

Reliable analysis for safe products.

Pesticide-analysis in honey by means of LC-MS/MS and GC-MS/MS – Investigation of the most appropriate technique

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Content

- About Intertek Food Services GmbH
- Legal background of pesticide residues in food
- Sample preparation and analytical measurement systems
- Investigation of the most appropriate technique
- Determination of pesticides in honey and hive products
- Conclusions



About us

Intertek Food Services GmbH (former APPLICA GmbH)

- Intertek provides **quality and safety services** to a wide range of global and local industries.
- Intertek Food Services GmbH has established itself as a well-known player within the field of **applied chemical analysis**.
 - well-known expertise in **honey, hive products**, fruits and vegetable foods.
- Innovative methods and state-of-the-art equipment coupled with a highly specialized know-how for excellent analytical performance and service to our customers

....we support our customers in their global trade

Pesticide residues: legal background

Honey and hive products

- Honey is defined as natural product (Honey Directive 2001/110/EC; HonigV)
- Maximum residue levels of pesticides (MRLs): Regulation (EC) 396/2005
- MRLs of bee treatment agents: Regulation (EC) 470/2009 + Annexes Regulation (EEC) 2377/90
- royal jelly and pollen are defined as food (annex I of Regulation (EC) 396/2005)

Default MRL: 0.01 mg/kg (Art. 18 Regulation (EC) 396/2005)

- for those products for which no specific MRLs are given

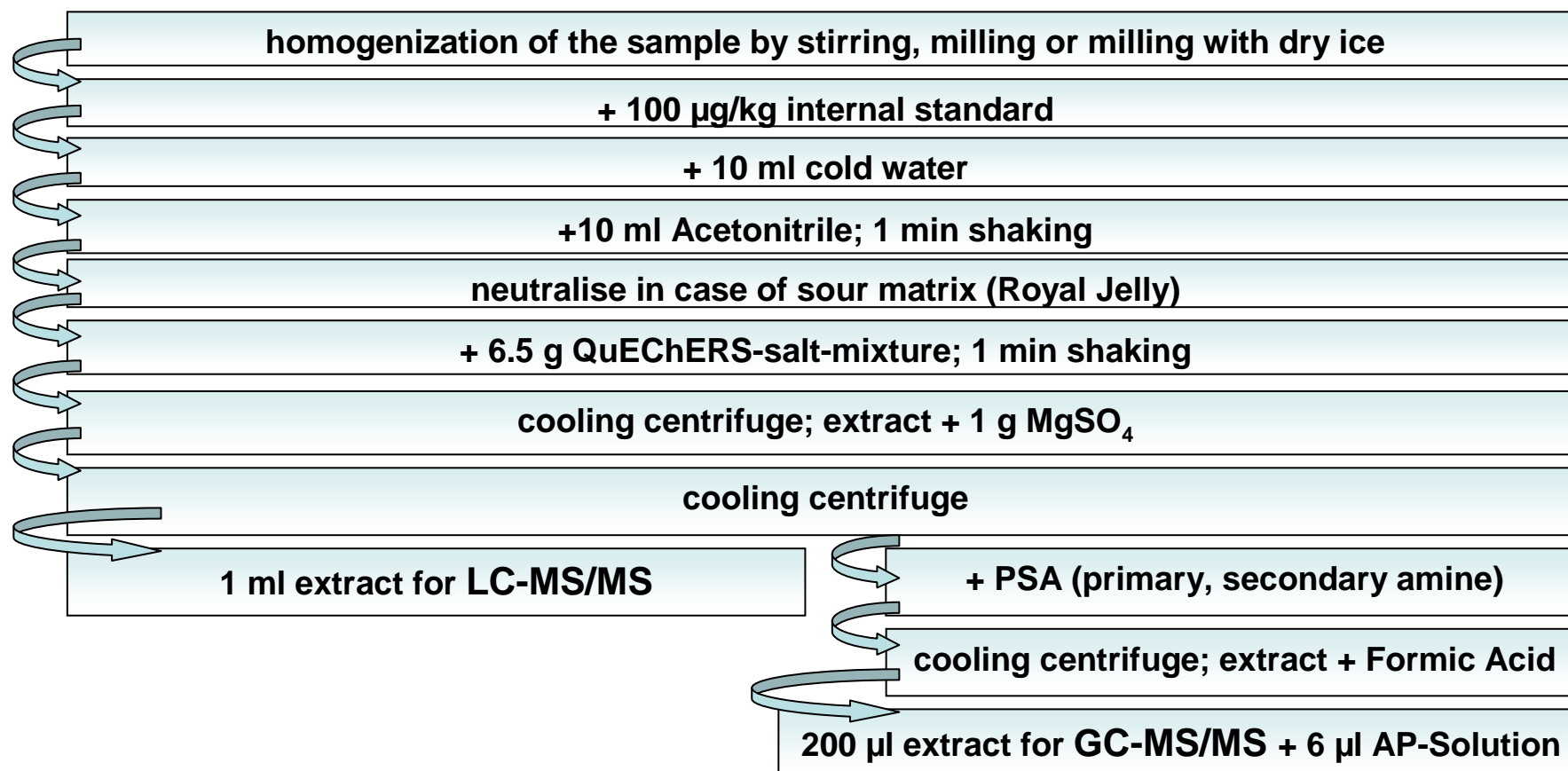
Organic products: guidance value 0.01 mg/kg (BNN^[1])



