Development of a Multi-residue Method for the Determination of Pesticides in Foodstuffs by LC-MS/MS on a Solid Core Particle C18 Column

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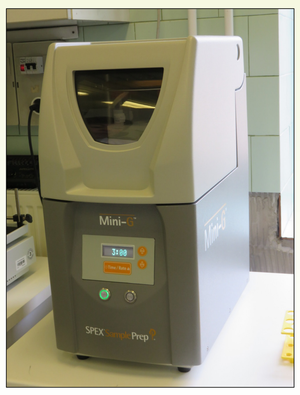
Introduction

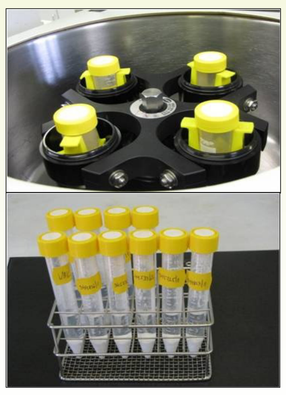
Today any laboratory who wishes to test for pesticide residues in foodstuffs must be able to carry out simultaneous trace level analysis of hundreds of compounds belonging to many classes in a single run. To meet the requirements for today`s LOQ levels and due to the more polar and/or thermally unstable nature of many pesticides, compounds ought to be analyzed more and more by LC-MS/MS.

For the purpose of extending the number of pesticides measured and lowering the LOQs of our previous LC-MS/MS screening method **we applied an ACE UltraCore SuperC18 2,5µm 2,1*100mm column and developed a HPLC-semi-UHPLC method. With the application of MSZ EN 15662:2009[1] method for sample preparation (well-known as QuEChERS) and with the use of this solid-core particle column we aimed to achieve an analysis that includes approximately 300 pesticides in a single run** using a Perking-Elmer Flexar FX-10 UHPLC system coupled with an ABSciex 4000QTrap LC-MS/MS system operated in QQQ Scheduled MRM mode. All compounds were monitored with at least two mass transitions.

A method validation was carried out regarding a cucumber matrix at two spiking levels: 0.01 mg/kg (LOQ 1x), and 0.10 mg/kg (LOQ 10x) for 300 compounds. For each spiking level we have prepared 5 parallel samples and measured them with our LC-MS/MS method. Two calibration series were prepared: one in methanol : water = 1: 1 (containing 5mM Ammonium formate) and the other one in 0.5 g/ml cucumber matrix. The calibration series were the following: 0.003, 0.005, 0.02, 0.05 and 0.075 ug/ml.

Sample preparation





Extraction

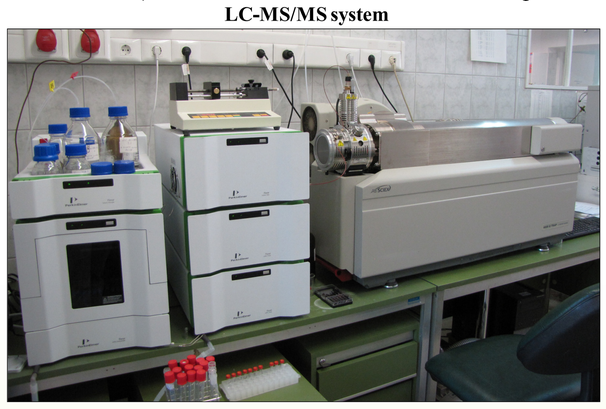
- Weight10 g sample into 50 ml centrifuge tubes
- Add standard mixture for recovery experiments
- Add 10 ml acetonitrile
- Shake with MiniG (3 min, 1500 rounds per minute)
- Add 6,5 g of salt mixture (4 g $MgSO_4$ + 1 g NaCl + 1 g tri-Sodium citrate dihydrate + 0,5 g di-Sodium hydrogen citrate 1,5-hydrate)
- Shake with MiniG (3 min, 1500 rounds per minute)
- Centrifuge (5 minutes, 3000 rounds per minute)

Cleanup:

