.23 min) in animal feed matrix at 10 ng/g; resolving power: 100,000.

R = 100000
± 2 ppm

→ 3 pics

Kellmann M. et al.,
J. Am. Soc. Mass Spectrom. (2009) 20, 1464

# Vitesse d'acquisition, résolution et précision

Nbre de points pour définition du pic chromatographique (quantification)

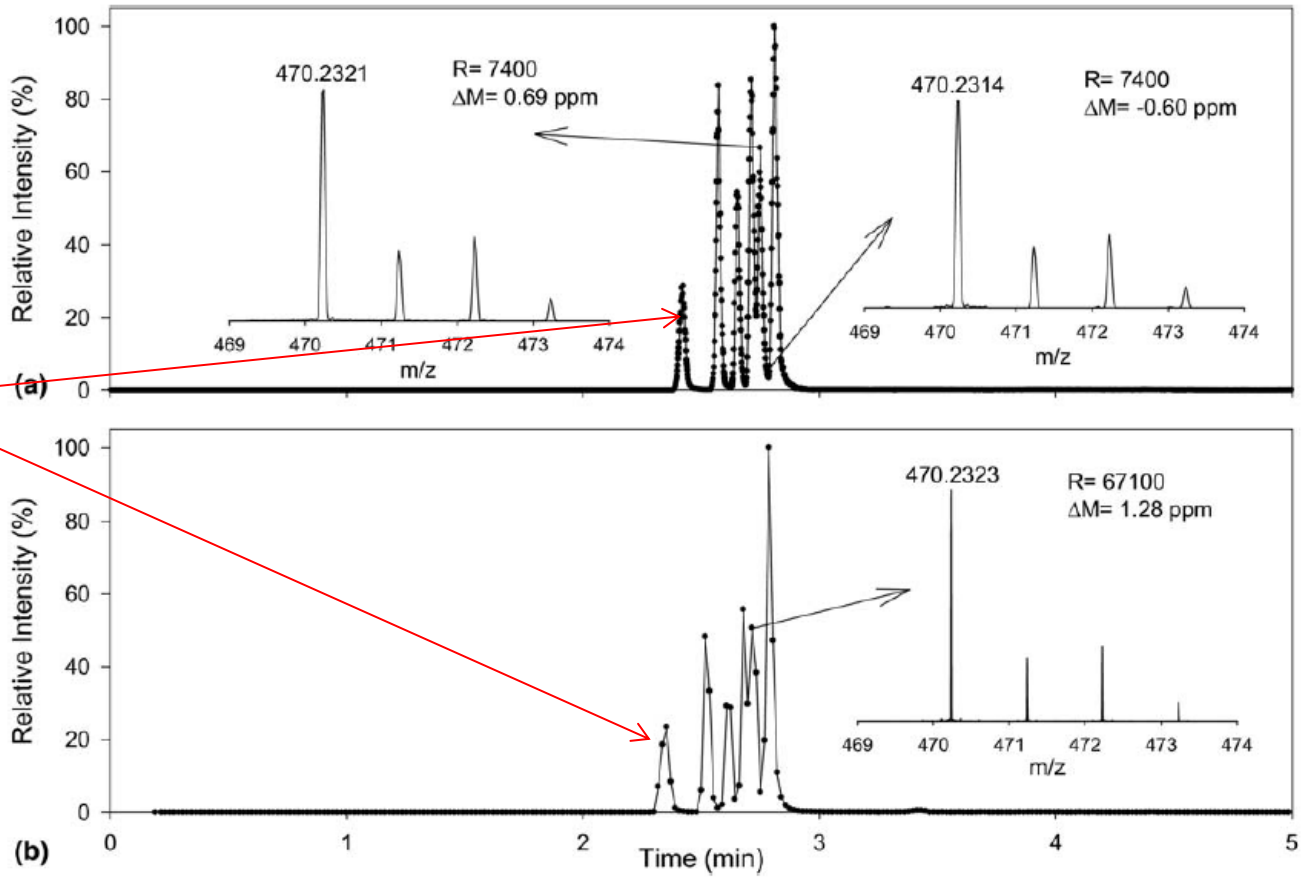
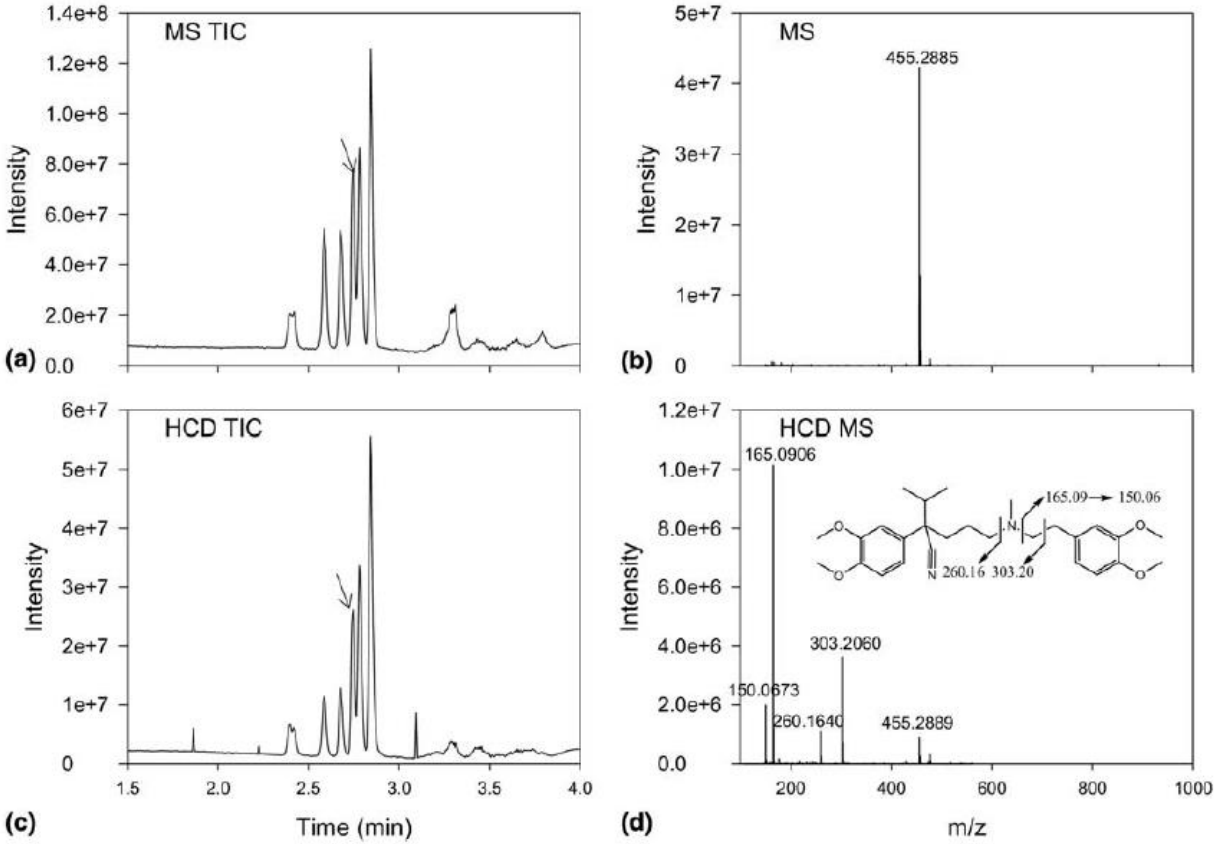



**Figure 1.** Comparison of (**a**) 10 Hz and (**b**) 1 Hz data acquisition rates using the Exactive™. Insets in (**a**) shows mass spectra taken from peak top and peak tail. Inset in (**b**) shows spectra taken from peak top.

Bateman M. et al.,
J. Am. Soc. Mass Spectrom. (2009) 20, 1441

# Full scan MS et MS/MS



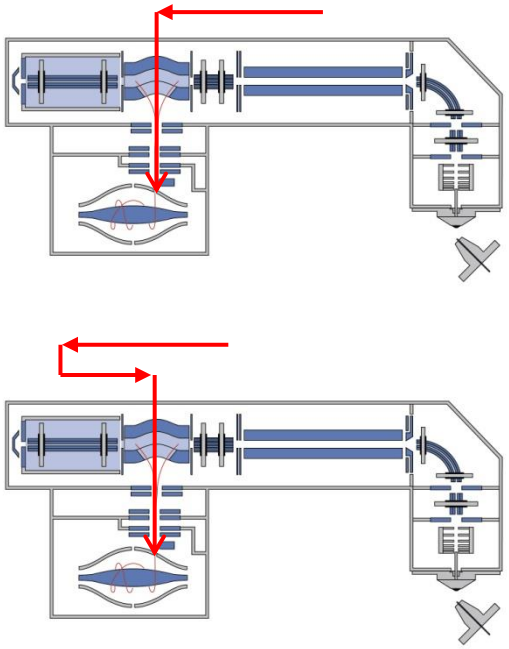
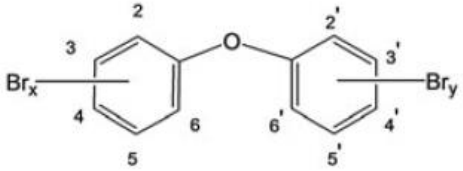



**Figure 2.** (a) MS and (c) HCD chromatograms for the analysis of a mixture of 6 compounds. (b) Mass spectrum extracted from peak labeled with arrow in (a) showing intact $[M + H]^+$ for verapamil. (d) HCD mass spectrum from peak labeled with arrow in (c). Structure in (d) shows assignment of fragment ions for verapamil.

