# Development of extraction and analytical methods by GC-MS2 and LC-MS2 in honey for 3 pesticide families (nicotinoids, pyrethroids and pyrazoles)

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# Development of extraction and analytical methods by GC-MS² and LC-MS² in honey for 3 pesticide families (nicotinoids, pyrethroids and pyrazoles)

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## *Introduction*

Honeybees play an essential role in pollination (75% of entomofaunic pollination). They are also considered as bioindicators because of their high sensitivity to pesticides. Since a dozen years, colonies worldwide have collapsed. Pesticides are highly suspected to participate to the collapse. But, analytical methods able to detect very low amounts of these molecules have to be developed.

The aim of this work was to develop extraction and analytical methods in honey and enabling quantification of 25 pesticides belonging to 3 chemical families.

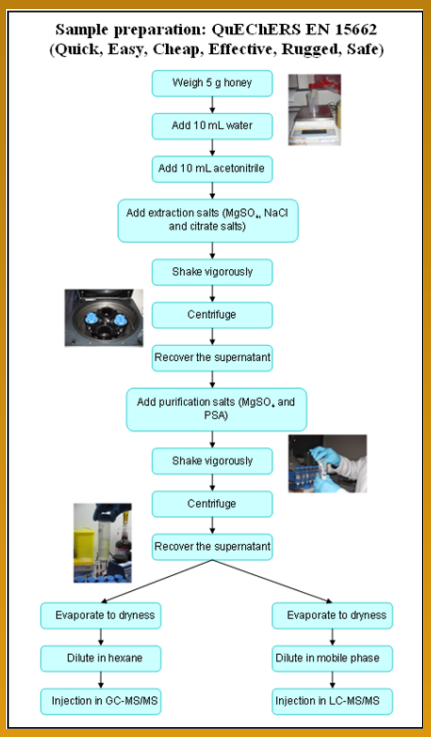
## *Methods validation*

To be validated analytically and statistically, methods have to be specific, reliable, accurate, repeatable and reproducible. *(Decision 2002/657/CE )*

### Search for 25 pesticides

| Neonicotinoids | Pyrethroids | Pyrazoles |
|---|---|---|
| Acetamiprid | Acrinathrin | Dimetilan |
| Clothianidin | Bifenthrin | Ethiprole |
| Imidacloprid | Cyfluthrin | Fipronil |
| Thiacloprid | Cypermethrin | Fipronil Desulfinyl |
| Thiametoxam | Deltamethrin | Fipronil Sulfide |
| | Fenvalerate | Fipronil Sulfone |
| | λ-cyhalothrin | Pyraclofos |
| | Permethrin | Tebufenpyrad |
| | Resmethrin | Tolfenpyrad |
| | Tefluthrin | |
| | τ-fluvalinate | |

### Sample preparation: QuEChERS EN 15662 (Quick, Easy, Cheap, Effective, Rugged, Safe)

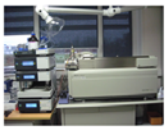



### Analytical conditions in GC-MS/MS

- Column: DB 5 ms, 30 m x 0.25 mm I.D., 0.25 µm
- Injection in splitless mode
- Carrier gas: Helium
- Injected volume: 2 µL
- Temperature program: 60°C to 300°C in 32 minutes
- EI source
- Triple quadrupole detector

GC Trace Ultra and TSQ Quantum (Thermo)

### Analytical conditions in LC-MS/MS



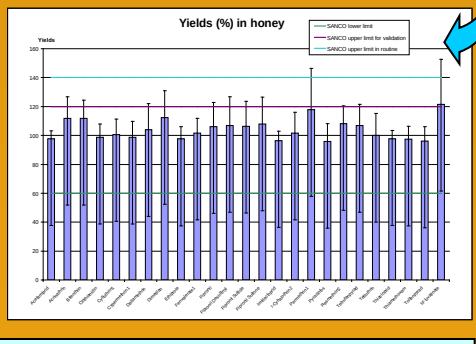
Ultimate 3000 (Dionex) and Api 4000 (AB Sciex)

- Column: Uptisphère C18 15 cm x 2.1 mm
- Injected volume: 20 µL
- Injection at 40°C
- Flow: 0.35 mL/minute
- Mobile phase: water-acetonitrile mixture
- ESI source
- Triple quadrupole detector

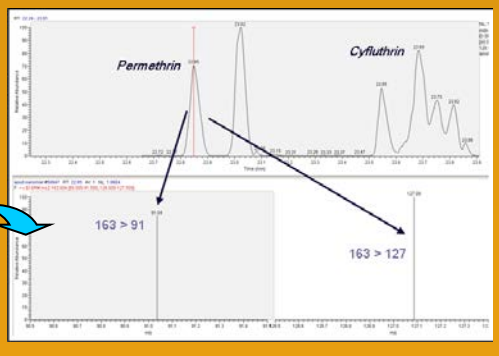
## *Recovery yields*

✓ The QuEChERS extraction was repeated twice at each spiking level (0.5, 2 and 10 ng/g) and each sample was injected twice.

✓ Mean yields were typically between 90 and 120% and CV were < 30%. Average yields are shown on the graph below.




✓ Mass spectrometry is performed in SRM (Selective Reaction Monitoring) mode.
✓ Each ion is characterized by a quantification transition and a confirmation transition.
✓ The graphs show the chromatographic signal [signal = f(t)] and the mass spectrum [intensity = f(m/z)]




## *Conclusions*

➢ Specific methods for the 4 types of honey tested (oilseed rape, acacia, chestnut and "all flowers")
➢ Recovery rates within the SANCO limits 2011
➢ Quantification at values of 1 ng/g for 21 pesticides and 2 ng/g for 4 pesticides
➢ Methods used in routine in an analysis laboratory

## *Perspectives*

➢ Analysis of more than 300 samples collected in Vendée during a 2009-2010 field study
➢ Development of similar methods in other hive products (pollen and bees)
➢ Availability of these methods for the study of pesticide impact on bee mortalities

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