- Take 5 ml upper layer for cleanup and add 880 mg cleanup mixture (PSA : $MgSO_4$ powder = 1:6)
- Shake with MiniG (3 min, 1500 rounds per minute)
- Centrifuge (5 minutes, 3000 rounds per minute)
- Filter through 0,45 µm PTFE syringe filter
- Stabilisation: add 10 µl acetonitrile containing 5% HCOOH / extract ml
- Evaporate the acetonitrile cautiously (avoid evaporation to absolute dryness since it can cause loss of volatile compounds) than reconstitute samples in methanol:water = 1:1 (containing 5mM Ammonium formate) to **0.5 g/ml sample ratio for LC-MS/MS analysis**

Measurement and instrumental conditions

Perkin-Elmer, Flexar FX-10 UHPLC and AB Sciex 4000 QTRAP LC-MS/MS system



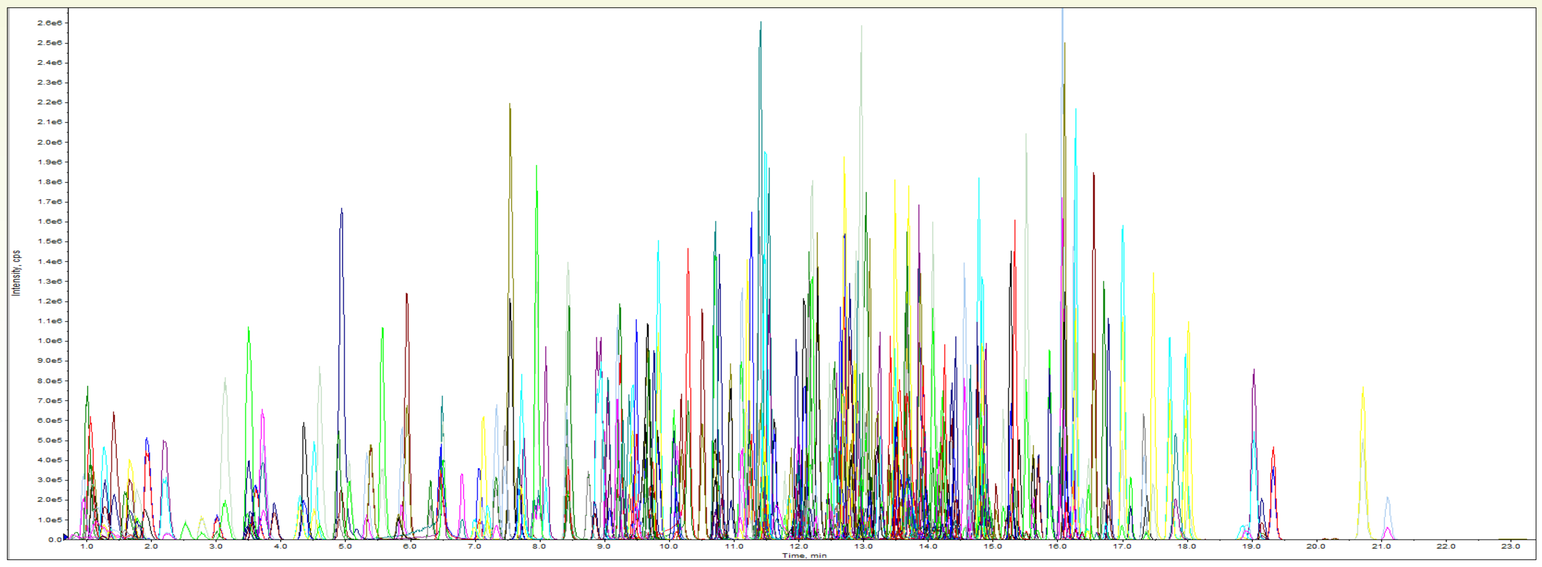
MS conditions and parameters

Ion-source:	Turbo V™
Acquisition type:	Scheduled MRM
Capillary:	TurboIonSpray® ESI
Polarity:	Positive
Pause between mass ranges:	2 msec
MRM detection window:	120 sec
Target Scan time (Cycle time):	1.2 sec
Curtain Gas:	20 psi
Collision Gas (CAD):	Medium
Capillary Voltage (IS):	5000 V
Heater gas temperature:	450 °C
Nebuliser Gas (Gas1):	50 psi
Heater Gas (Gas2):	50 psi
Entrance potential:	10 V
Injection volume:	6 µl
Autosampler rack temperature:	7 °C
Flush/Wash solution:	Acetonitrile : Methanol = 1:1
Flush/Wash volume:	250 µl
Flush/Wash event:	2 pre injection and 2 post injection
Software:	Analyst 1.6.1