[1] Bundesverband Naturkost Naturwaren Herstellung und Handel e.V.:
http://www.n-bnn.de/html/img/pool/BNN_orientationvalue_JVL_English.pdf

Sample preparation

QuChERS (Quick, easy, cheap, effective, rugged and safe^[1])



[1] Anastassiades, M., S. J. Lehotay, D. Stajnbaher and F. J. Schenck (2003). "Fast and easy multiresidue method employing acetonitrile extraction/partitioning" and "dispersive solid-phase extraction for the determination of pesticide residues in produce." Journal of AOAC International 86(2): 412-31.

Analytical systems for Pesticide Testing

Analytical systems and instrument parameters

- LC-MS/MS:

TSQ Quantum Ultra (Thermo Fisher Scientific)

- Autosampler: Finnigan Surveyor Plus (Thermo-Fisher)
- Pump: Finnigan Surveyor MS Pump Plus (Thermo-Fisher)
- Column: Hypersil GOLD C8, 150 x 2.1 mm, 5 μ m
- Temperature: 35°C
- Solvent A: Water + 0.01 % Acetic acid
- Solvent B: Methanol + 0.01 % Acetic acid
- Gradient:

Time [min]	Solvent A [%]	Solvent B [%]	Flow [ml/min]
0.00	90	10	0.200
1.00	90	10	0.200
6.00	10	90	0.200
19.00	10	90	0.200
19.10	90	10	0.200
25.00	90	10	0.200

Analytical systems for Pesticide Testing

Analytical systems and instrument parameters

- GC-MS/MS (test for routine analysis):

TSQ Quantum GC (Thermo Fisher scientific)

- Autosampler: CTC Combi PAL
- PTV: Optic3 (ATAS, Axel Semrau)
- Column : VF-5ms, 30 m x 0.25 mm x 0.25 μ m
- Temperature-Progr.: 100°C (1 min), 4°C/min, 280°C (3.5 min)
- Transferline: 280°C

- GC-MS (routine analysis)

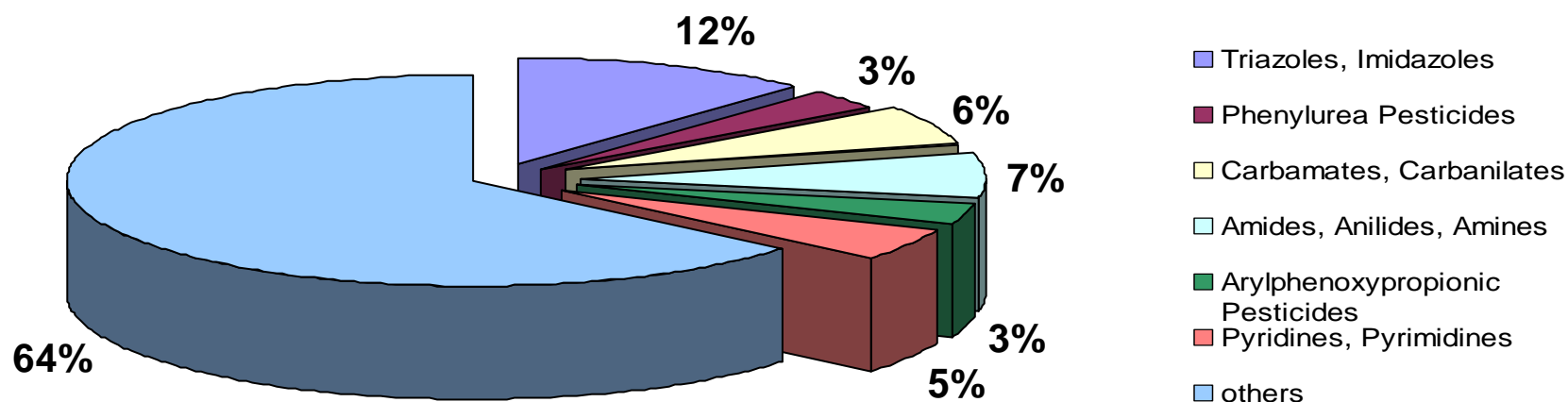
GC-MSD (Agilent)