Bateman M. et al.,
J. Am. Soc. Mass Spectrom. (2009) 20, 1441

# Utilité de la FT-MS pour la confirmation de métabolites : exemple des PBDE




$1 < x + y < 10$

**PBDEs**

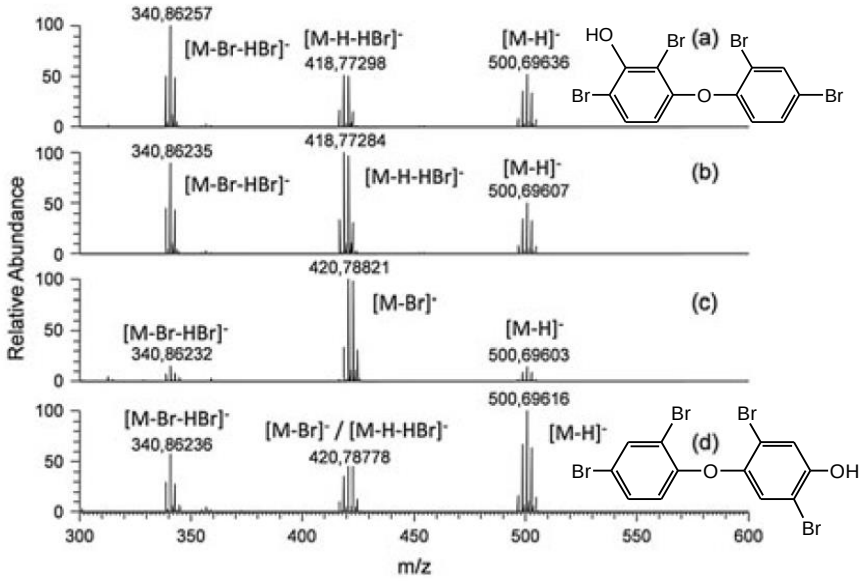



**Figure 3.** Negative APPI mass spectra of (a) 3-OH-BDE-47, (b) 5-OH-BDE-47, (c) 6-OH-BDE-47, and (d) 4′-OH-BDE-49.

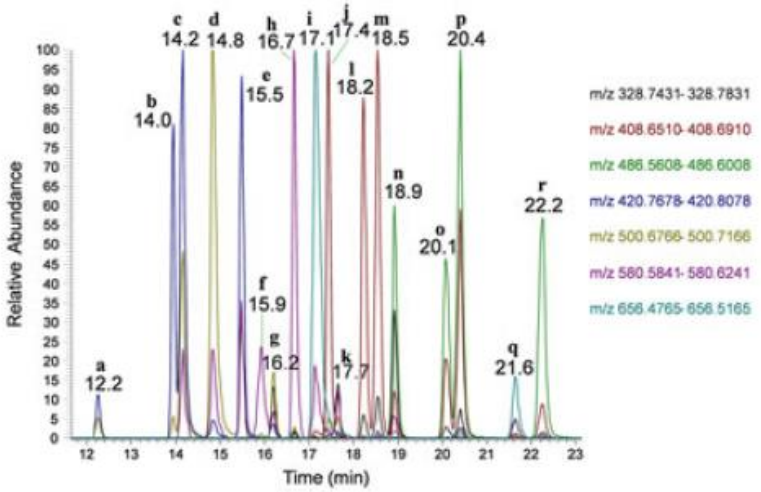
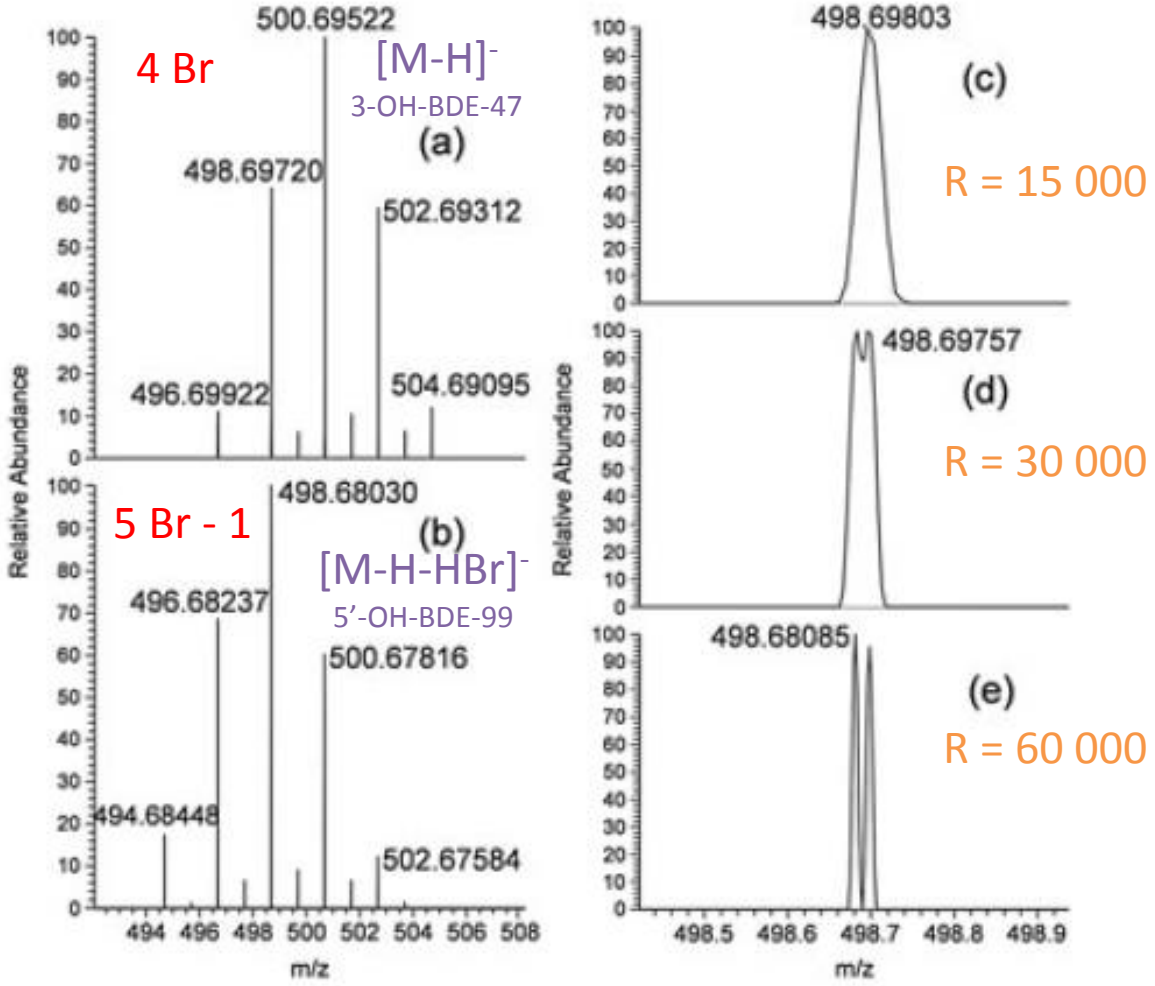



**Figure 4.** Separation of a PBDE/OH-PBDE mixture (50 pg on-column each) on a PFP column using optimised conditions: (a) 3-OH-BDE-47, (b) 6-OH-BDE-47, (c) 4′-OH-BDE-49 + 3-OH-BDE-100, (d) 3′-OH-BDE-154 + BDE-47, (e) 5′-OH-BDE-99 + 6-OH-BDE-180, (f) BDE-100, (g) BDE-154, (h) BDE-99, (i) 4′-OH-BDE-201, (j) BDE-184, (k) BDE-153, (l) BDE-202, (m) BDE-201, (n) BDE-204, (o) BDE-208, (p) BDE-207, (q) BDE-206, and (r) BDE-209.

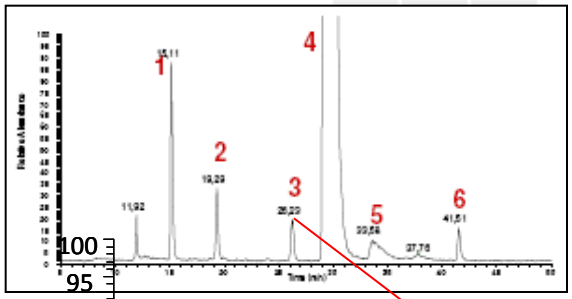
C. Marteau, L. Debrauwer et al.,
*Rapid Commun. Mass Spectrom.* (2012) 26, 599

# Utilité de la FT-MS pour la confirmation de métabolites : exemple des PBDE




C. Marteau, L. Debrauwer et al.,
*Rapid Commun. Mass Spectrom.* (2012) 26, 599

# Autre exemple : métabolisme du tétrabromobisphenol-A chez le têtard de Xénope




Métabolite 3
(NI-ESI-MS)

[M-H]⁻

542,65

544,62

546,66

540,64

526,90

500,83

518,02

555,44

591,95

581,96

597,77

624,46

641,67

646,61

610,70

573,85

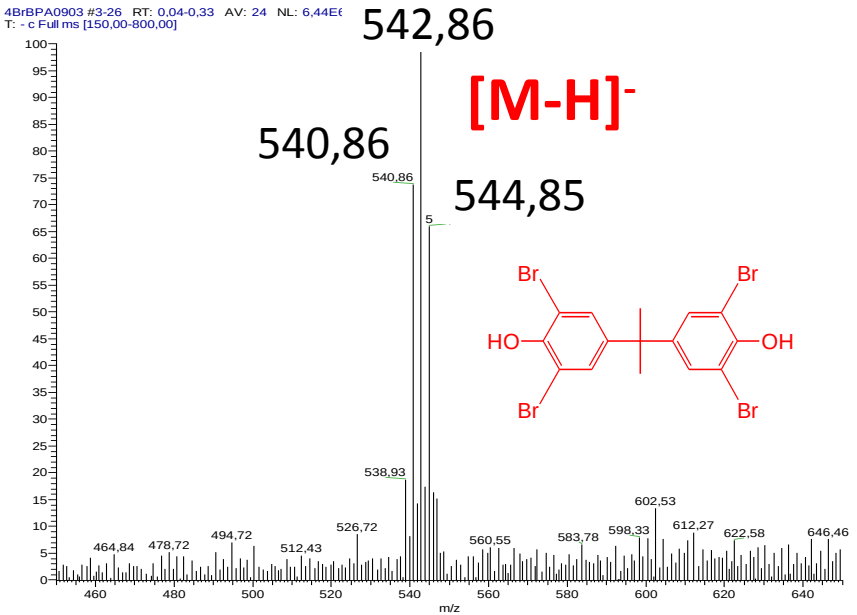
458,62

479,90

493,59

Relative Abundance

m/z

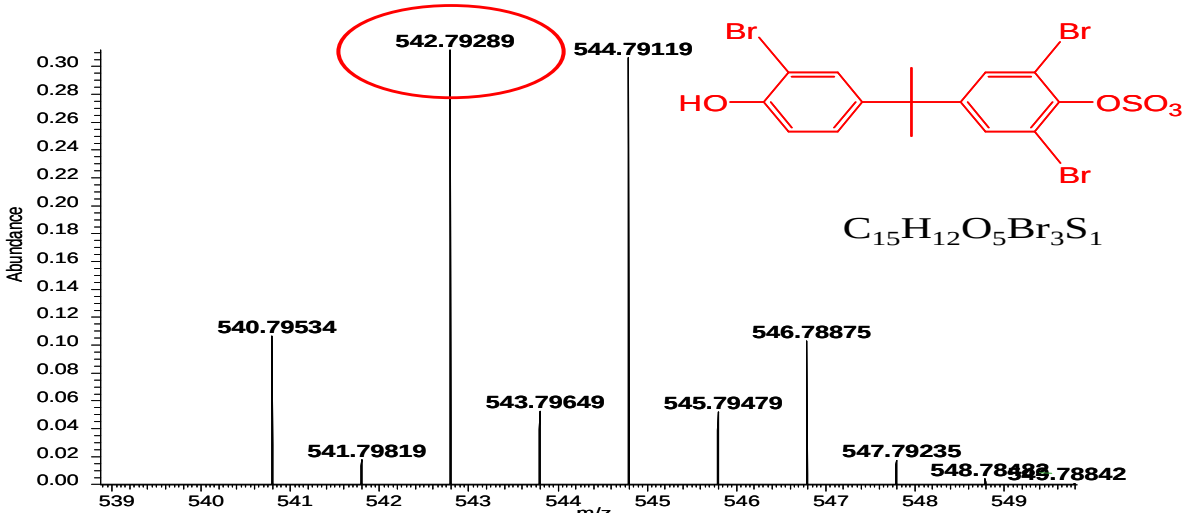






J.B. Fini, L. Debrauwer et al.,
*Tox. Sci.* (2012)155, 359.

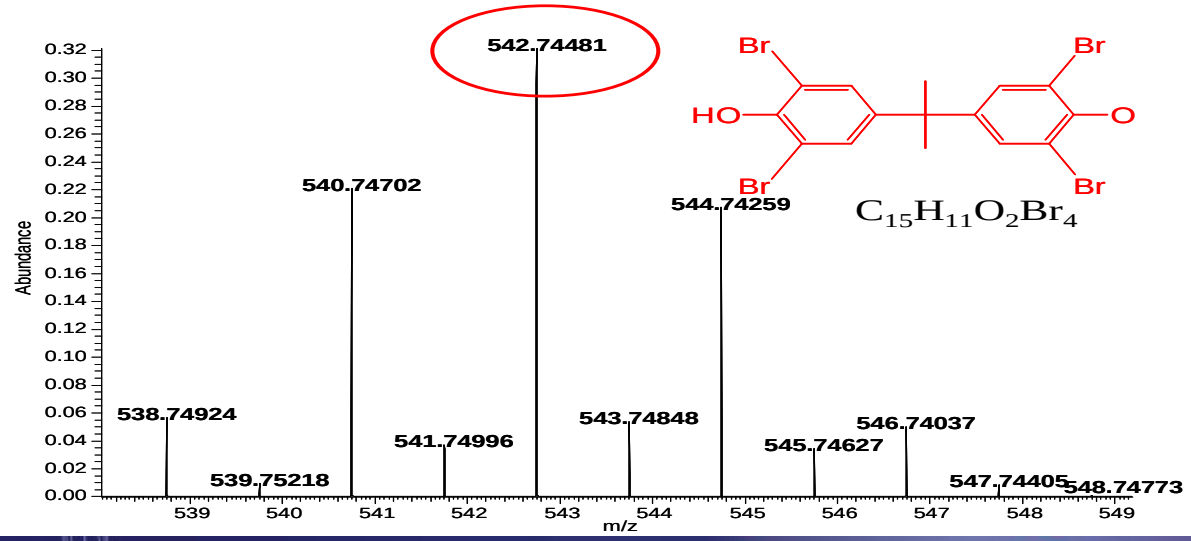
# Autre exemple : métabolisme du tetrabromobisphenol-A chez le têtard de Xénope




Exp. 542,79289

$C_{15}H_{12}O_5Br_3S$
Calc. 542,79296
$\Delta$ 0.13 ppm

$C_{15}H_{11}O_2Br_4$
Calc. 542,74461
$\Delta$ 89.1 ppm




Exp. 542,74481

$C_{15}H_{11}O_2Br_4$
Calc. 542,74461
$\Delta$ 0.37 ppm

$C_{15}H_{12}O_5Br_3S$
Calc. 542,79296
$\Delta$ 88.8 ppm

J.B. Fini, L. Debrauwer et al.,
*Tox. Sci.* (2012)155, 359.

