

# Analysis of Neonicotinoid Insecticides in Fruits and Vegetables using LC-MS(MS)

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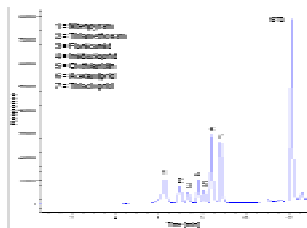
## Introduction

The increasing incidence of single- and multiple-resistance to many conventional insecticides has led to the development of a large number of new active compounds, such as the neonicotinoids, which were introduced as an alternative to the organophosphates, N-methyl-carbamates and pyrethroid insecticides.

Despite their increasing use in crop protection, so far only limited analytical data (mainly on imidacloprid) exist to elucidate the residue situation in crops and to assess the exposure of consumers to these pesticides. The main goal of our study was therefore to develop an analytical methodology, which would allow us to integrate the analysis of neonicotinoids in the routinely employed multi-residue method of our laboratory using LC-MS or LC-MS/MS.

## Experimental

This study describes a LC-MS or LC-MS/MS method for the simultaneous determination of seven neonicotinoids (acetamiprid, clothianidin, flonicamid, imidacloprid, nitenpyram, thiacloprid and thiamethoxam) in fruits and vegetables. Sample preparation is based on the recently published QuEChERS multi-residue method [1], that consists of an extraction with acetonitrile followed by liquid-liquid partitioning induced by the addition of salts. Cleanup is performed by dispersive SPE using PSA (primary/secondary amine) sorbent. After separation by reversed-phase liquid chromatography using C8 column the seven neonicotinoid insecticides are detected by single mass spectrometry or tandem mass spectrometry in the positive ion mode.

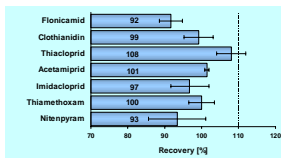


LC-MS chromatogram for a standard mixture of neonicotinoids (c = 1.0 µg/ml)

	Sample preparation (QuEChERS)	LC-MS(MS) determination
Extraction and liquid/liquid partition	<b>Extraction with ACN</b> 10 g sample + 10 ml ACN → intensively shaking (1 min)	<b>Equipment</b> 1) LC/MSD Agilent 1100 series 2) LC-MS/MS system: Agilent 1100 liquid chromatograph and an Applied Biosystems API 3000
	<b>Liquid/liquid partition</b> Addition of 4 g MgSO <sub>4</sub> + 1 g NaCl → intensively shaking (1 min)	<b>Column</b> Zorbax Eclipse XDB C8, 4.6 x 150 mm, 3.5 µm
	<b>Addition of internal standard</b> 100 µl → shortly shaking	<b>Mobile phase</b> A: 10 mM HCOONH <sub>4</sub> :MeOH (95/5), pH = 4.2, B: MeOH Gradient: 30 % B → 100 % B in 8 min Flow: 0.4 ml/min; Temp.: 40 °C; Injection volume: 5 µl
Clean-up	<b>Centrifugation</b> 5 min, 3000 rpm	<b>MS-parameters</b> API electrospray, SIM mode Fragmentor voltage var. Drying gas: N <sub>2</sub> 10 l/min, 330 °C V <sub>cap</sub> : +4000 V Neb. Pres.: 45 psig
	<b>Dispersive SPE</b> 1 ml aliquot + 25 mg PSA + 150 mg MgSO <sub>4</sub> → intensively shaking (1 min)	<b>MS/MS-parameters</b> ESI, Turbo IonSpray Scan type: MRM Interface Neb. Gas.: zero air Potential: +3000 V Temp.: 500 °C Curtain gas: N <sub>2</sub> 8 l/min Collision gas: N <sub>2</sub> 4 l/min Turbo gas: N <sub>2</sub> 8 l/min
	<b>Centrifugation</b> 1 min, 5300 rpm → supernatant subjected to LC-MS(MS)	

## Recoveries

Numerous recovery tests have been conducted by spiking blank homogenized samples with each pesticides. The recoveries achieved lay between 70 and 110 % (usually between 90 and 100 %). Quantitation of all seven analytes was performed in SIM mode by the standard addition methodology.



Recoveries of the studied neonicotinoids: spiked apple (0.1 mg/kg, n = 5)

## Limit of detection and quantitation

Using LC-MS, the limits of detection and quantitation determined following the DFG-procedure, which requires quadruplicate fortifications at four concentration levels near the LOQ, were ≤ 6 µg/kg and ≤ 10 µg/kg, respectively. With LC-MS/MS lower limits of detection and quantitation could be achieved (LOD ≤ 3 µg/kg and LOQ ≤ 5 µg/kg). Even at such low concentration levels the recoveries and the precision of the method were excellent (RSD ≤ 7.6; mean recoveries 84-98 %).

## References

- Anastassiades M., Lehoty S.J., Stajnbaher D., Schenck F.J., Journal of AOAC Int. 86 (2), 412-431 (2003)
- Zywitz, D., Anastassiades M., Scherbaum E., Deutsche Lebensmittel-Rundschau 99 (5), 188-196 (2003).

