UPLC®-MS/MS, an analytical tool for the accurate and rapid quantification of phytoestrogen metabolites in milk

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2015 IDF/ISO Analytical Week and OptiMir Symposium

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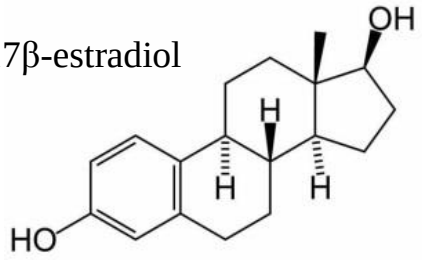
❖ Phytoestrogens [a, b]

17β-estradiol



- These are nonsteroidal compounds present in plants which contribute to the plants development,
- These polyphenolic compounds are structurally or functionally similar to mammalian estrogens which allows them to have estrogenic activity,
- They are essentially present in Leguminosae (soybean, clover, etc.),
- They may be found in dairy milk and their concentrations varied according to feed rations and individuals,

They are considered as endocrine disruptors, indicating that they have the potential to cause adverse health effects (infertility or cancer).

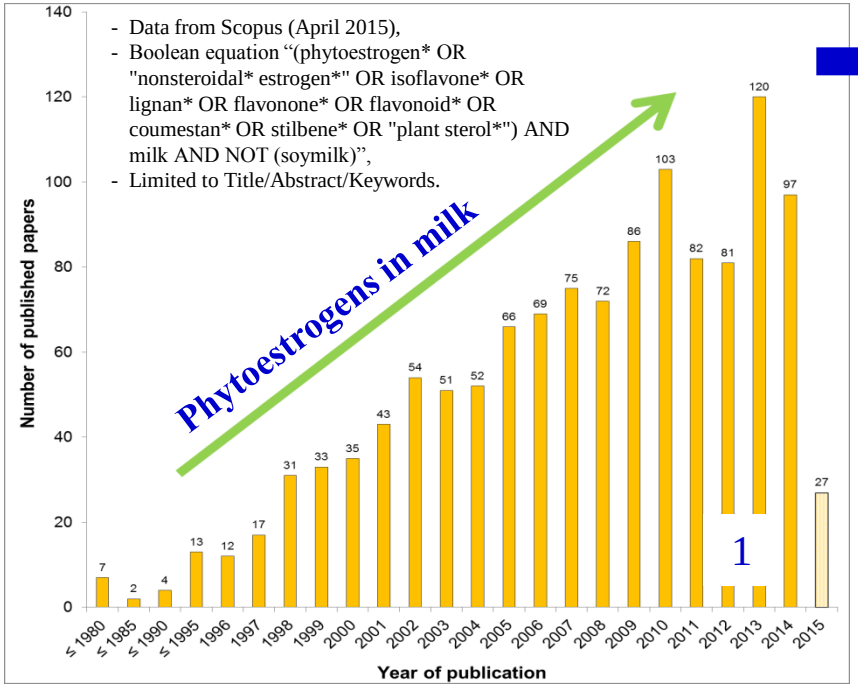
Several health benefits including a lowered risk of osteoporosis, heart diseases, breast cancer and menopausal symptoms, are attributed to these compounds.