# Identification structurale : masse exacte et composition élémentaire

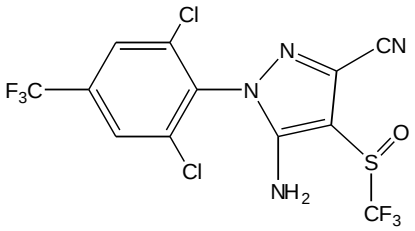
FTMSfipro0109 #7-46 RT: 0,06-0,41 AV: 40 NL: 5,80E6
F: FTMS - p ESI Full ms [150,00-600,00]

434,92948

$C_{12}H_4{}^{35}Cl_2F_6N_4OS$

$M_{calc.}$ 434,93033

$\delta$ -1,9 ppm

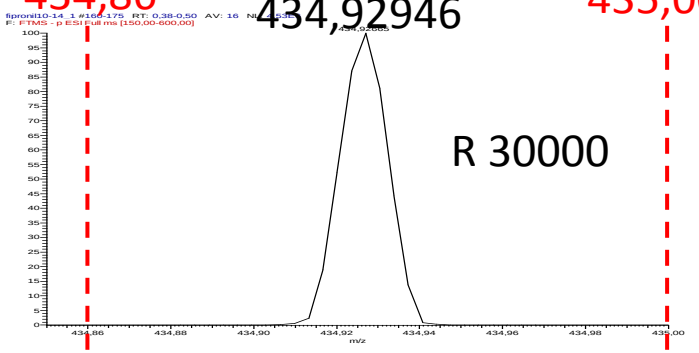



$C_{12}H_4{}^{35}Cl^{37}ClF_6N_4OS$

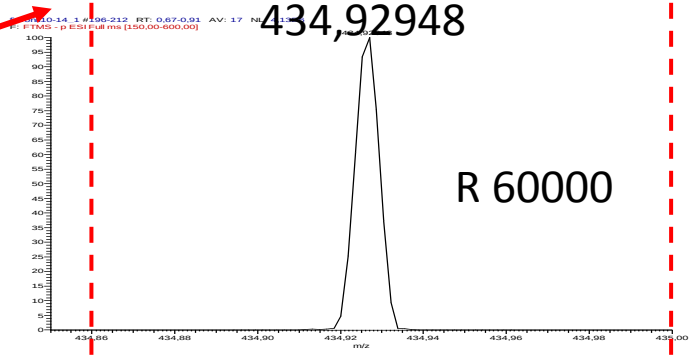
436,92641



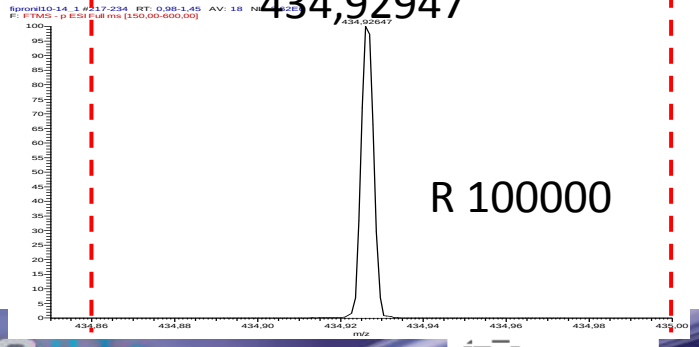

434,86 434,92946 435,00




434,92948

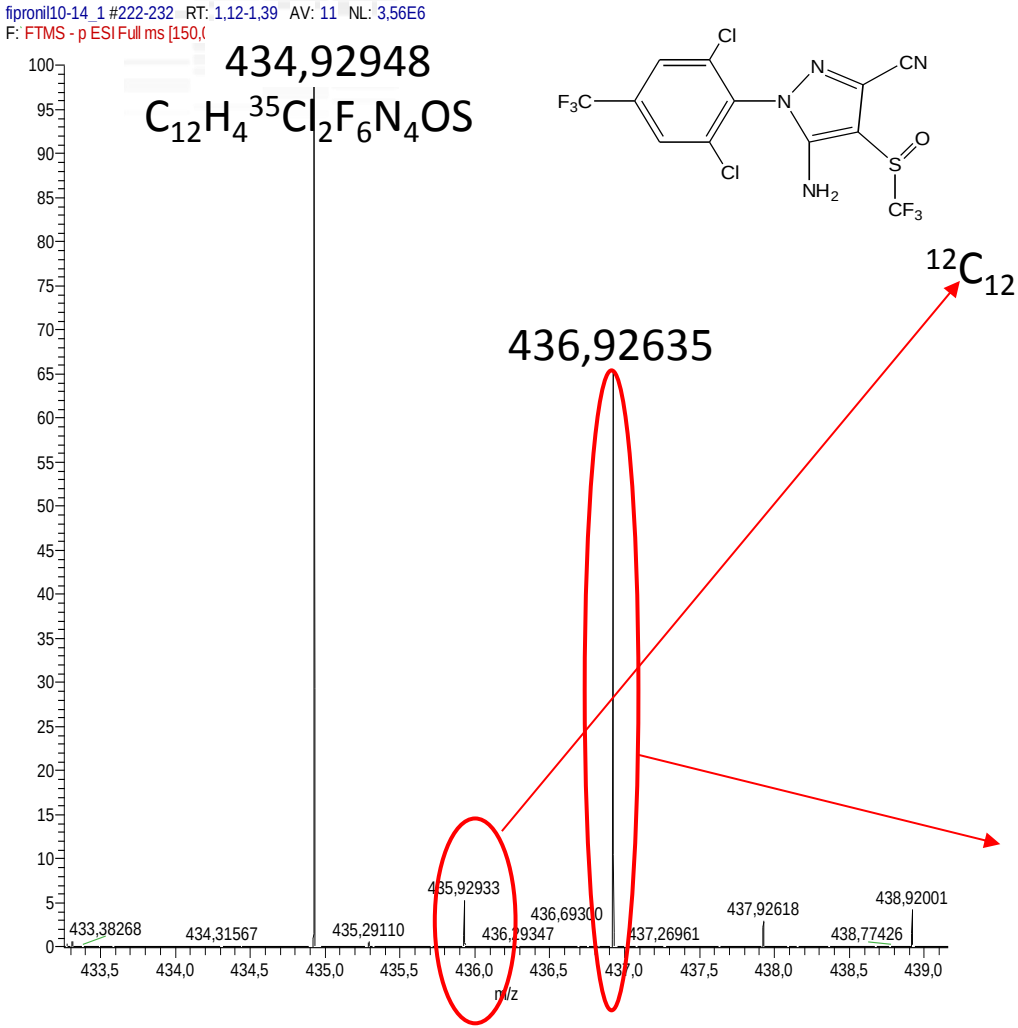



434,92947



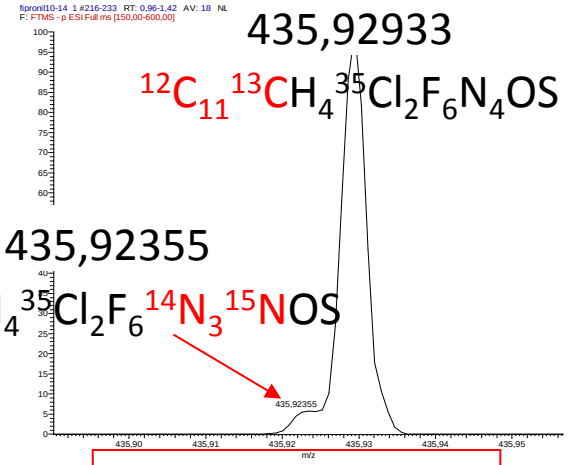

J.P. Cravedi, L. Debrauwer et al.,
*Chemosphere* (2013)93, 2276.

# Identification structurale : résolution des massifs isotopiques

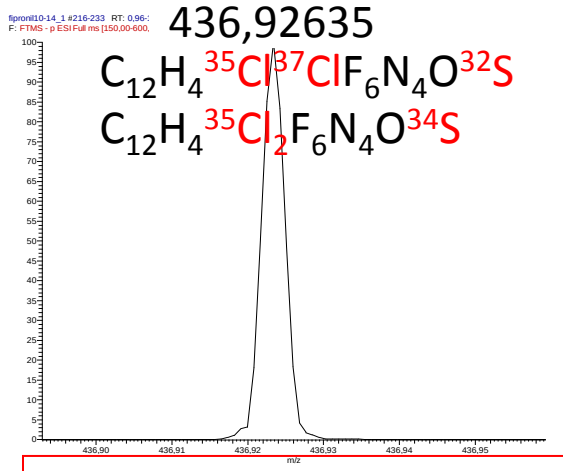









$R_{min}$ = 70000 → OK




$R_{min}$ = 345000 → FT-ICR !!!

J.P. Cravedi, L. Debrauwer et al.,
*Chemosphere* (2013)93, 2276.

# Utilisation de la FT-MS pour l'identification de métabolites fongiques

## Application : métabolites secondaires de *Aspergillus fumigatus*

**Stratégie : marquage uniforme par isotopes stables ($^{13}C$ $^{15}N$)**
**→identification de nouveaux métabolites (potentiellement mycotoxines)**
**→Discrimination de composés non biologiques**

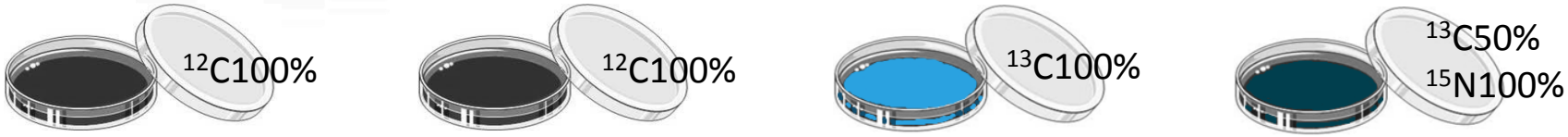
| Blé 12C | Blé 12C | Blé 13C | Blé 13C15N |
|---|---|---|---|
| 99% $^{12}C$ | 99% $^{12}C$ | 3% $^{12}C$ | 53% $^{13}C$ |
| 1% $^{13}C$ | 1% $^{13}C$ | 97% $^{13}C$ | 96% $^{15}N$ |

Culture blanche

Culture *Aspergillus fumigatus* sur blé

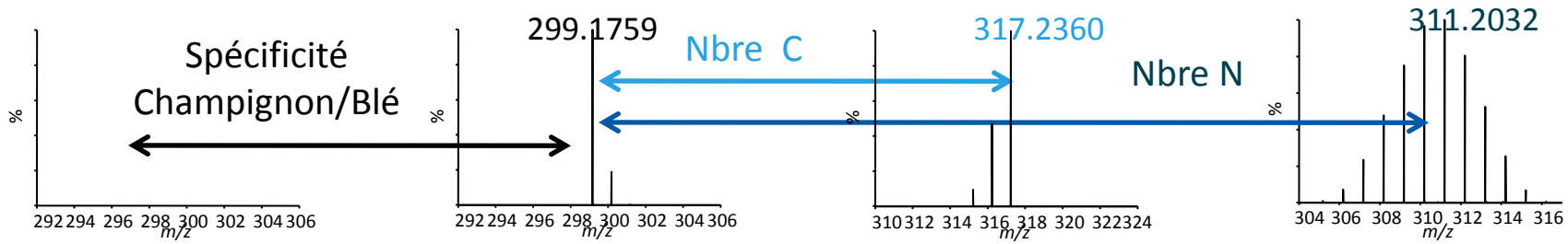
P. Cano, L. Debrauwer et al.,
*Anal. Chem.* (2012) 85, 8412

# Utilisation de la FT-MS pour l'identification de métabolites fongiques



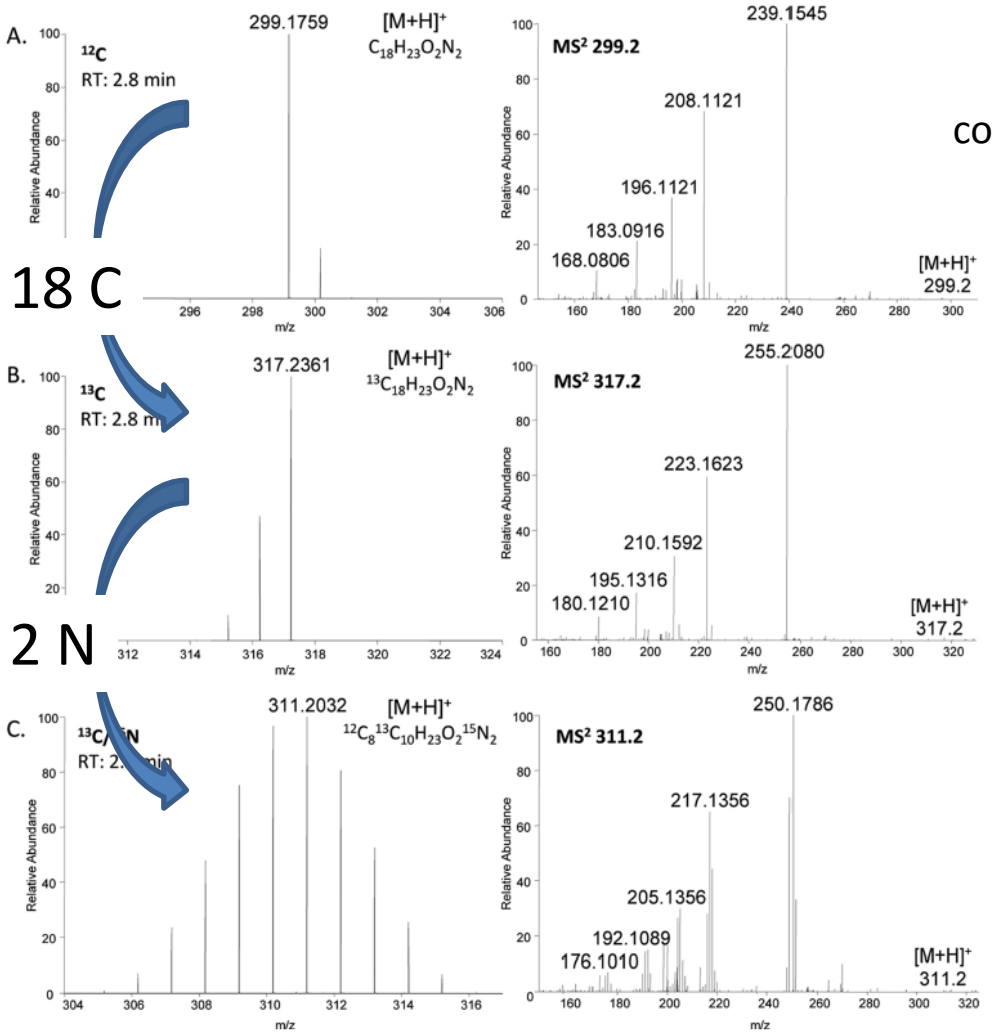

Blé + Culture blanche

Blé + Culture *Aspergillus fumigatus*




Logiciel "MassCompare" (home-made ): comparaison listes formules brutes + simulation massif isotopique