Chromatographic conditions and gradient program

Coloumn	ACE UltraCore SuperC18 2,5µm 2,1*100mm		
Eluent A	Methanol : Water = 1 : 9 (containing 5mM Ammonium formate)		
Eluent B	Methanol : Water = 9 : 1 (containing 5mM Ammonium formate)		
Time (min)	**Flow rate (µl/min)**	**A%**	**B%**
0	300	70	30
0.5	300	70	30
15	300	0	100
22	300	0	100
22.1	300	70	30
27	300	70	30

Total MRM chromatogram - 50 ng/ml standard mixture containing 300 compounds

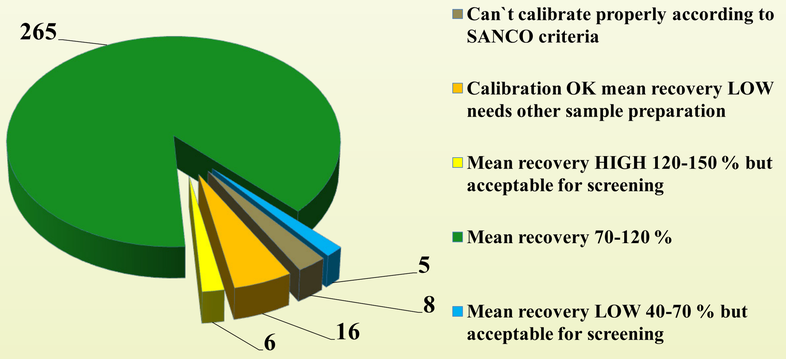


List of compounds and retention time of their respective time windows

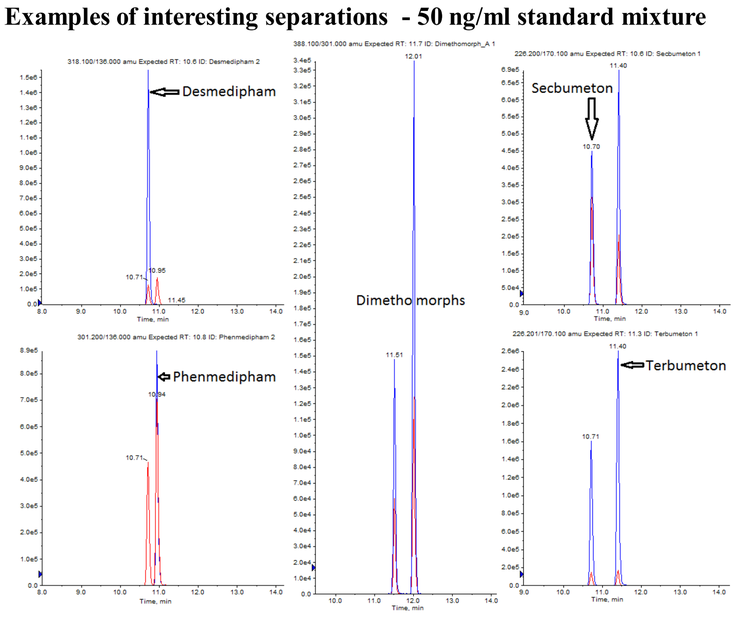
Background colors represent the performance of the analytes described on the diagram below
Note that in some cases pesticides may appear in two time windows