## Analysis of real samples

Since the introduction of this methodology more than 2500 samples of fruits and vegetables obtained from the German market have been analyzed for neonicotinoid residues in our laboratory. All measurements were performed by LC-MS in the ESI (pos.) mode. In some cases positive findings were confirmed by LC-MS/MS. Fruiting vegetables were the common group with the highest frequency of findings, with almost every second sample containing residues of neonicotinoids and almost every fifth sample containing multiple residues (up to 5).

### Spectrum of samples analyzed

Group	Commodities analyzed	Conventionally grown food		
		No. of samples	No. of positive samples	No. of samples > MRL
Citrus fruits	lemon, orange, mandarin, grape fruit	177	2	0
Stone fruits	peach, nectarine, apricot, cherry	111	5 (4.5 %)	0
Pome fruits	apple, pear	175	5 (2.9 %)	0
Berries	strawberry, raspberry, currant, blueberry, grape	556	12 (2.2 %)	3 (0.5 %)
Tropical and subtropical fruits	pineapple, kiwi, kaki, mango, kumquat	101	1	1
Leafy vegetables and fresh herbs	lettuce, cress, spinach, dill, chives, parsley	231	24 (10.4 %)	3 (1.3 %)
Fruiting vegetables	tomato, pepper, aubergine, courgette, melon, cucumber, chili pepper	540	252 (46.7 %)	104 (19.3 %)
Brassica vegetables	cauliflower, Chinese cabbage, Brussels sprout, kohlrabi, white cabbage	47	1	0
Root and tuber vegetables	carrot, radish, swede	39	0	0
Legume and stem vegetables	asparagus, bean, pea, celery	33	0	0
Dietary foods, cereals and cereal products	maize, wheat, cornmeal, maize semolina, bran, rice and other	50	0	0
Miscellaneous	rape, tea, dried fruit, leek, must, mash, potato, (concentrated) fruit juice and other	64	0	0
<b>Sum</b>		<b>2124</b>	<b>302 (14.2 %)</b>	<b>111 (5.2 %)</b>
Group	Commodities analyzed	Organically grown food		
		No. of samples	No. of positive samples	No. of samples > MRL
Citrus, tropical and subtropical fruits	lemon, orange, clementine	61	0	0
Stone fruits	apricot, peach, cherry, plum	5	0	0
Pome fruits	apple, pear	65	0	0
Berries	strawberry, blueberry, currant, grape	65	0	0
Leafy vegetables	lettuce, spinach	29	1	0
Fruiting vegetables	tomato, pepper, cucumber, courgette, melon	102	2	0
Brassica and root vegetables	Chinese cabbage, carrot	10	0	0
Dietary foods, cereals and cereal products	corn meal, maize semolina, bran, dietary foods for infants and young children, dietary supplement	31	1	0
Micellaneous	Sultana, raisin, dried apricot, must, mash, potato, banana pulp, (concentrated) fruit juice	84	0	0
<b>Sum</b>		<b>452</b>	<b>4 (0.9 %)</b>	<b>0</b>

### Multiple neonicotinoid residues in conventionally grown fruiting vegetables

Country of origin	No. of samples	No. of residues per samples					
		0	1	2	3	4	5
Spain	270	110	83	49	19	7	2
Turkey	89	37	37	15			
Germany	51	51					
The Netherlands	39	34	3	1	1		
Italy	25	21	3	1			
Israel	19	7	11				
Morocco	9	4	3	1	1		
Unknown	19	4	3	4	2	1	
Others	19	15	4				
<b>Sum</b>	<b>540</b>	<b>288</b>	<b>147</b>	<b>72</b>	<b>23</b>	<b>8</b>	<b>2</b>

### Comparison of domestic and imported samples as regards neonicotinoid residues

Samples of plant origin	Imported samples		Domestic samples		Sum	
	Number	%	Number	%	Number	%
<b>Sum</b>	<b>1736</b>	<b>67</b>	<b>840</b>	<b>33</b>	<b>2576</b>	<b>100</b>
Thereof: with residues	296	17.1	10	1.2	306	11.9
below the MRL	187	10.8	8	1.0	195	7.6
above the MRL	109	6.3	2	0.2	111	4.3

## Conclusion

With the presented method, we succeeded in integrating the analysis of seven neonicotinoid insecticides in multi-class analysis of pesticide residues in fruits and vegetables. Sample preparation was performed following the QuEChERS method, which is simple, fast and highly economic allowing the processing of 8 samples in less than 30 minutes. By employing LC-MS no extensive sample clean-up procedure is required. The recoveries and limits of detection and quantitation achieved meet the needs of regulatory control. The findings of neonicotinoid insecticides in real samples demonstrate the importance of including this compound class in routine analysis.

## Acknowledgement

The project has been financially supported by the Landesstiftung Baden-Württemberg GbmbH in Germany.