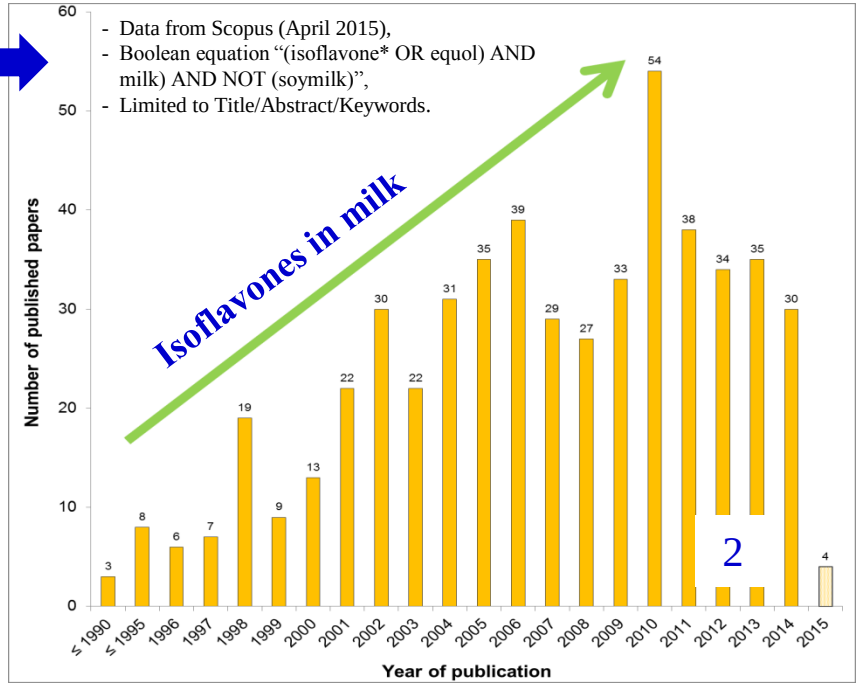
[a] M. Mostrom, T.J. Evans. Veterinary Toxicology –Basic and Clinical Principles (2nd). Ramesh C. Gupta (ed.), Netherlands, 2012, Chapter 76.

[b] Patisaul H.B., Jefferson W., Front. Neuroendocrin. 31 (2010) 400-419.

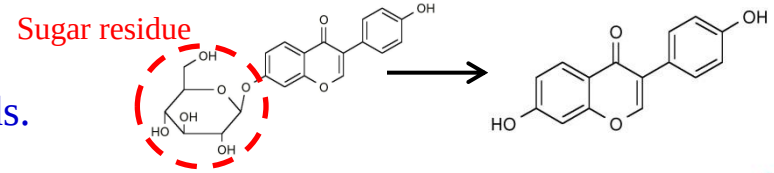
❖ Phytoestrogens *(→ focus on isoflavones in milk)*

- Growing interest since the mid 1990's,





- Present principally as conjugated forms (in milk: glucuronides or sulfates),
  - ➢ Sample preparation often requires an hydrolysis step
    (aglycones → biologically active forms which can be absorbed by the organism)
- LC-MS is the most widely used method,
- Lack of validation process for quantitative methods.