Compound names A to Z	RT (min) of time window	Compound names A to Z	RT (min) of time window	Compound names A to Z	RT (min) of time window	Compound names A to Z	RT (min) of time window	Compound names A to Z	RT (min) of time window
3-Hydroxycarbofuran	3,5	Cyproconazole	12,3	Flumethrin	20,2	Metribuzin	7,1	Pyraclostrobin	14,5
Acephate	1,0	Cyprodinil	14,1	Flumetsulam	2,1	Mevinphos	3,4 and 4,9	Pyrazophos	14,8
Acetamiprid	3,6	Demeton-S-methyl	7,7	Flumioxazin	10,7	Molinate	12,0	Pyridaben	18,0
Aclonifen	13,9	Demeton-S-methylsulfone	1,6	Fluometuron	8,9	Monocrotophos	1,8	Pyridapenthion	12,4
Alachlor	12,9	Desmedipham	10,7	Fluopicolide	11,9	Monolinuron	8,7	Pyridate	19,1
Aldicarb	5,4	Desmethyl-pirimicarb	5,8	Fluopyram	12,5	Myclobutanil	12,2	Pyrifenox	13,0
Aldicarb-sulfone	1,2	Diafenthiuron	17,4	Fluoxastrobin	12,8	Napropamide	12,9	Pyrimethanil	11,3
Aldicarb-sulfoxide	1,1	Diazinon	14,2	Fluquinconazole	12,6	Nitenpyram	1,3	Pyriproxyfen	16,7
Ametryn	11,1	Dichlofluanid	12,8	Flusilazole	13,3	Novaluron	15,6	Pyroxsulam	5,6
Aminopyralid	0,8	Diclobutrazol	13,7	Flutolanil	12,0	Nuarimol	11,2	Quinalphos	13,9
Amitrole	0,8	Dicrotophos	2,1	Flutriafol	9,7	Ofurace	7,6	Quinoclamine	6,8
Atrazine	9,3	Diethofencarb	11,1	Fomesafen	11,3	Omethoate	1,0	Quinoxyfen	16,4
Atrazine-desethyl	4,4	Difenoconazole	14,8	Fonofos	14,3	Oxadiazon	16,2	Rotenone	13,4
Atrazine-desisopropyl	2,4	Diflubenzuron	13,5	Fosthiazate	8,9	Oxadixil	6,4	Sechumeton	10,7
Avermektin B1a	18,2	Diflufenican	15,4	Fuberidazole	6,9	Oxamyl	1,2	Silthiofam	13,5
Avermektin B1b	19,1	Dimethachlor	10,2	Furathiocarb	15,9	Oxycarboxin	4,5	Simazine	7,2
Azamethiphos	6,9	Dimethenamid	11,3	Heptenophos	10,1	Oxydemeton-methyl	1,4	Simetryn	9,4
Azinphos-ethyl	13,0	Dimethoate	3,6	Hexaconazole	14,3	Paclobutrazol	11,8	Spinosyn A	17,3
Azinphos-methyl	10,9	Dimethomorph	11,8	Hexaflumuron	15,5	Paraoxon	9,4	Spinosyn D	18,3
Aziprotryne	11,8	Dimoxystrobin	13,7	Hexazinone	7,3	Paraoxon-methyl	6,1	Spirodiclofen	17,4
Azoxystrobin	11,4	Diniconazol	14,8	Hexythiazox	16,6	Parathion	13,8	Spiromesifen	16,8
Benalaxyl	14,0	Disulfoton	15,0	Imazalil	13,6	Penconazole	13,7	Spirotetramat	12,8
Benfuracarb	15,7	Disulfoton-sulfone	9,6	Imidacloprid	2,7	Pencycuron	14,8	Spiroxamine	13,3
Benthiavalicarb-isopropyl	12,0	Disulfoton-sulfoxide	9,2	Indoxacarb	15,2	Pendimethalin	16,9	Sulfotep	14,0
Bifenazate	12,5	Ditalimfos	13,1	Ipconazole	15,3	Pethoxamid	12,7	Tau-fluvalinate	18,9
Bifenox	14,9	Diuron	10,0	Iprodione	13,3	Phenmedipham	10,9	Tebuconazole	13,9
Bifenthrin	21,0	DMST	8,0	Iprovalicarb	12,6	Phenthoate	13,9	Tebufenozide	13,5
Bitertanol	14,6	Dodine	13,6	Isofenphos	14,7	Phorate-sulfone	9,6	Tebufenpyrad	15,9
Bixafen	13,6	Epoxiconazole	12,9	Isofenphos-methyl	13,8	Phorate-sulfoxide	9,2	Teflubenzuron	16,3