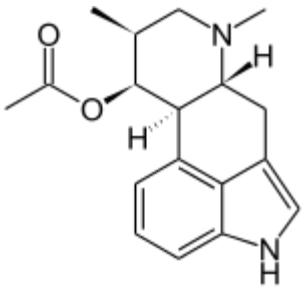
# Identification de la Fumigaclavine A




Logiciel “MassCompare” (home-made ):
comparaison listes formules brutes + simulation massif isotopique + règle de l’azote

**1 formule brute** ([M+H]$^+$: $C_{18}H_{23}O_2N_2$)

Spectres MS/MS



Fumigaclavine A

# Identification de métabolites chez *Aspergillus fumigatus*

Table I. Detected Known Metabolites of *A. fumigatus* in 15 Day Old Cultures on Wheat Grains

| chemical family | metabolite | formula[a] | ion[a] | RT (min)[b] | $^{12}C$ $m/z$[c] | $^{13}C$ $m/z$[c] | $^{13}C/^{15}N$ $m/z$[c] |
|---|---|---|---|---|---|---|---|
| trypacidins | trypacidin | $C_{18}H_{16}O_7Na$ | $[M + Na]^+$ | 27.74 | 367.0785 | 385.1388 | 376.1090 |
| | | | | | 0.7 ppm | 1 ppm | 0.1 ppm |
| | questin | $C_{16}H_{11}O_5$ | $[M - H]^-$ | 27.82 | 283.0620 | 299.1159 | 292.0922 |
| | | | | | 3 ppm | 3.6 ppm | 3.1 ppm |
| | monomethylsulochrin | $C_{18}H_{17}O_7$ | $[M - H]^-$ | 28.07 | 345.0989 | 363.1599 | 355.1321 |
| | | | | | 3 ppm | 4.2 ppm | 1.6 ppm |
| anthraquinones and anthrones | emodin | $C_{15}H_9O_5$ | $[M - H]^-$ | 36.20 | 269.0461 | 284.09650 | 495.2653 |
| | | | | | 2.2 ppm | 2.2 ppm | 1.2 ppm |
| pseurotins | pseurotin A | $C_{22}H_{25}NO_8Na$ | $[M + Na]^+$ | 17.25 | 454.1469 | 476.2205 | 467.1827 |
| | | | | | 0.7 ppm | 1.1 ppm | 3.9 ppm |
| helvolic acid | helvolic acid | $C_{33}H_{43}O_8$ | $[M - H]^-$ | 37.96 | 567.2975 | 600.4079 | 584.3544 |
| | | | | | 2.1 ppm | 1.4 ppm | 1.8 ppm |
| fumigadavins | fumigadavine A | $C_{18}H_{23}N_2O_2$ | $[M + H]^+$ | 2.8 | 299.1759 | 317.2360 | 311.2032 |
| | | | | | 1.8 ppm | 0.9 ppm | 0.6 ppm |
| | fumigadavine B | $C_{16}H_{21}N_2O$ | $[M + H]^+$ | 2.5 | 257.1651 | 273.2191 | 268.1889 |
| | | | | | 1.0 ppm | 2.1 ppm | 0.6 ppm |
| | festuclavin | $C_{16}H_{21}N_2$ | $[M + H]^+$ | 4.17 | 241.1699 | 257.2236 | 252.1939 |
| | | | | | 0.1 ppm | 0.2 ppm | 1.1 ppm |
| fumitremorgins | fumitremorgin B | $C_{27}H_{32}N_5O_4$ | $[M - H_2O + H]^+$ | 37.3 | 462.2385 | 489.3285 | 480.2793 |
| | | | | | 0.4 ppm | 1.6 ppm | 1.7 ppm |
| | fumitremorgin C | $C_{22}H_{26}N_3O_3$ | $[M + H]^+$ | 23.47 | 380.1968 | 402.2692 | 392.2168 |
| | | | | | 0.1 ppm | 3.7 ppm | 3.3 ppm |
| | verruculogen | $C_{27}H_{32}N_5O_6$ | $[M - H_2O + H]^+$ | 37.7 | 494.2279 | 521.3183 | 512.2681 |
| | | | | | 1.2 ppm | 1.5 ppm | 3.6 ppm |
| | | $C_{27}H_{33}N_5O_7Na$ | $[M + Na]^+$ | 37.7 | 534.2202 | 561.3097 | 551.2567 |
| | | | | | 1.6 ppm | 3.8 ppm | 4.4 ppm |
| | | $C_{27}H_{32}N_5O_7$ | $[M - H]^-$ | 35.41 | 510.2257 | 537.3164 | 527.2636 |
| | | | | | 2.3 ppm | 2.4 ppm | 2 ppm |
| | TR-2 | $C_{22}H_{26}N_3O_6$ | $[M - H]^-$ | 6.86 | 428.1840 | 450.2579 | 443.2150 |
| | | | | | 3.0 ppm | 3 ppm | 2.2 ppm |
| | cyclotryprostatin A | $C_{22}H_{24}N_3O_5$ | $[M - H]^-$ | 10.94 | 410.1734 | 432.2471 | 425.2043 |
| | | | | | 3.1 ppm | 3 ppm | 2.0 ppm |
| | spirotryprostatin A | $C_{22}H_{26}N_3O_4$ | $[M + H]^+$ | 15.04 | 396.1900 | 418.2638 | 409.2173 |
| | | | | | 4.4 ppm | 4.1 ppm | 2.3 ppm |
| | spirotryprostatin B | $C_{21}H_{22}N_3O_3$ | $[M + H]^+$ | 22.61 | 364.1618 | 385.2344 | 376.186 |
| | | | | | 3.3 ppm | 4.2 ppm | 1.1 ppm |
| | compound 1[d] | $C_{23}H_{24}N_3O_6$ | $[M - H]^-$ | 13.21 | 426.1684 | 448.2423 | 440.1960 |
| | | | | | 3.2 ppm | 3.2 ppm | 2.2 ppm |
| | | $C_{23}H_{25}N_3O_6Na$ | $[M + Na]^+$ | 13.21 | 450.1633 | 472.2377 | 464.1894 |
| | | | | | 0.4 ppm | 0.9 ppm | 4.6 ppm |
| fumiquinazolines | fumiquinazoline C | $C_{24}H_{22}N_3O_4$ | $[M + H]^+$ | 23.56 | 444.1664 | 468.2463 | 462.1945 |
| | | | | | 0.4 ppm | 1.6 ppm | 2 ppm |
| tryptoquivalines | tryptoquivaline F | $C_{23}H_{19}N_4O_4$ | $[M + H]^+$ | 20.12 | 403.1405 | 425.2135 | 419.1685 |
| | | | | | 0.1 ppm | 0.8 ppm | 0.1 ppm |
| fumigallins | fumagillin | $C_{26}H_{34}O_7Na$ | $[M + Na]^+$ | 37.00 | 481.2193 | 507.3061 | 495.2653 |
| | | | | | 0.8 ppm | 1.6 ppm | 1.2 ppm |

≈ 20 métabolites identifiés

# Identification d'un métabolite « artefact »

métabolite à RT=16min présentant 1 $^{12}C$ et 22 $^{13}C$ dans les échantillons 100% $^{13}C$.

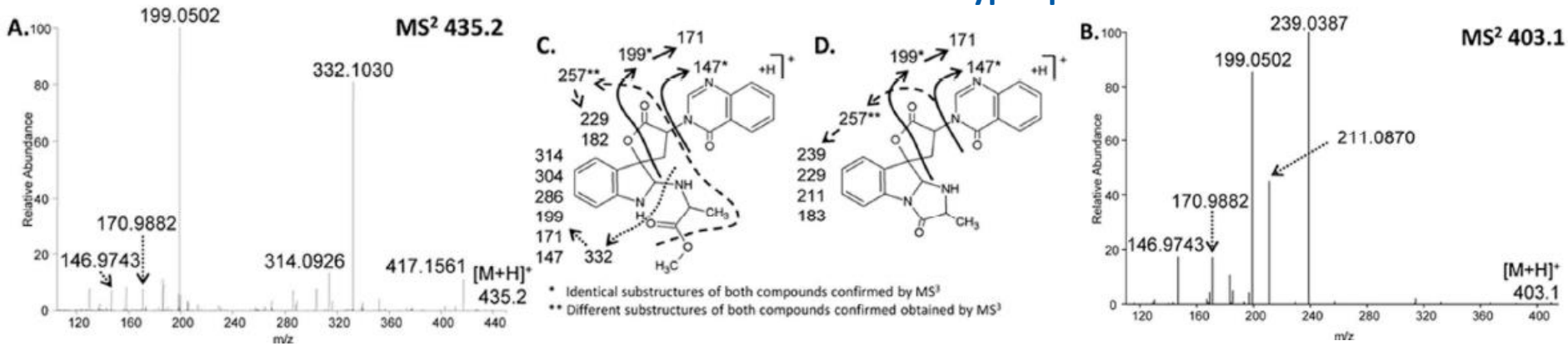
$$C_{23}H_{22}O_5N_4 \longrightarrow {}^{12}C^{13}C_{22}H_{22}O_5N_4$$

→ Incorporation d'un atome de carbone post-biosynthèse?

1ère info : non détecté dans les échantillons dissous dans $CH_3CN$ au lieu de $CH_3OH$

2ième info : $MS^n$ :

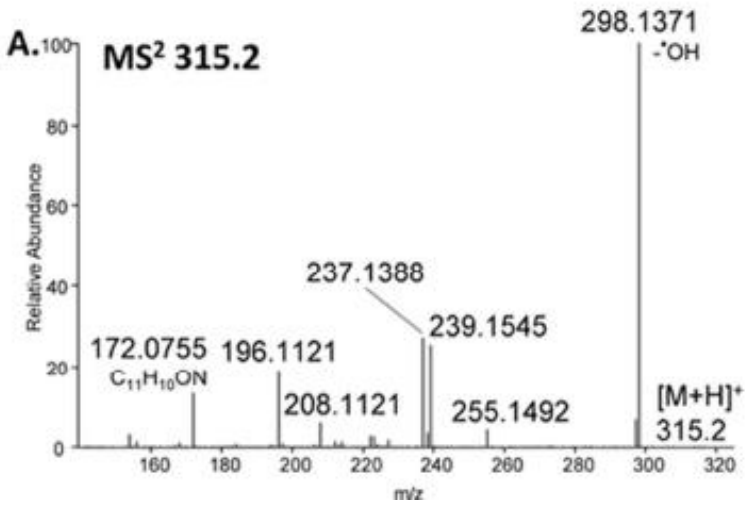
**Inconnu** **Tryptoquivaline F**




Dégradation de la tryptoquivaline F par hydrolyse d'une fonction amide cyclique + méthylation de l'acide carboxylique formé

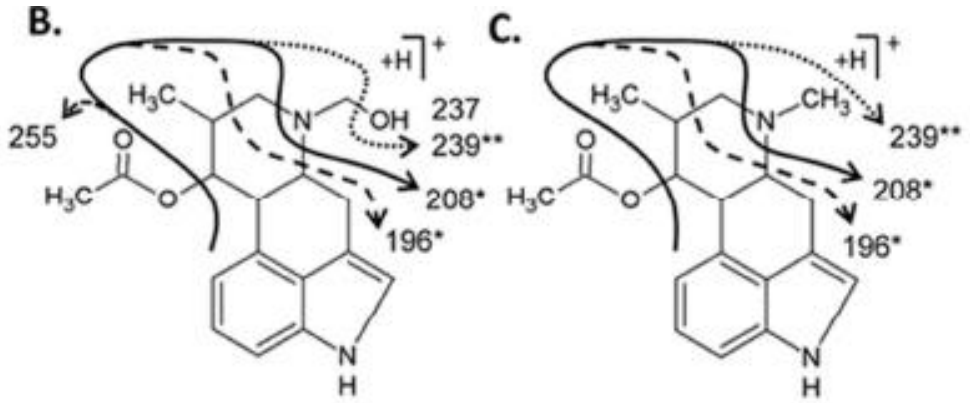
# Identification de nouveaux métabolites fongiques

## Nouveau métabolite : la « Fumigaclavin D »




Inconnue

Fumigaclavin A




* Identical substructures of both compounds confirmed confirmed by MS³

** Different substructures of both compounds confirmed obtained by MS³

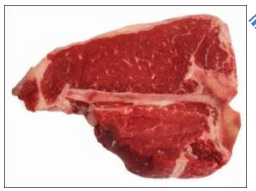
P. Cano, L. Debrauwer et al.,
*Anal. Chem.* (2012) 85, 8412

# Analyse de métabolites réactifs : exemple des alcénals

Régime pro-oxydant (viande rouge) et promotion du cancer colorectal (CRC)

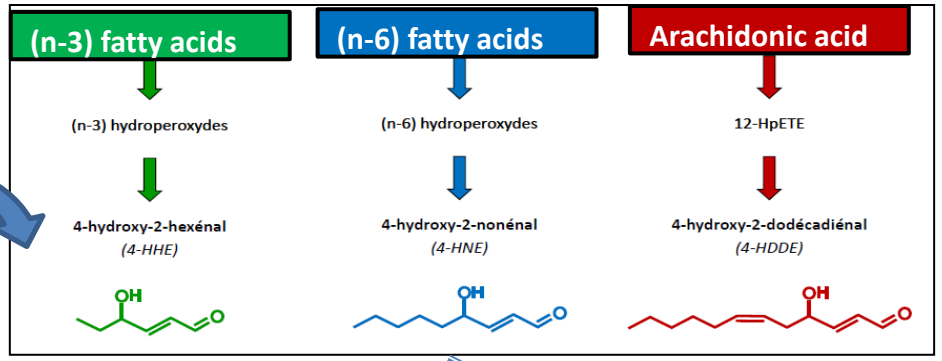
Gras

Viande rouge



Heme

Catalyse




Composés réactifs : carbonylation des protéines cellulaires / adduits ADN

Propriétés cytotoxiques des alcénals (HNE / HHE)

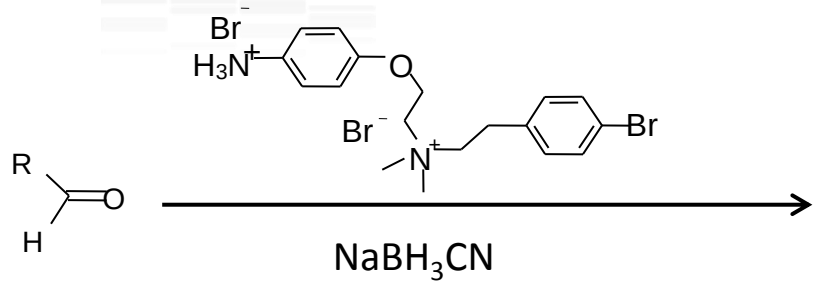
**Autres aldéhydes (inconnus ?) formés dans la lumière intestinale ?**

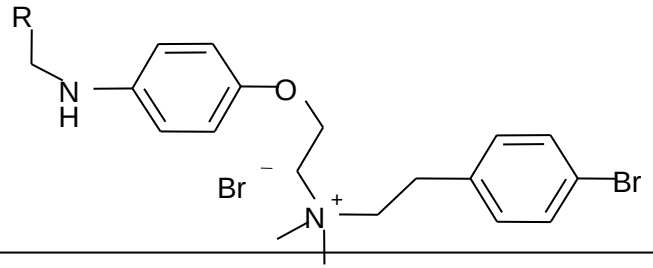
**Chaînon manquant entre viande rouge et risque de CRC mis en évidence dans les études épidemiologiques ?**

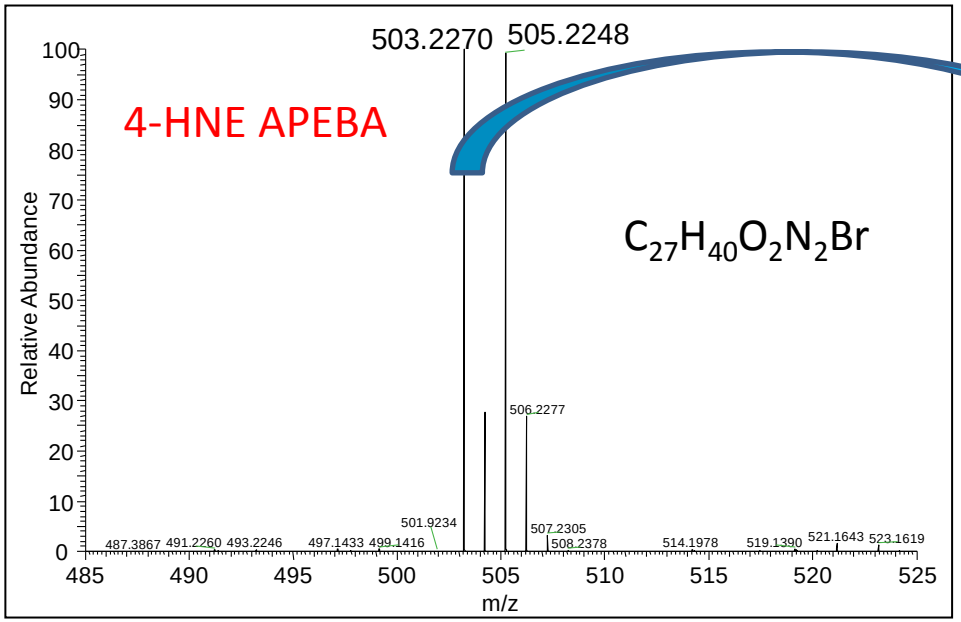
M. Baradat, L. Debrauwer et al.
*Chem. Res. Toxicol.*, 2011, 24, 1984.

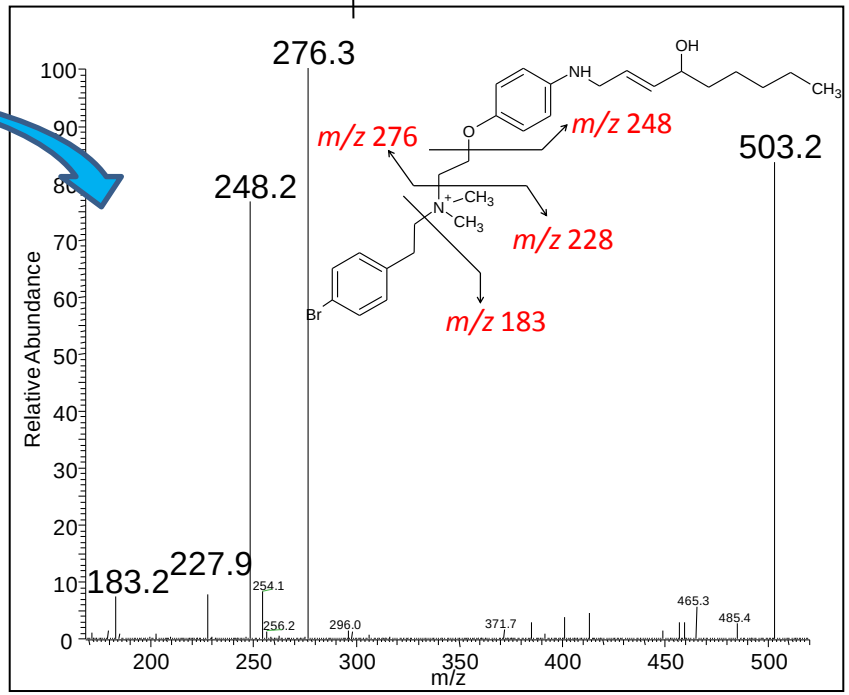
# Piégeage et analyse des aldéhydes libres

4-(2-((4-bromophenethyl)dimethyl ammonio) ethoxy)benzenaminium dibromide (4-APEBA)



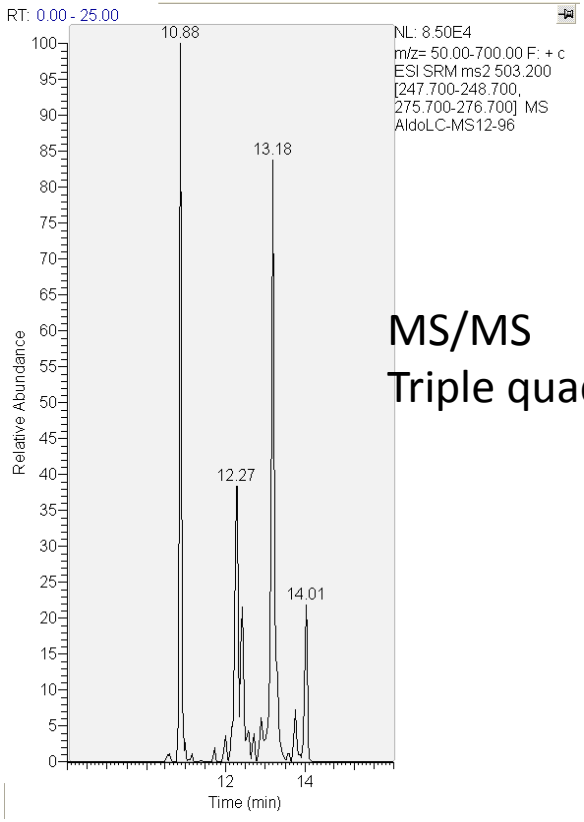



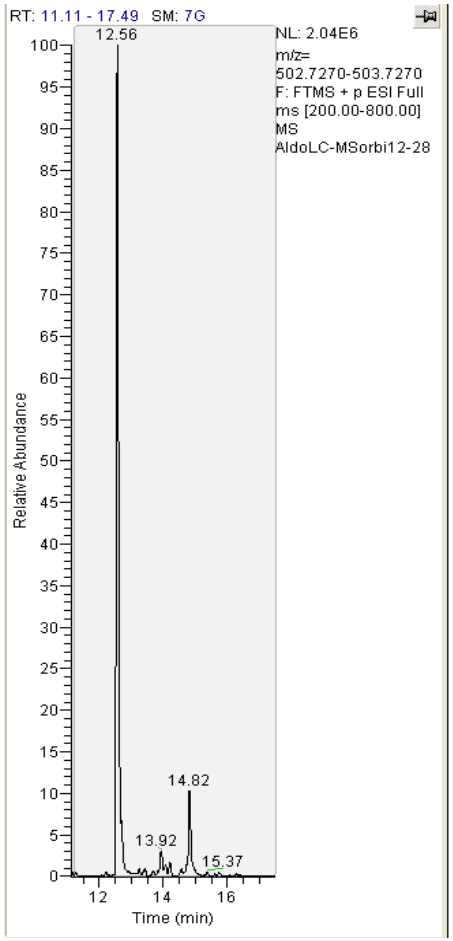

M. Eggink et al. (2010)
**Anal. Bioanal. Chem.,** 397; 665-675

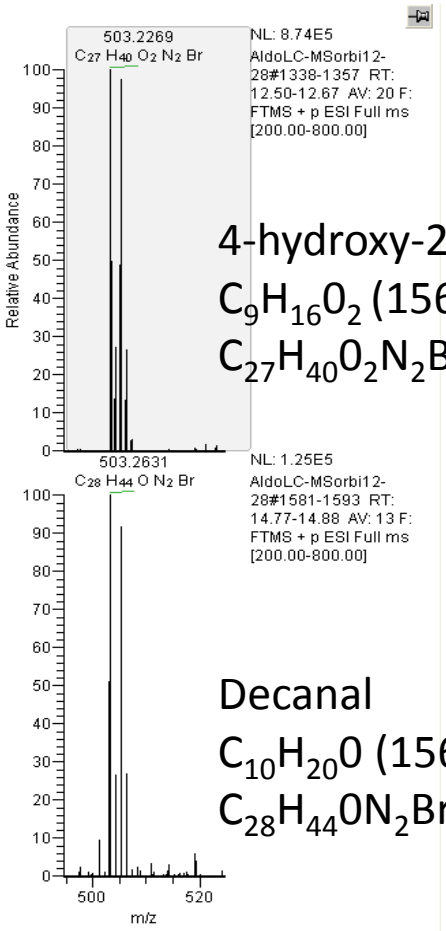
# FT-MS et différentiation d'isobares

HNE (Mw156)
Decanal (Mw 156)




MS/MS
Triple quad.




4-hydroxy-2-nonenal
$C_9H_{16}0_2$ (156.11503)
$C_{27}H_{40}0_2N_2Br$ (503.2270)

Decanal
$C_{10}H_{20}0$ (156.15145)
$C_{28}H_{44}0N_2Br$ (503.2634)

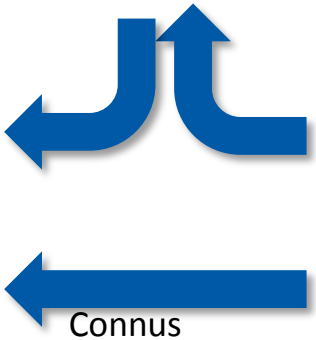
# Stratégie : « aldéhydomique » des eaux fécales

Eaux fécales

Dérivés APEBA synthétisés

LC-(+)ESI-HRMS

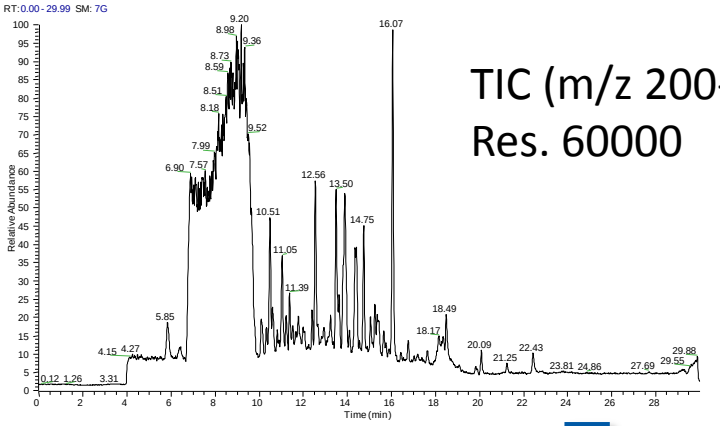
Identification $MS^n$



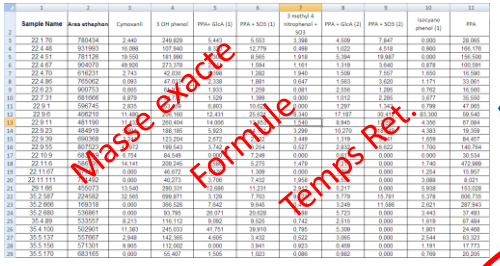
Inconnus

Connus
(standard disponible)

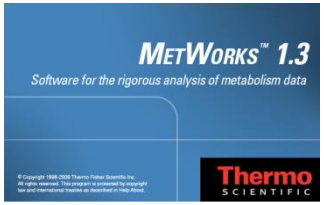
Liste d'aldéhydes libres dans les eaux fécales




TIC (m/z 200-800)
Res. 60000




Liste de dérivés bromés



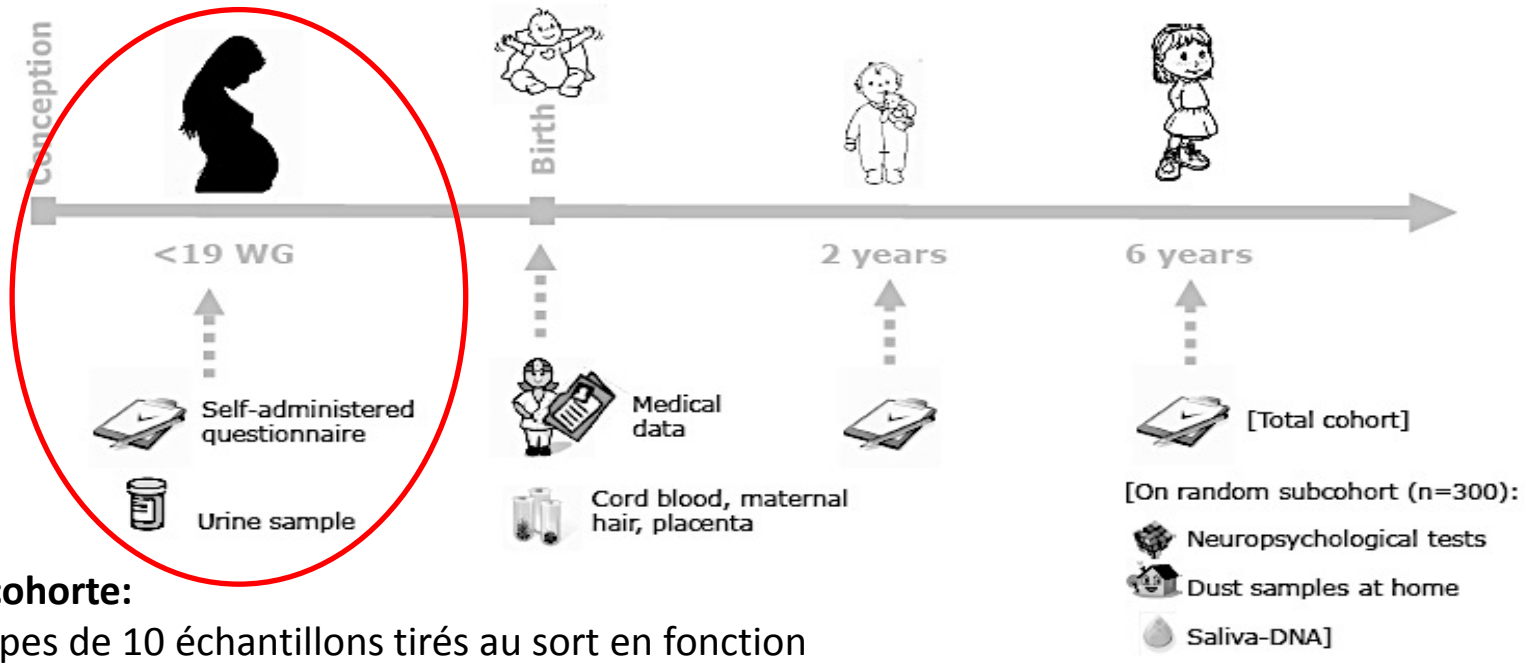

Filtration du signal massif isotopique
$\Delta$ = 1,9979 u.m.a.
Défaut de masse

# Recherche de métabolites urinaires marqueurs d'exposition

## Cohorte PELAGIE : Perturbateurs Endocriniens Etude Longitudinale sur les Anomalies de la Grossesse, l'Infertilité et l'Enfance

INSERM (Coord. Sylvaine Cordier)

environ 3500 femmes enceintes, région Bretagne , entre 2002 et 2006



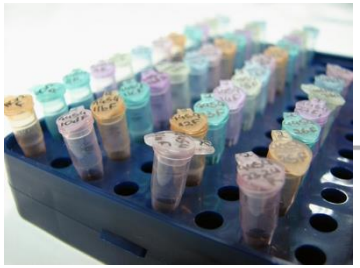

**Sous-cohorte:**

4 groupes de 10 échantillons tirés au sort en fonction
de la part de culture de céréale (%) dans la commune de résidence

**urbain / peu exposé / exposé / très exposé**

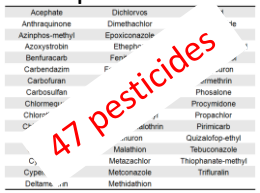
# Recherche de métabolites urinaires marqueurs d'exposition

urines

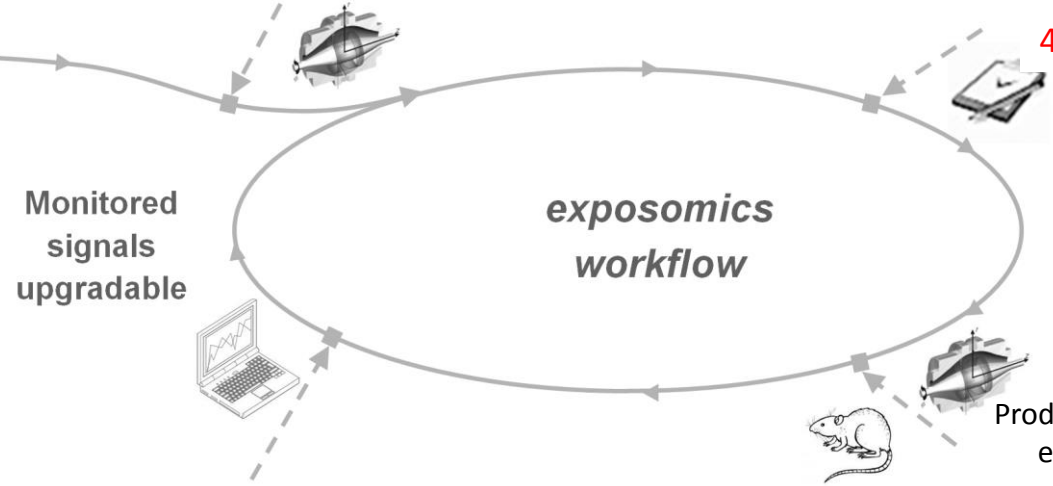


LC-ESI-HRMS

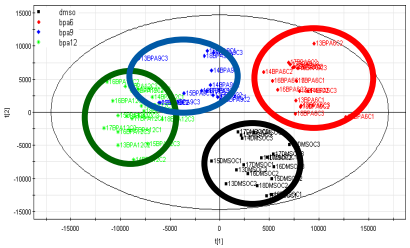
Cartographie des cultures
Pratiques culturales…

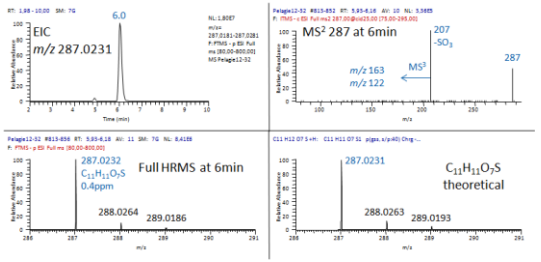



In silico + in biblio
460 métabolites




Production de « standards » par expérimentation animale






E. Jamin, L. Debrauwer et al.
*Anal. Bioanal. Chem.*, 2014, 406, 1149.

# Recherche de métabolites urinaires marqueurs d'exposition

## > Mode négatif

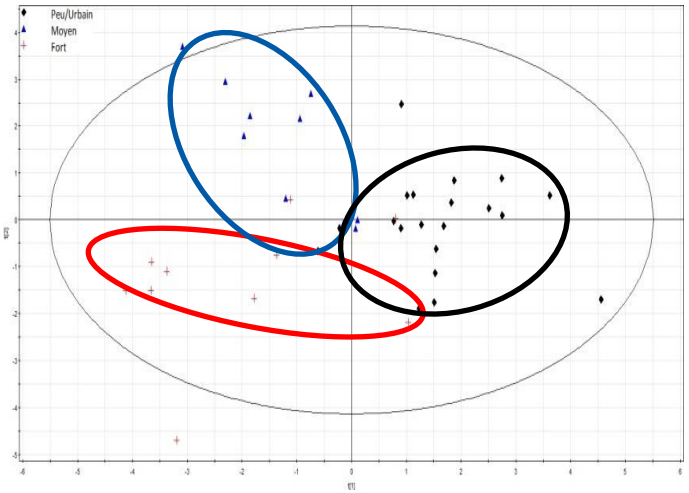
**128** molécules « suspectes » détectées

**24** métabolites pertinents → analyse statistique

**7** pesticides correspondant :

*Cymoxanil, Quizalofop-éthyl, Procymidone, Fenpropimorphe, Carbofuran, Chlorprophame, Azoxystrobine*

**> PLS-DA (OSC)** sur les 24 métabolites:




## > Mode Positif

**33** molécules « suspectes » détectées

**0** molécule pertinente

- *nombre de variables trop faible*
- *Pesticides = identiques mode négatif*

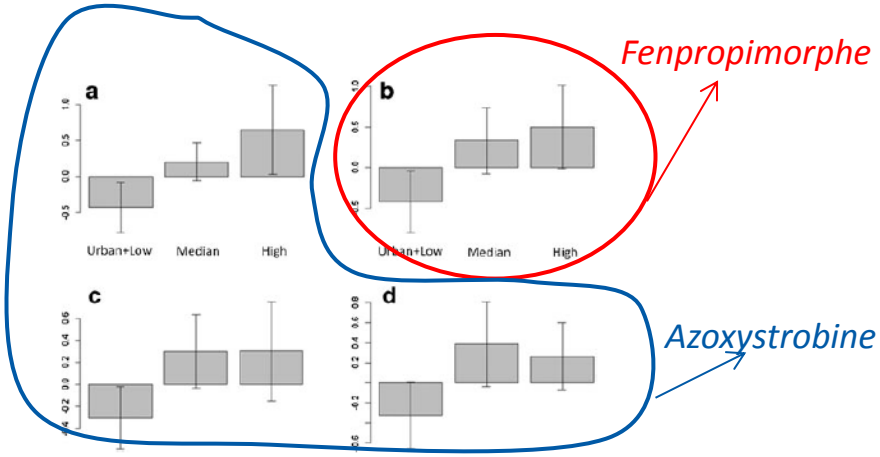


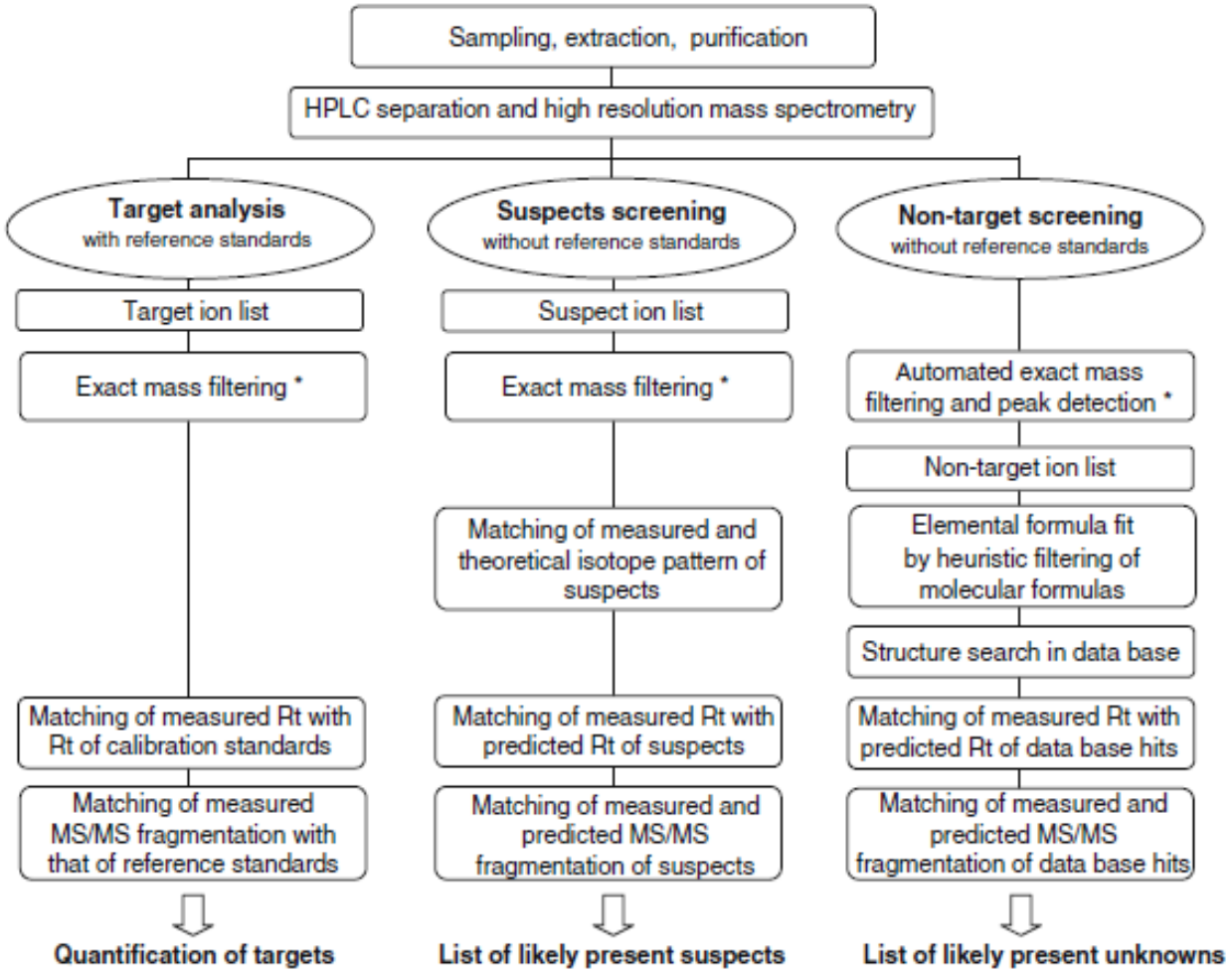

**Fig. 5** Variation of filtered normalized signals of (**a**) methyl-2-(2-hydroxyphenyl)-3-methoxyacrylate sulfate, (**b**) 2-methyl-2-phenylpropanoic acid, (**c**) methyl-2-(2-hydroxyphenyl)-3-methoxyacrylate glucuronide (1; *E* or *Z*), and (**d**) methyl-2-(2-hydroxyphenyl)-3-methoxyacrylate glucuronide (2; *E* or *Z*) in human urine samples

- Mise en évidence de marqueurs pertinents pour du biomonitoring (screening ciblé)
- Application au screening multi-résidus (familles chimiques, polluants, contaminants ≠)

E. Jamin, L. Debrauwer et al.
*Anal. Bioanal. Chem.*, 2014, 406, 1149.

# Comment gérer les inconnus ?

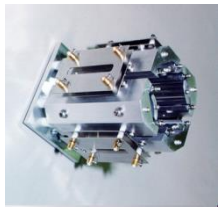
**Fig. 1** Comparison of systematic workflows for (i) quantitative target analysis with reference standards, (ii) suspects screening without reference standards, and (iii) non-target screening of unknowns in environmental samples by using LC–high resolution (tandem) mass spectrometry.
*Note that the *m/z* range of the extraction window for the exact mass filtering depends on the mass accuracy and the resolving power of the mass spectrometer used




M. Krauss, H. Singer, J. Hollender.
*Anal. Bioanal. Chem.*, 397 (2010) 943-951.

# Screening non-ciblé de contaminants par FT-MS

**Spectrométrie de masse**
- **Spectres Full MS**
- **MS/MS**
- **MS$^n$**
- **Haute résolution**



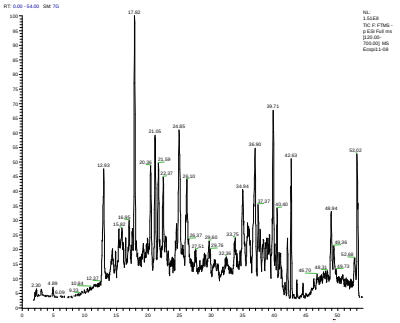
Trappe d'ions

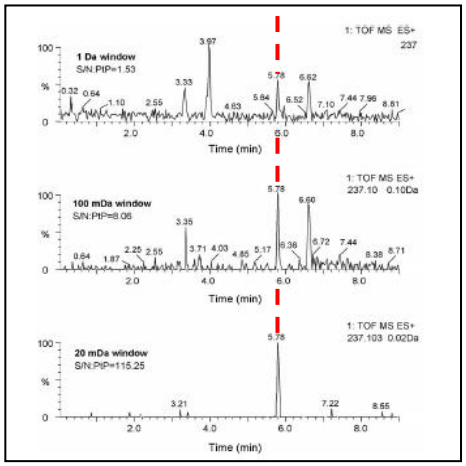
LTQ-Orbitrap

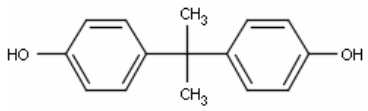
Schéma de fragmentation

Filiation ionique

Accès aux compositions élémentaires






# Exposition aux perturbateurs endocriniens : approches analytiques

Composés connus
Approche ciblée

→ Extraction
Purification

→ LC-(HR)MS/MS

LC-(HR)MS/MS

Colonne affinité
Récepteur ERα

(estrogénomimétiques)

Extraction (ASE)
(hexane-acetone 75-25)

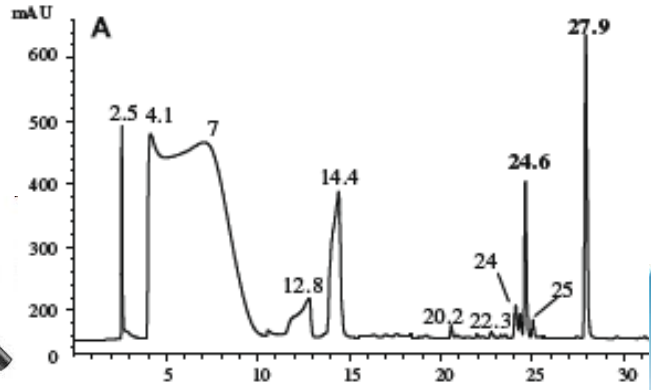
Composés inconnus
Approche non ciblée

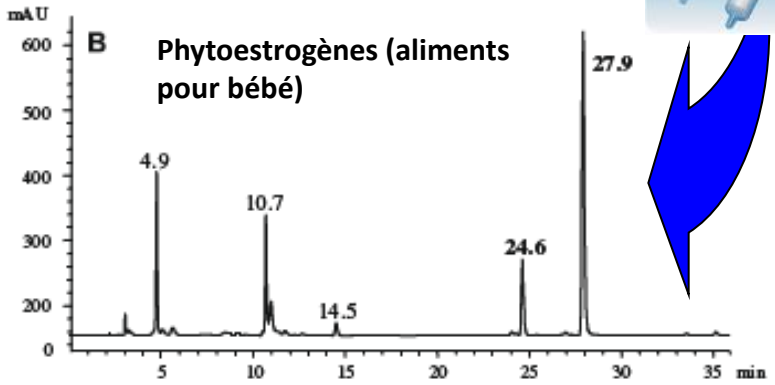
Extraction

Fractionnement

LC-(HR)MS/MS

Test activité biol.



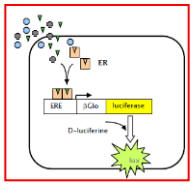




→ Identification structurale

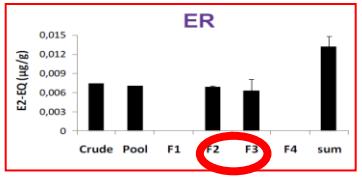
J.P. Antignac et al., Anal. Chim. Acta (2009) 637, 55
A. Riu, L. Debrauwer et al., *Food Chem. Tox.* (2008) 46, 3268.

# Application : recherche de perturbateurs endocriniens dans des sédiments de rivière

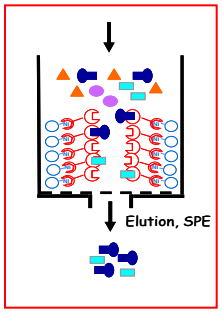
Fractionnement



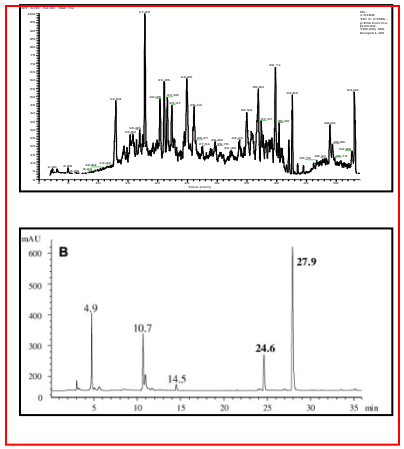

Test d'activité

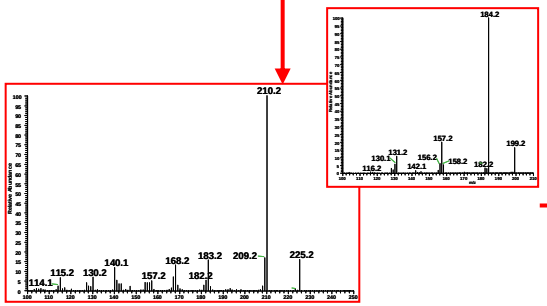



Analyse LC-HRMS (MS/MS)

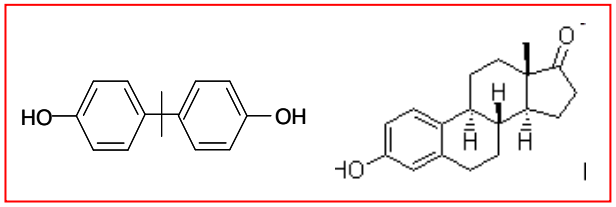



Colonne d'affinité RN




Analyse structurale

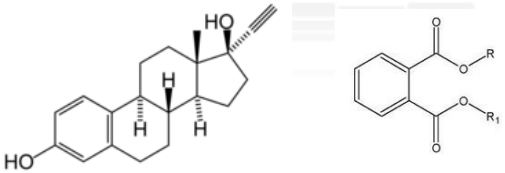



Identification molécules bioactives

Quantification

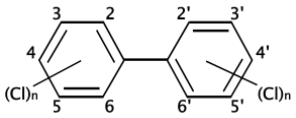
A. Jondeau-Cabaton, L. Debrauwer *et al.* *Environ. Sci. Pollut. Res.*(2013) 20, 2705

# Stratégie expérimentale

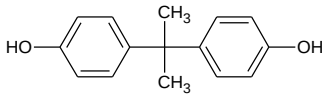


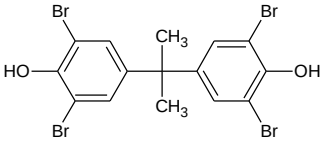


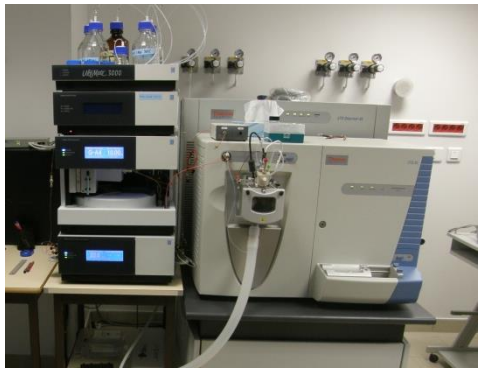





?



HPLC: C18 Uptisphere (250×2mm, 5 µm) 35°C
(A) H2O/CH3CN/CH3CO2H 95/5/01 (B) CH3CN
0min 0%B, 30-40min 100%B

MS: LTQ Orbitrap XL
ESI + et – (paramètres globaux)
full HRMS résolution 60000 (m/z 100 à 700)

**Data processing:**

Comparaison des échantillons avec un extrait blanc (« background subtraction »)

Proposition de formule brute ± 5ppm (C 0–30, H 0–60, N 0–6, O 0–15, P 0–3, I 0–6, and F 0–6), règle de l'azote, massif isotopique, MS/MS.

Interrogation base de données « Chemspider »

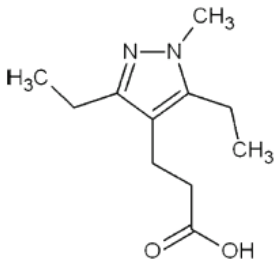
Identification par LC-HRMS$^n$ ou LC-ITMS$^n$, comparaison avec standards

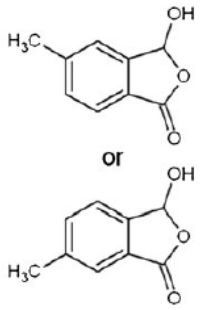
A. Jondeau-Cabaton L. Debrauwer *et al.*
*Environ. Sci. Pollut. Res.*(2013) 20, 2705

# Quelques résultats

| HRMS [M-H]$^-$ ($m/z$) | $R_T$ (min) | Molecular formula[a] | MS/MS fragment ions ($m/z$) | Number of candidates[b] | Pre-fractions |
|---|---|---|---|---|---|
| 313.1762 | 9.3 | $C_{15}H_{26}O_5N_2$ | 265<br>209 | 155 | F2+F3 |
| 209.1292[c] | 10.2 | $C_{11}H_{18}O_2N_2$ | 191.1187<br>165.1396<br>59.0141 | 893 | F2+F3 |
| 205.1342 | 10.7 | $C_{12}H_{18}N_2O$ | ND | 920 | F2 |
| 165.0418 | 11.3 | $C_6H_6O_2N_4$ | ND | 145 | F2 |
| 341.2075 | 12.0 | $C_{17}H_{30}O_5N_2$ | 293 | 103 | F2 |
| 168.0778 | 12.5 | $C_7H_{11}O_2N_3$ | 97.0773 | 479 | F3 |
| 185.0353 | 14.9 | $C_{10}H_6O_2N_2$ | 142 | 126 | F2+F3 |
| 377.2436[c] | 17.6 | $C_{21}H_{34}O_4N_2$ | ND | 291 | F2+F3 |
| 363.2648[c] | 18.6 | $C_{21}H_{36}O_3N_2$ | 295<br>249 | 163 | F2+F3 |
| 187.0510 | 19.0 | $C_{10}H_8O_2N_2$ | ND | 718 | F2+F3 |
| 267.0299 | 20.6 | $C_{15}H_8O_5$ | ND | 44 | F3 |
| 288.0661 | 21.6 | $C_{18}H_{11}O_3N$ | 260.0714<br>232.0767<br>182.0247 | 125 | F3 |
| 227.1077 | 22.6 | $C_{15}H_{16}O_2$ | 212.0834<br>133.0654 | 976 | F2+F3 |
| 285.1492 | 23.4 | $C_{13}H_{22}O_2F_4$<br>$C_{18}H_{22}O_3$ | ND | 2<br>961 | F3 |
| 313.2384 | 24.6 | $C_{18}H_{34}O_4$ | 295<br>277<br>269 | 244 | F2+F3 |
| 193.0866 | 25.0 | $C_{11}H_{14}O_3$ | 137.0244<br>136.0162 | 1858 | F3 |
| 163,0401 | 25.4 | $C_9H_8O_3$ | 135.0513<br>119.0503 | 314 | F2 |
| 267.1391 | 25.6+27.5 | $C_{18}H_{20}O_2$ | ND | 1 | F2+F3 |
| 253.0870 | 25.95 | $C_{10}H_{14}O_3$<br>$C_9H_{11}O_2N_6F$ | ND | 1276<br>1 | F3 |
| 249.1858 | 26.8 | $C_{16}H_{26}O_2$ | 205.1601<br>192.1158 | 801 | F3 |
| 269.1537 | 27.3 | $C_{18}H_{22}O_2$ | 171.1389 | 920 | F3 |
| 205.1592 | 32.2 | $C_{14}H_{22}O_2$ | ND | 1436 | F2+F3 |
| 283.0396 | 35.0 | $C_{16}H_9O_4F$ | 163 | 27 | F2 |

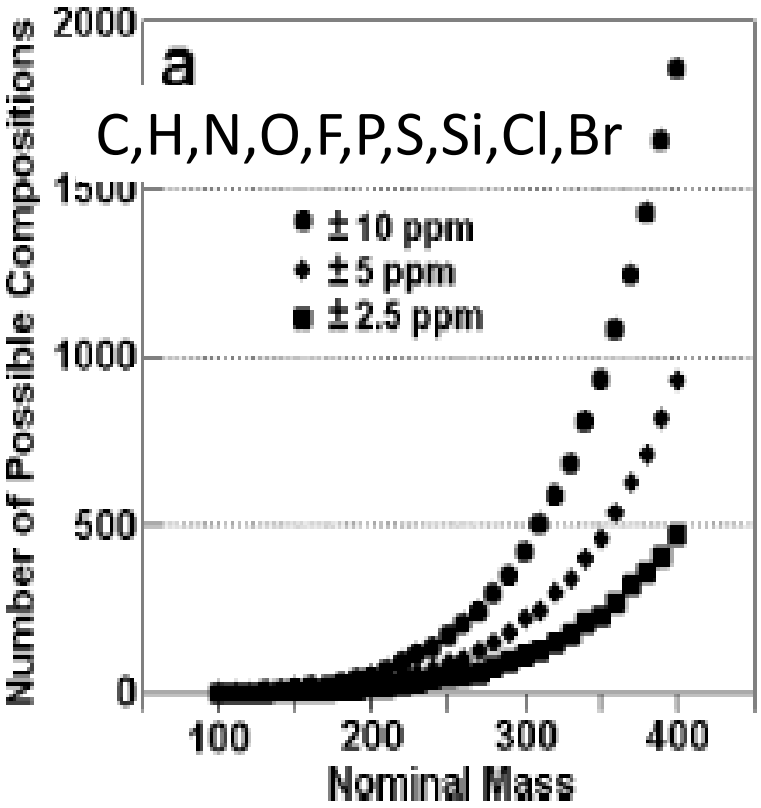
- 30 substances détectées
Seulement 7 uniquement en ESI+
- 4 PE connus identifiés : BPA, *n*butylparaben, 4OP-monoethoxylate , 4OP (level1)
- 1 PE connu suspecté : DES (level2)
- 4 composés suspects infirmés
- 2 inconnus caractérisés (level 1 et 2)

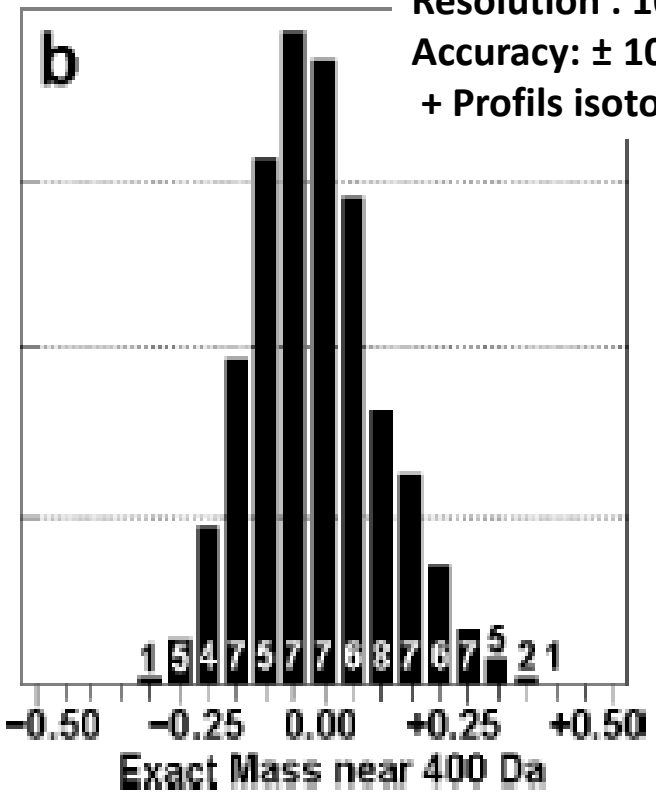



A. Jondeau-Cabaton L. Debrauwer *et al.* *Environ. Sci. Pollut. Res.*(2013) 20, 2705

# Comment aller plus loin ?
# Performance instrumentale




**Resolution : 10000**
**Accuracy: ± 10 ppm**
**+ Profils isotopiques**

M 400.00000

± 2.5 ppm → 470 compositions

± 5.0 ppm → 932 compositions

± 10 ppm → 1860 compositions

Nombre maximum de compositions possibles pour 399.95

A. Grange, F. Genicola, G. Socovol,
*Rapid Commun. Mass Spectrom.*, (2002) 12, 2356

# Comment aller plus loin ?
# Performance instrumentale

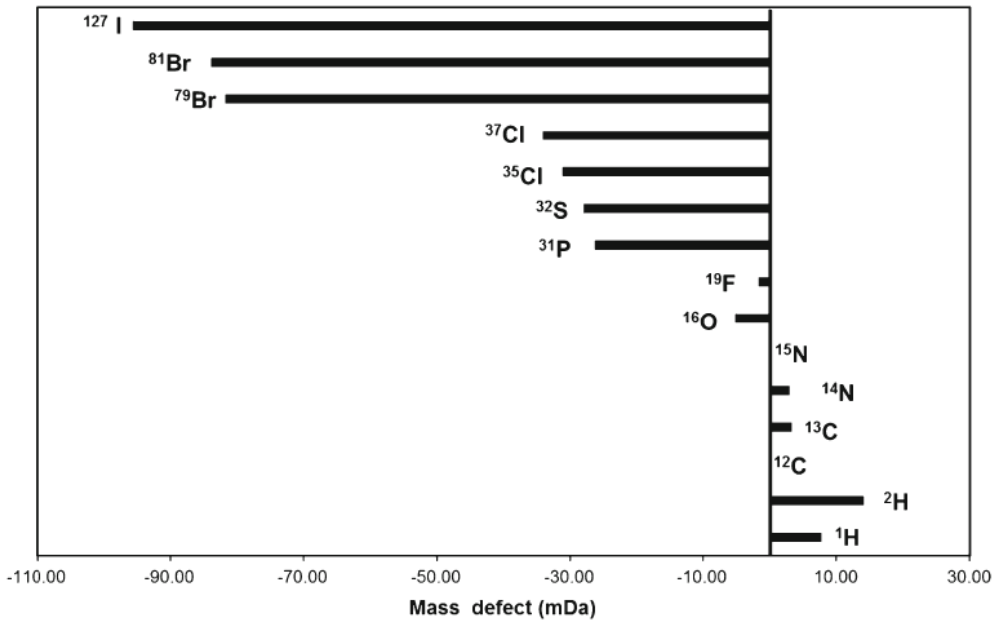



**Fig. 1** The different mass defects of elemental isotopes enable a unique elemental composition for any molecule to be determined from a sufficiently accurate mass measurement. (Adapted from [32])

Objectif une seule formule brute possible
Précision nécessaire 0,1mDa y compris en mélange complexe

| | |
|---|---|
| ToF / Orbitrap | 2-10 mDa |
| FT-ICR | ≈ 1 mDa |

M. Zedda et al.,
*Anal. Bioanal. Chem*. (2012) 403, 2493

# Comment aller plus loin ?
# Recherche dans les bases de composés chimiques

Table 1: Example of a molecular formula search for $C_{15}H_{12}O_7$ in different chemical databases. Search date: July 2007

| Database name | Compounds found | Total database entries |
|---|---|---|
| Chemical Abstracts (CAS) | 181 | 24,000,000 |
| Beilstein Database (MDL) | 166 | 8,000,000 |
| Dictionary of Natural Products (DNP) | 129 | 170,000 |
| PubChem (NIH) | 19 | 800,000 |
| Available Chemicals Directory (MDL) | 6 | 400,000 |
| ChemIDplus (NIH) | 6 | 370,000 |
| KEGG (Kyoto University) | 3 | 13,000 |
| NIST05 (NIST mass spectral database) | 2 | 163,000 |
| MOLGEN molecular isomer generator (allowing 2 benzene groups; 1 ether group, 1 keto group; 5 hydroxy groups) | 788,000 | - |

Kind T., Fiehn O
*BMC Bioinformatics* (2006) 7, 234)

# Comment aller plus loin ?
# Recherche dans les bases de composés chimiques

**Table 3: Number of possible molecular formulas at different levels of mass accuracy and the impact of isotopic abundance accuracy. A mass spectrometer capable of 3 ppm but with 2% correct isotopic pattern outperforms even a (non-existing) mass spectrometer with 0.1 ppm mass accuracy! The results are computed for randomly selected targets, so single results vary but the trend remains. LEWIS and SENIOR check was applied. Candidates with unrelated high element counts were already excluded**

| | without isotope abundance information | | | | | 2% isotopic abundance accuracy | 5% isotopic abundance accuracy |
|---|---|---|---|---|---|---|---|
| molecular mass [Da] | 10 ppm | 5 ppm | 3 ppm | 1 ppm | 0.1 ppm | 3 ppm | 5 ppm |
| 150 | 2 | 1 | 1 | 1 | 1 | 1 | 1 |
| 200 | 3 | 2 | 2 | 1 | 1 | 1 | 1 |
| 300 | 24 | 11 | 7 | 2 | 1 | 1 | 6 |
| 400 | 78 | 37 | 23 | 7 | 1 | 2 | 13 |
| 500 | 266 | 115 | 64 | 21 | 2 | 3 | 33 |
| 600 | 505 | 257 | 155 | 50 | 5 | 4 | 36 |
| 700 | 1046 | 538 | 321 | 108 | 10 | 10 | 97 |
| 800 | 1964 | 973 | 599 | 200 | 20 | 13 | 111 |
| 900 | 3447 | 1712 | 1045 | 345 | 32 | 18 | 196 |

Kind T., Fiehn O
*BMC Bioinformatics* (2006) 7, 234)

# Manque de bases de spectres MS/MS comparables

Anal Bioanal Chem (2012) 403:2493–2502
DOI 10.1007/s00216-012-5893-y

REVIEW

## Is nontarget screening of emerging contaminants by LC-HRMS successful? A plea for compound libraries and computer tools

Marco Zedda · Christian Zwiener