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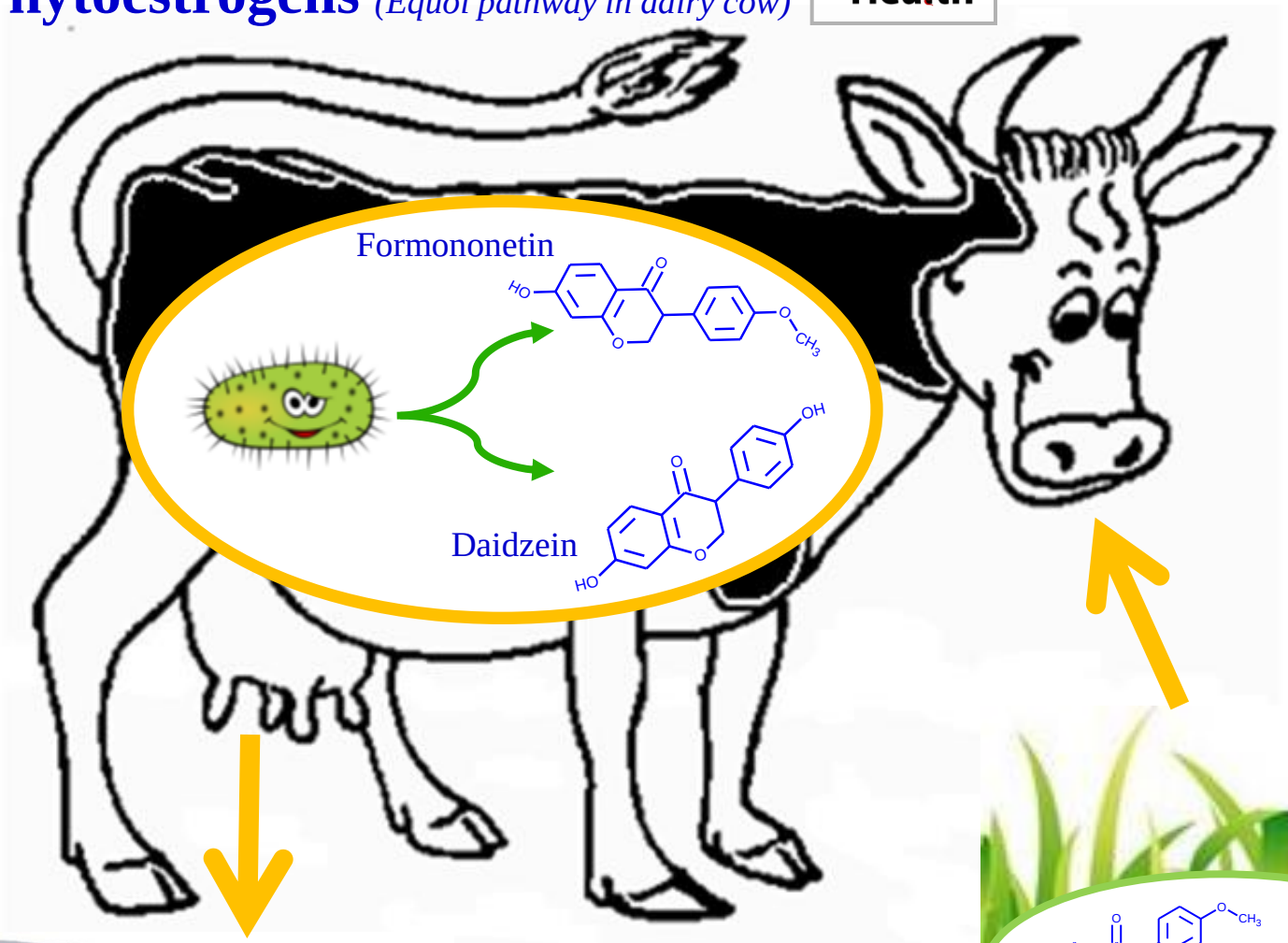
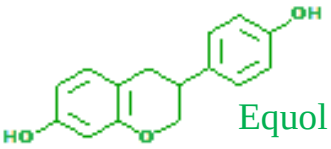
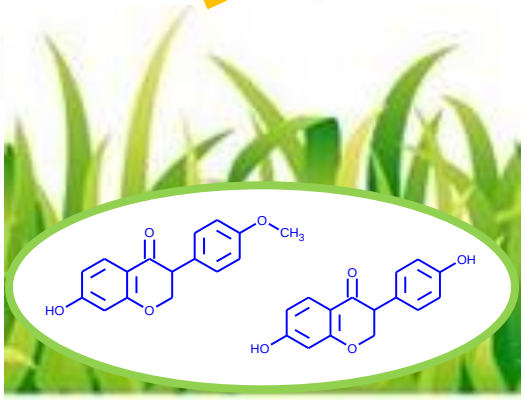
❖ Phytoestrogens *(Equol pathway in dairy cow)*

Phyto Health [a]

cra-w







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[a] Daems *et al.* (2014) 19th NSABS (Poster) *(http://orbi.ulg.ac.be/handle/2268/162932)*

❖ Analytical method developed and validated [a]

Internal Standard
(daidzein-d_4)

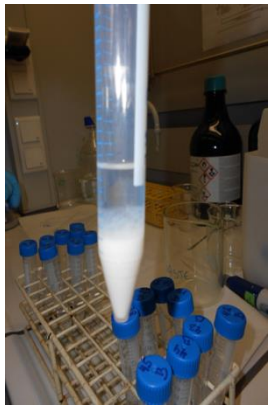
Random sampling & storage at -18°C

Enzymatic hydrolysis

Double extraction Liq./Liq.
(Hexane → remove fat & ethyl acetate → recover interest compounds)

Evaporation & reconstitution in solvent

UPLC®-MS/MS analysis



System:
Waters Acquity™ UPLC

Column:
Acquity UPLC® HSS T3
(2,1×100 mm, 1,8 µm)

Mobile phases (gradient):
(A) H_2O/ acetonitrile *(95/5 , v:v with 0,01% formic acid)*
(B) acetonitrile/H_2O *(95/5, v:v with 0,01% formic acid)*

Run time: 5 min

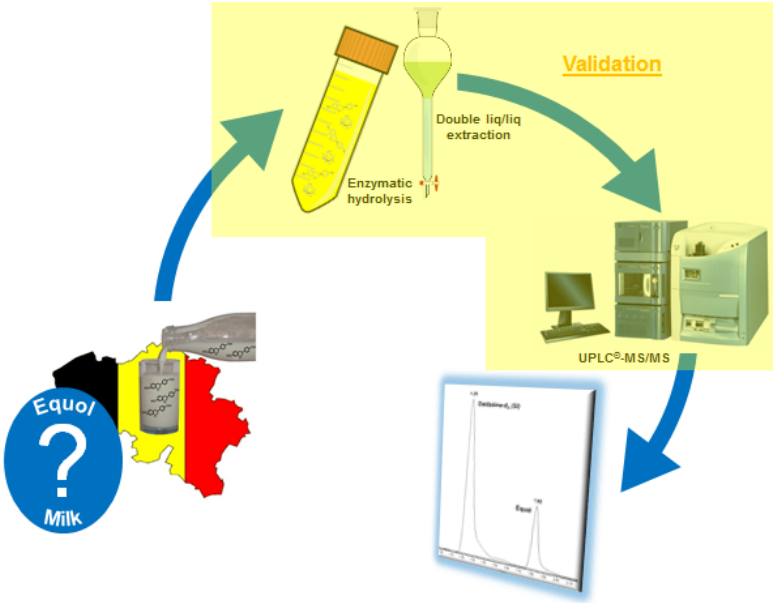
Flow rate: 0,6 mL.min^{-1}

Detector: Quattro Premier XE
(ESI $^+$, MRM mode)

[a] Daems *et al.*, Dairy Sci. & Technol. 95:3 (2015) 303-319.

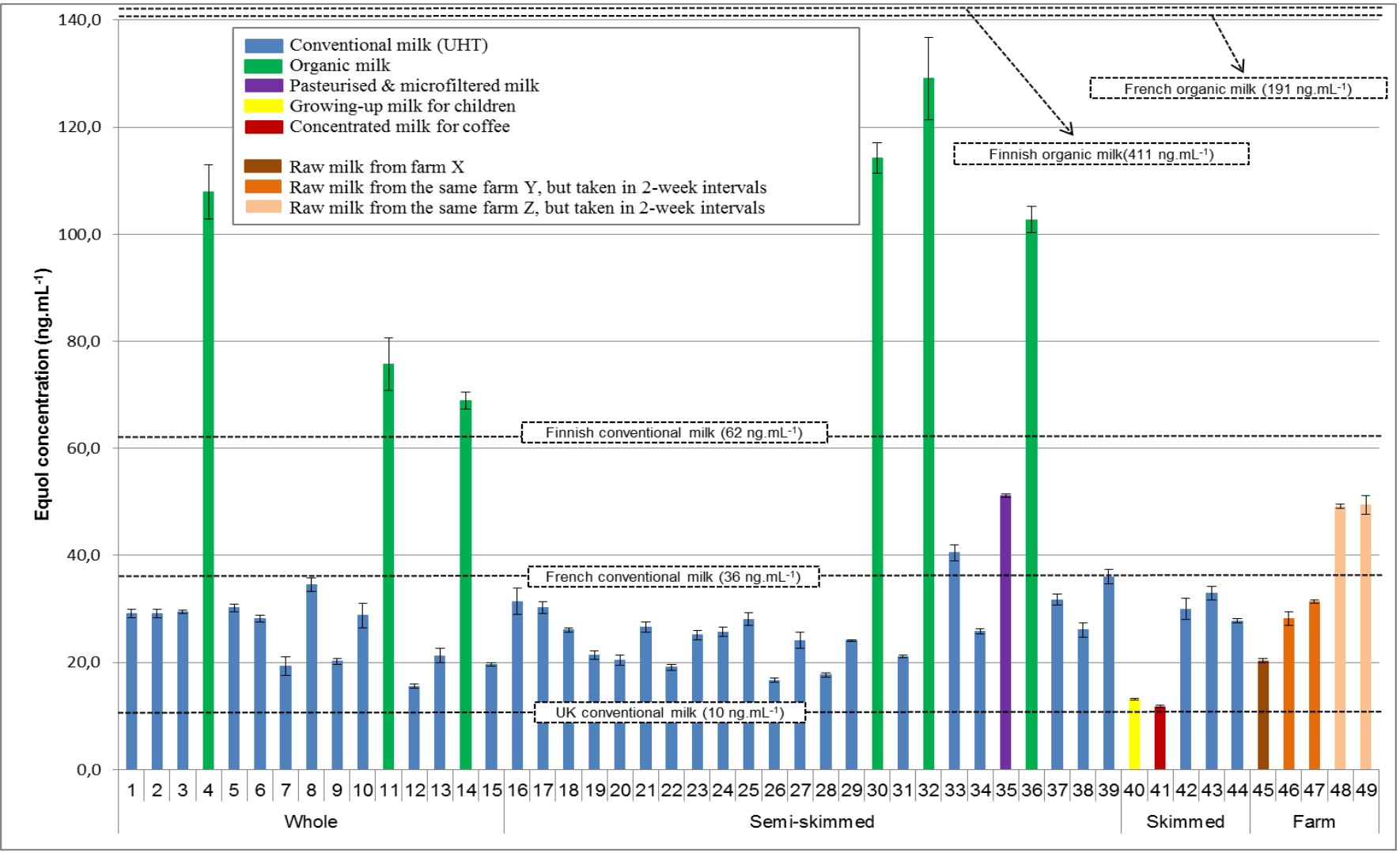
❖ Analytical method developed and validated [a]

Validation parameters						
Selectivity	Range Linearity	LOD/LOQ	Accuracy	Precision	Stability	Robustness
V	5 - 1000 ng.mL^{-1}	0,3 / 5 ng.mL^{-1}	V	V	V	V



[a] Daems *et al.*, Dairy Sci. & Technol. 95:3 (2015) 303-319.

❖ Equol in Belgian cow's milk [a]



[a] Daems *et al.*, Dairy Sci. & Technol. 95:3 (2015) 303-319.

❖ Conclusion and perspectives

UPLC®-MS/MS

- Powerful tool for analysis of complex samples.
- Pushes the limits of quantification to lower levels while giving reliable results.
- More environmentally friendly, less expensive and less time-consuming for the development and the validation.

- Equol is present in milk commercialized in Belgium.

  → *Milk as a potential source of EQ*

- Equol content is higher in organic milk than in the conventional one.

  → *Impact of agricultural practices*

- More studies are needed to better understand the impact of phytoestrogens in human and animal health.
- Development and validation of a quantification method with more compounds could also lead to more reliable monitoring of these compounds in dairy production. *(Reduce the time of samples preparation !)*

Thank you for your attention

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