Boscalid	11,7	Ethion	16,5	Isoprocarb	9,4	Phosalon	14,6	Tembotrione	5,9
Bromfenvinfos	14,3	Ethirimol	9,7	Isoprothiolane	12,1	Phosmet	11,1	Terbufos	16,1
Bromuconazole	12,2 and 13,5	Ethofumesate	11,3	Isoproturon	9,7	Phosphamidon	6,4	Terbufos-sulfone	11,1
Bupirimate	13,5	Ethoprophos	12,7	Isoxadifen-ethyl	13,9	Phoxim	14,7	Terbufos-sulfoxide	11,0
Buprofezin	16,1	Ethoxyquin	10,7 and 12,9	Isoxaflutole	10,0	Picloram	1,2	Terbumeton	11,4
Cadusafos	14,8	Etofenprox	20,6	Kresoxim-methyl	13,9	Picolinafen	16,2	Terbuthylazine	11,4
Carbaryl	8,3	Etrimfos	14,2	Lenacil	9,5	Picoxystrobin	13,6	Terbutryn	12,9
Carbendazim	4,7	Famoxadone	14,4	Linuron	11,3	Piperonyl butoxide	16,2	Tetrachlorvinphos	13,5
Carbofuran	7,4	Fenamidone	11,5	Lufenuron	16,4	Pirimicarb	9,0	Tetraconazole	12,9
Carbosulfan	19,3	Fenamiphos	13,4	Malaoxon	7,9	Pirimiphos-ethyl	16,3	Thiabendazole	6,2
Carboxin	8,3	Fenamiphos-sulfone	8,4	Mandipropamid	11,9	Pirimiphos-methyl	14,8	Thiacloprid	4,7
Carfentrazone-ethyl	13,8	Fenamiphos-sulfoxide	7,9	Mecarbam	13,0	Prochloraz	14,4	Thiamethoxam	1,7
Chlorantraniliprole	10,7	Fenarimol	12,7	Mepanipyrim	12,9	Profenofos	15,6	Thiencarbazone-mehyl	2,3
Chlorbromuron	11,7	Fenazaquin	18,0	Mepronil	12,1	Prometryn	12,6	Thiodicarb	9,2
Chlorfenvinphos	14,3	Fenbuconazole	13,2	Mesotrione	1,2	Propachlor	9,6	Thiophanate-methyl	7,6
Chloridazon	3,7	Fenbutatin oxide	22,9	Metaflumizone	16,1	Propamocarb	1,1	Tolclofos-methyl	14,9
Chlorpyrifos	16,8	Fenhexamid	12,6	Metalaxyl	9,8	Propaquizafop	16,0	Tolylfluanid	13,9
Chlorpyrifos-methyl	15,2	Fenoxycarb	13,6	Metamitron	3,4	Propargite	17,0	Topramezone	1,6
Chlortoluron	9,1	Fenpropathrin	17,3	Metazachlor	9,6	Propazine	11,0	Triadimefon	12,1
Cinidon-ethyl	16,3	Fenpropidin	10,8	Metconazole	14,4	Propetamphos	12,4	Triadimenol	12,4
Clethodim	10,1 and 12,8	Fenpropimoph	18,7	Methacrifos	10,7	Propham	9,4	Tri-allate	16,7
Clofentezine	15,1	Fenpyroximate	17,4	Methamidofos	0,9	Propiconazole	14,0	Triazophos	12,6
Clomazone	10,7	Fensulfothion	10,0	Methiocarb	11,4	Propisochlor	14,0	Trichlorfon	3,4
Cloquintocet-mexyl	16,1	Fensulfothion-sulfone	10,4	Methiocarb-sulfone	4,1	Propoxur	7,2	Tricyclazole	5,2
Clothianidin	2,9	Fenthion-sulfon	9,0	Methiocarb-sulfoxide	3,0	Propyzamide	11,9	Trifloxystrobin	15,3
Coumaphos	14,3	Fenthion-sulfoxide	8,4	Methomyl	1,6	Proquinazid	17,7	Triflumizole	15,3
Cyanazine	6,7	Flonicamid	1,7	Methoxyfenozide	12,2	Prosulfocarb	15,5	Triflumuron	14,6
Cyazofamid	13,2	Flubendiamide	13,8	Metobromuron	9,4	Prosulfuron	9,0	Triforine	10,6
Cycloate	14,9	Fludioxinil	11,8	Metolachlor	13,0	Prothioconazole	14,1	Triticonazole	10,9 and12,7
Cycloxydim	8,4 and 13,1	Flufenacet	12,8	Metoxuron	5,7	Prothioconazole-desthio	13,0	Vamidothion	3,4
Cymoxanil	4,2	Flufenoxuron	17,1	Metrafenone	14,8	Pymetrozin	1,5	Zoxamide	14,2