- GC-FPD (routine analysis; flame photometric detector) (Thermo Fisher scientific)

LC-MS/MS Methods

2 LC-MS/MS screening methods including ~150 substances

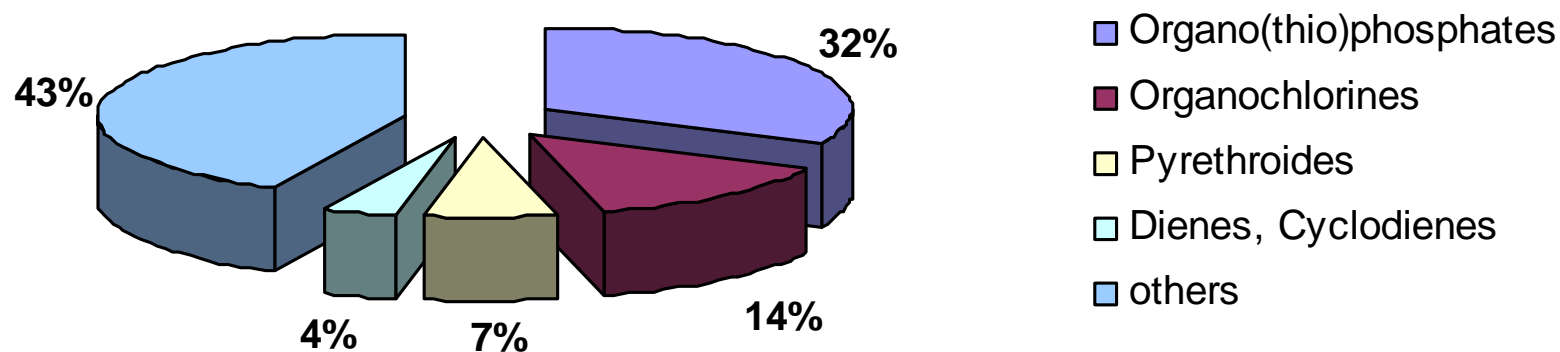
Pesticide Spectrum (LC methods)



GC Methods

3 methods including ~250 substances

Pesticide Spectrum (GC methods)



general difficulties:

- Pesticides belong to different chemical classes
- Confirmation of target compounds in food is often very difficult
- Retention time shifting
- Linearity over a wide range is needed

Investigation of the most appropriate technique

Examples of relevant pesticides in honey detectable with LC-MS/MS and GC-MS/MS

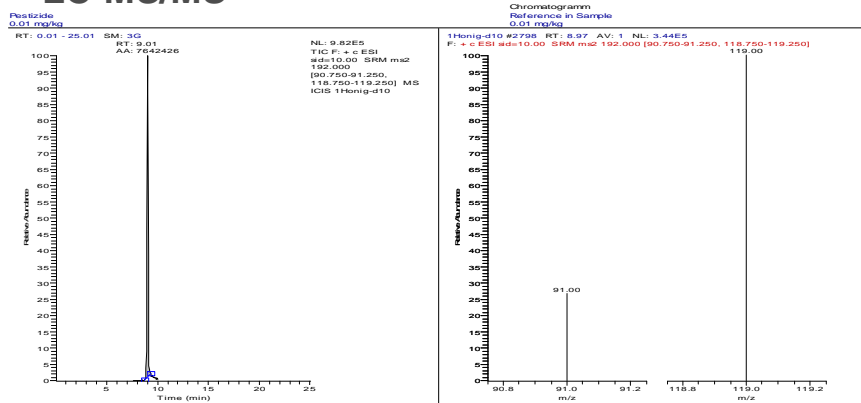
<p>N,N-diethyl-m-toluamide (DEET)</p> <p><chem>CC(=O)N(CC)Cc1cccc(C)c1</chem></p>	<p>Insect repellent Autan® (used also as bee repellent, but not registered; causes residues)</p>
<p>Cymiazole</p> <p><chem>CC1=CN(C)S1=Nc2ccc(C)cc2C</chem></p>	<p>Acaricide Apitol® (not registered anymore since 2001)</p>



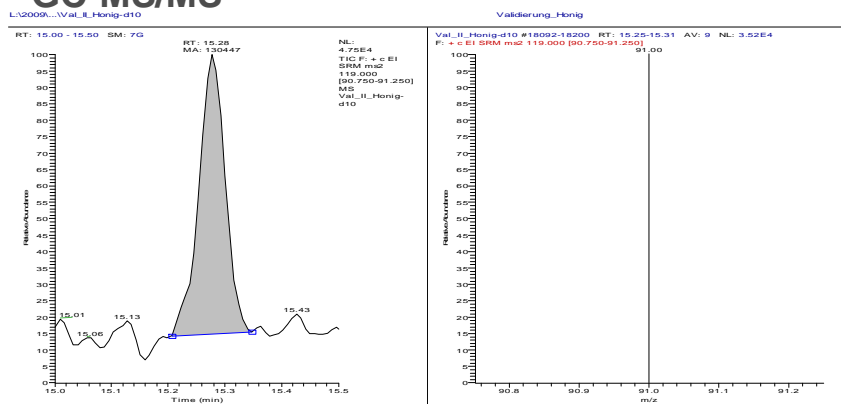
Investigation of the most appropriate technique

Examples of relevant pesticides in honey detectable with LC-MS/MS and GC-MS/MS

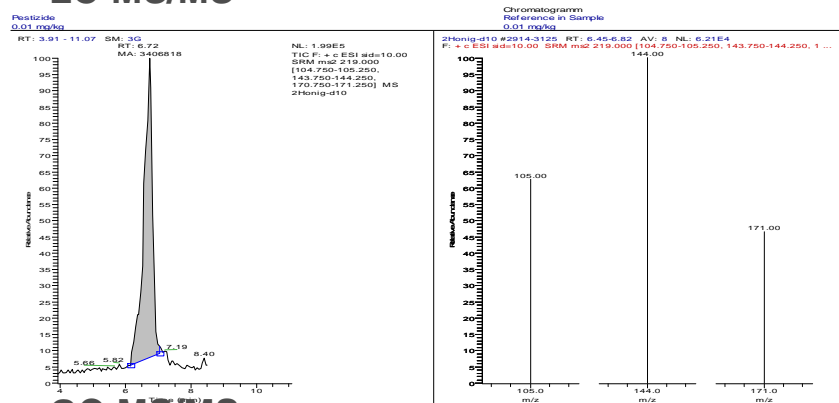
N,N-diethyl-m-toluamide (DEET) LC-MS/MS



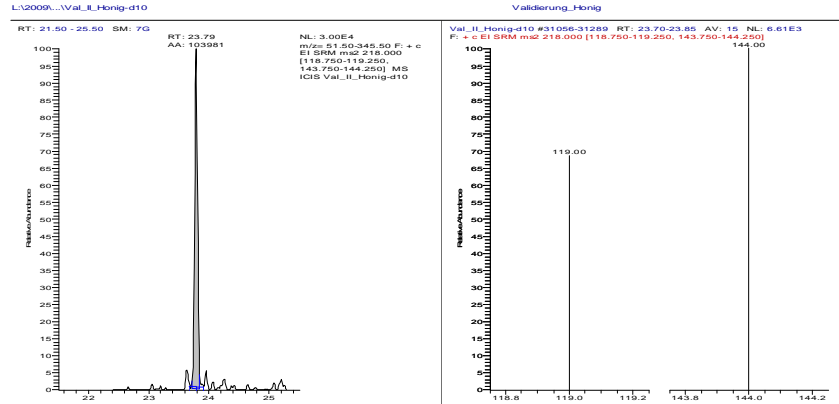
GC-MS/MS



Cymiazole LC-MS/MS



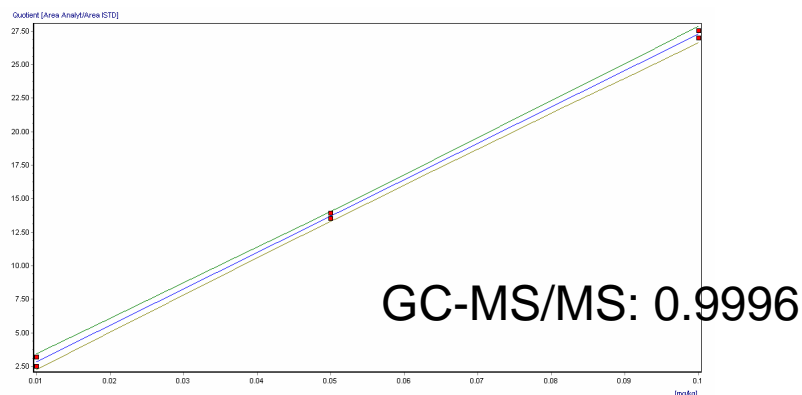
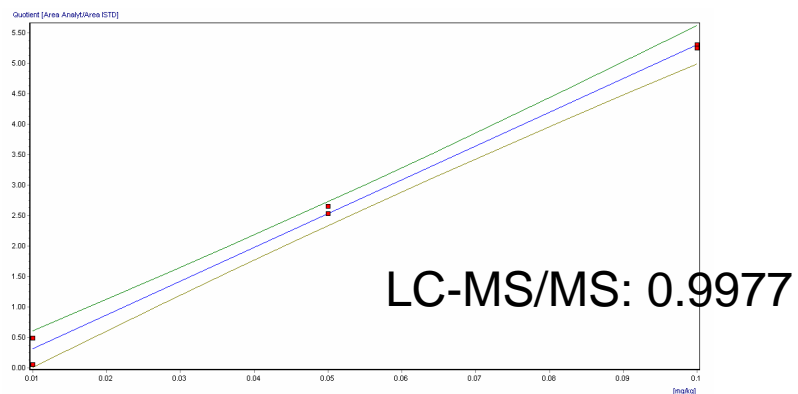
GC-MS/MS



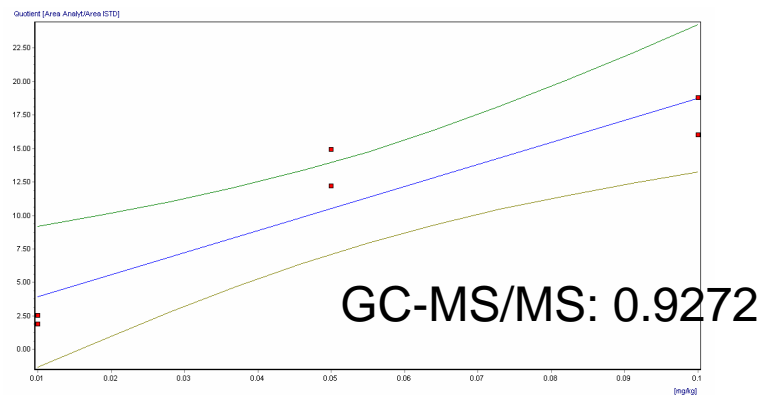
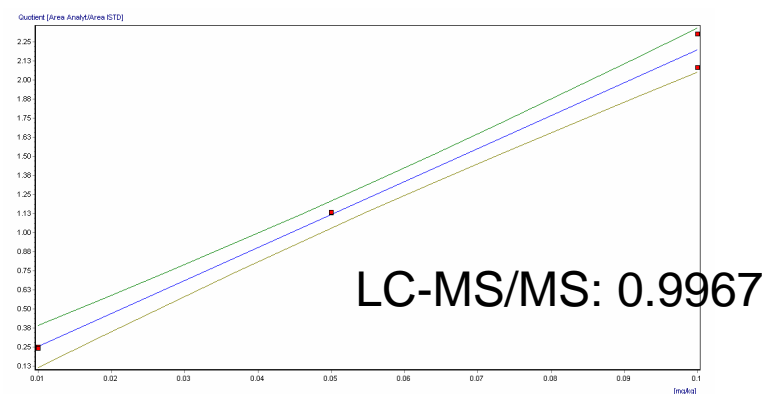
Investigation of the most appropriate technique

Comparison of Linearity

N,N-diethyl-m-toluamide (DEET)

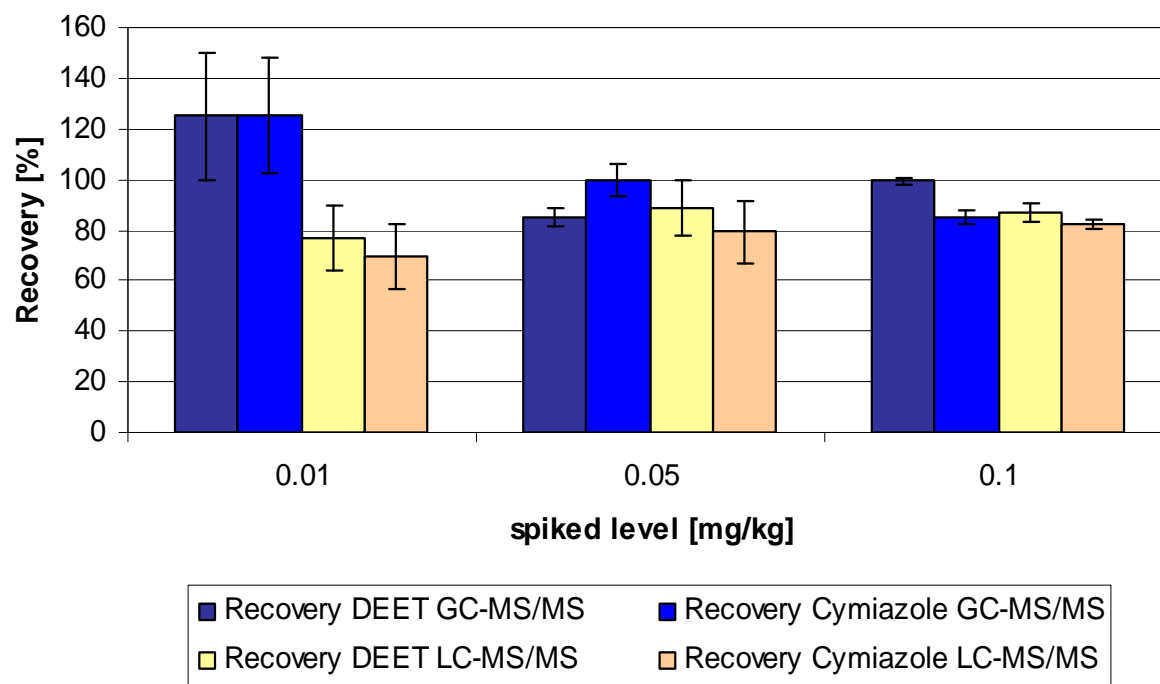


Cymiazole



Investigation of the most appropriate technique

Comparison of Recoveries and Sensitivities



DEET:

LC-MS/MS: LOD = 0.04 µg/kg

GC-MS/MS: LOD = 7.5 µg/kg

Cymiazole:

LC-MS/MS: LOD = 1.9 µg/kg

GC-MS/MS: LOD = 1.8 µg/kg

Investigation of the most appropriate technique

Conclusions

- DEET: LC-MS/MS better sensitivity
 GC-MS/MS better linearity

 - Cymiazole: LC-MS/MS better linearity
 GC-MS/MS better sensitivity
-
- substance dependend
 - it is useful to have a complimentary system available in order to get good sensivity and reliable results (result confirmation)

Determination of pesticides in different matrices

Honey, royal jelly, bee pollen and beeswax

Examples of relevant pesticides in hive products detectable with LC-MS/MS

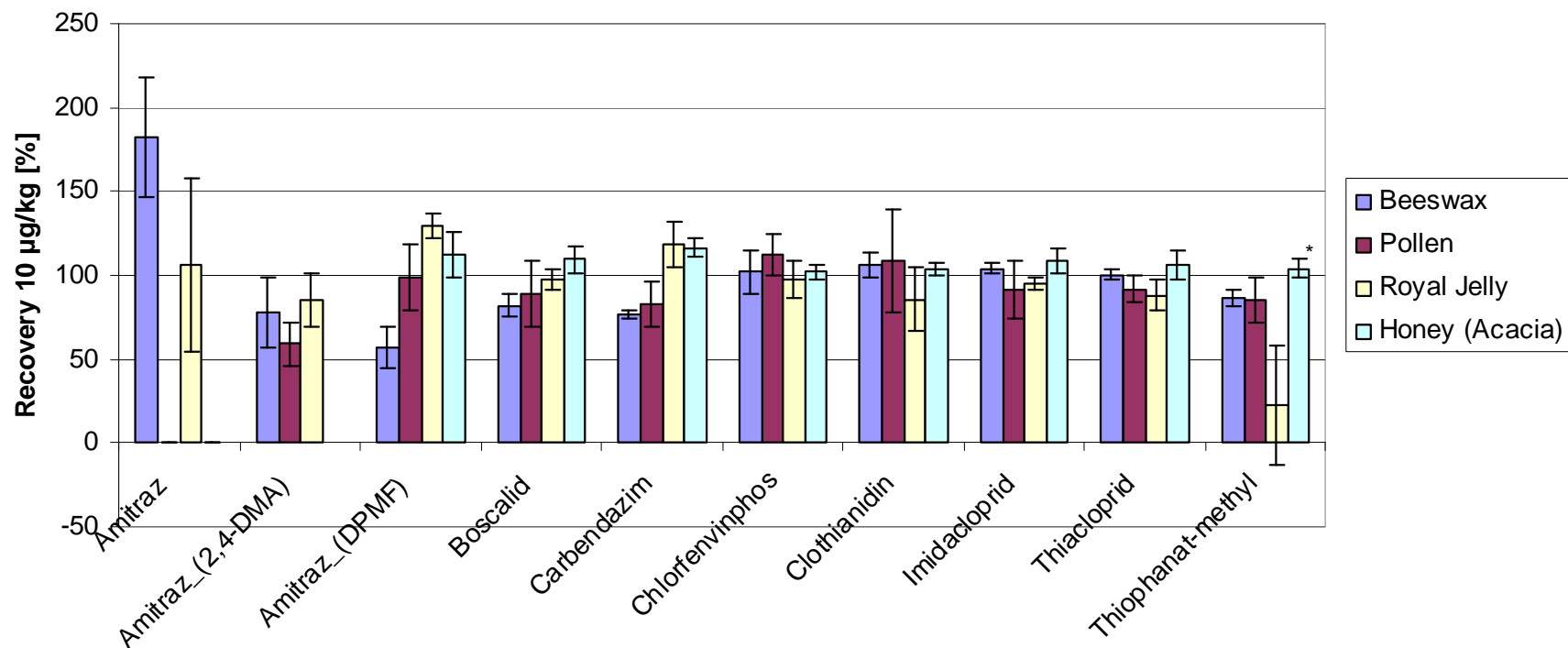
Amitraz (2,4-DMA, DPMF)	Formamidine Acaricide
Boscalid	Anilide/Pyridine Fungicide
Carbendazim	Benzimidazolylcarbamate Fungicide
Chlorfenvinphos	Organophosphate Acaricide not permitted in the EU
Clothianidin	Nitroguanidine/Thiazole Insecticide
Imidacloprid	Pyridylmethylamine Insecticide
Thiacloprid	Pyridylmethylamine Insecticide
Thiophanat-methyl	Carbamate Fungicide



Determination of pesticides in different matrices

Honey, royal jelly, bee pollen and beeswax

Recoveries [10 µg/kg; n = 5], SANCO/2007/3131: 70 – 120% (RSD ≤ 20%):

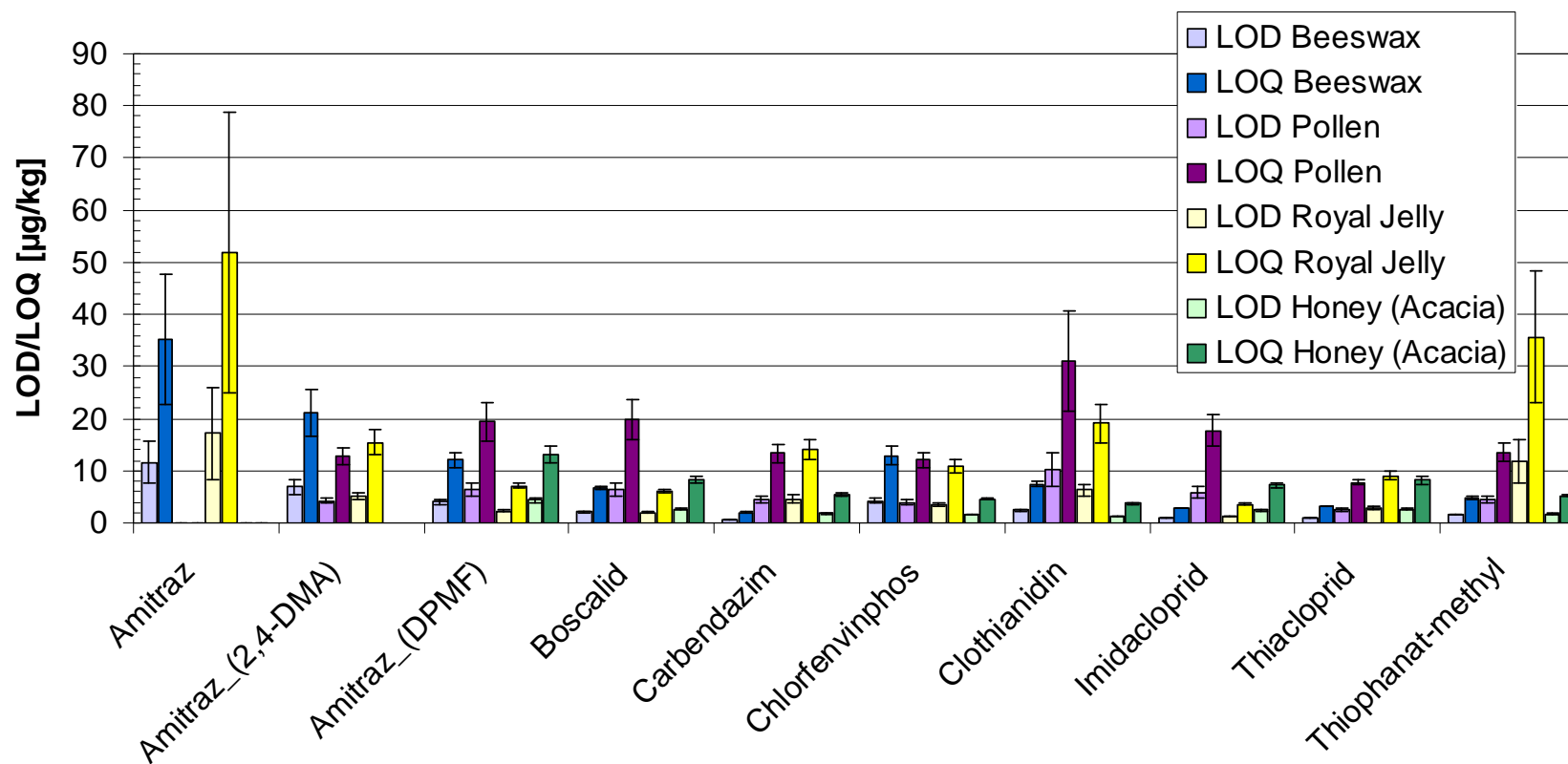


*recovery Thiophanat-methyl in Honey (Acacia) 50 µg/kg

Determination of pesticides in different matrices

Honey, royal jelly, bee pollen and beeswax

Limits of detection (LOD), limits of quantification (LOQ):



Determination of pesticides in different matrices

Honey, bee pollen and beeswax

Examples of relevant pesticides in hive products detectable with GC-MS/MS

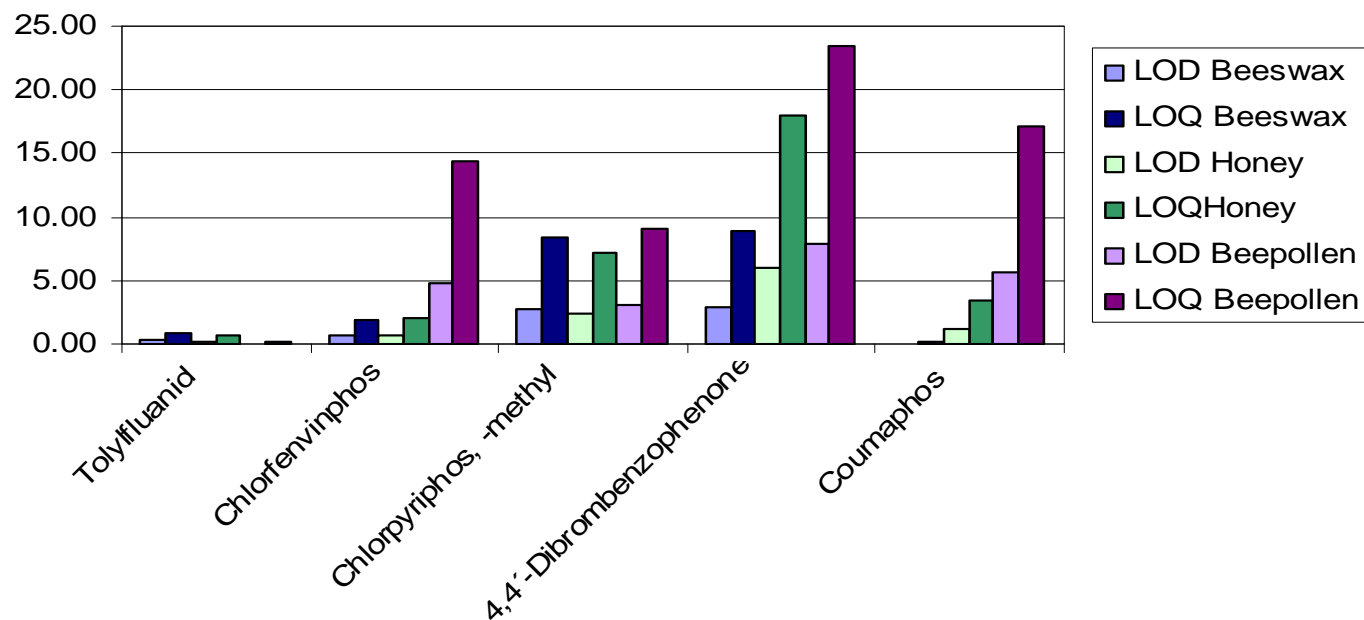
Tolyfluanid	Phenylsulfamide Fungicide
Chlorfenvinphos	Organophosphate Acaricide not permitted in the EU
Chlorpyrifos, -methyl	Pyridine Organothiophosphate Insecticide
4,4´-Dibrombenzophenone	Metabolite of Brompropylate (Acaricide)
Coumaphos	Organothiophosphate



Determination of pesticides in different matrices

Honey, bee pollen and beeswax

Limits of detection (LOD), limits of quantification (LOQ):



Recoveries: 80 – 120%, (SANCO/2007/3131: 70 – 120% (RSD ≤ 20%))

Conclusions

- Pesticide-analysis in food is challenging
 - but quick and easy sample preparation and fast measurement is needed for high throughput
- Sensitive and robust screening methods are necessary
 - QuEChERS in combination with LC-MS/MS and GC-MS/MS is suitable for this purpose
- complimentary methods are necessary for result confirmation
- LC-MS/MS and GC-MS/MS are complimentary methods
 - but it depends on the analyte which is the most appropriate technique

Intertek

APPLICA

Thank you very much



for your attention!