Performance of the method at 0.01 and 0.1 mg/kg recovery levels regarding cucumber matrix from 300 compounds



Examples of interesting separations - 50 ng/ml standard mixture



Experiences and recommendations

•The mixture containing the 300 compounds of interest was constituted from 7 sub mixes in acetone. **When exchanging solvents for any reason it is crucial to avoid high temperatures and evaporation to total dryness since this can cause loss of volatile compounds.** It`s better to use small volumes of higher concentrations from working solutions than fill it up to volume with the desired diluting agents (for example in case of a standard series dilution) .

•**Fresh eluents must be prepared before starting a sequence** for successful measurements, since the retention times can alter (especially for pH sensitive compounds) with the age of eluents due to pH variations. Retention times should be checked from time to time to ensure analytes of interest stay in the time window ranges.

•**The column should be equilibrated with the starting gradient until pressure reaches approximately 5000-5200 PSI** (10 minutes should suffice) and **it is highly advisable to run gradient 4-5 times before measurements** to reach the most stabile condition of the column. This will ensure that no analytes fall out of their time window and the retention shifts should be less than 0.2 minutes.

•**Normal HPLC equipment is also suitable to make the method work.** We have used PEEK tubing of ID 1/16" OD 0.005" and finger tight PEEK fittings (Supelco) which are usable up to a pressure of 5700 PSI. During a gradient run minimum pressure was 3500-3600 PSI while maximum pressure was 5600 PSI.

•The method has its limits, since when the gradient is closing towards the 100% B solution a lot of apolar pesticides elute, so many transitions must be measured. The narrowest peaks here are at least 12-14 seconds wide at their bases and despite the given cycle time a minimum dwell time must be maintained. This result in loss of points taken from the gauss curves but a minimum of 7-8 points is always taken and that is suitable enough in a screening method. This problem can be solved by setting individual time windows and dwell times, however this option is only available in a later version of the Analyst software.

Results

Results were evaluated according to the Document SANCO/12571/2013[2] - Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed. All successful calibrations and validated compounds fulfilled the SANCO document`s requirements. **From 300 compounds calibration was successful for 292 thus the measuring instrumental method is suitable for them**. No significant matrix effects observed regarding cucumber when solvent calibration was compared to matrix matched calibration. **From the 292 pesticides measurable we validated 265 compounds at both LOQ levels** (mean recovery 70-120%)**. Additional 6 pesticides showed high while 5 showed low but consistent recoveries, thus the method is suitable for the screening of 276 compounds. Other 16 tested pesticides require different sample preparation.** We are continuously expanding our method with new analytes of interest as well as test its performance especially regarding other matrices and their matrix effects. **With this new method** applied we have extended the scope of our laboratory since our previous LC-MS/MS screening method measured 130 compounds in 3 separate methods, each lasting 27 minutes thus **we approximately doubled the number of compounds measured by LC-MS/MS**. **In addition we decreased solvent consumption by 2.5 times and shortened measurement time to third of the previous screening method.**

Literature:
[1]MSZ EN 15662:2009: Foods of plant origin. Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE. QuEChERS-method
[2]Document N° SANCO/12571/2013 - Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed.