Overcoming sensitivity issues associated with analytes ionizing in negative LC-ESI in multiresidue methods using dual-channel chromatography

EPRW 2022 BOLOGNA

Francisco J. Díaz-Galiano, Łukasz Rajski, Amadeo R. Fernández-Alba

European Union Reference Laboratory for Pesticide Residues in Fruits & Vegetables, University of Almería, Department of Chemistry and Physics.
Agrifood Campus of International Excellence (ceiA3), Ctra. Sacramento s/n, La Cañada de San Urbano, Almeria, 04120, Spain. E-mail: diaz-galiano@ual.es

Co-funded by the European Union

Overview

Optimal mobile phase modifiers are not equivalent for positive and negative ionisation modes in electrospray. For this reason, it is complicated to **achieve high sensitivity** in multiresidue methods which combine the analysis of compounds which ionise **in the positive and negative polarity modes**.

This issue can be overcome by independently injecting a sample twice. **Dual-channel chromatography allows for sample multiplexing**, thus achieving a sample throughput equivalent to single-channel, single-mobile phase instruments, while performing two analyses at a time.

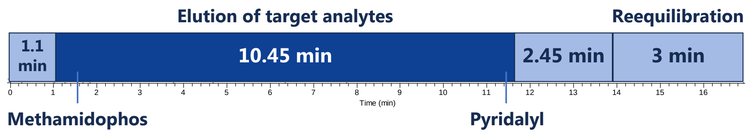
The **total number of pesticides was 264**, out of which 238 were analysed with the methanol gradient and 26 with the acetonitrile gradient. The validated **limit of quantitation was 0.003 mg/kg for 97 %** of the evaluated compounds.

The validation study was followed by a **42 real baby food sample survey**, during which 16 positive results were found at concentrations between 0.003 mg/kg and 0.020 mg/kg.

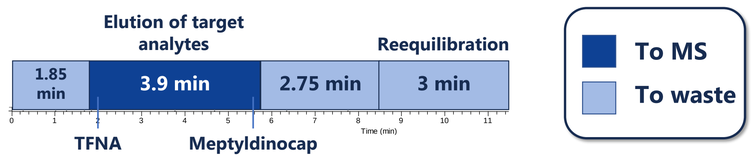
Methods

Channel-independent mobile phases

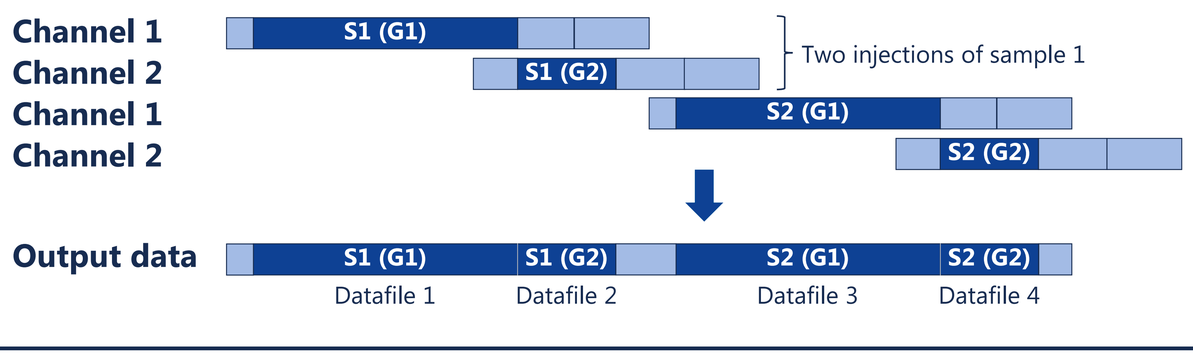
Gradient 1
Water:MeOH (98:2, V/V; A)
MeOH:Water (98:2, V/V; B)
Formic acid (0.1 %)
Ammonium formate (5 mM)



Gradient 2
Water (A)
AcN (B)
Acetic acid (0.05 %)



Analytical workflow



After a QuEChERS-based extraction, the samples were analysed using a dual-channel Thermo Scientific™ Transcend™ DUO LX-2 instrument coupled to a TSQ Altis™ triple quadrupole mass spectrometer (**Figure 1**).

In the **first injection,** samples are analysed with a mobile phase optimal for the positive polarity ionisation (water, methanol, formic acid, and ammonium formate).

For the **second injection** the mobile phase was optimised for the negative polarity ionisation (water, acetonitrile, acetic acid).

In a single-channel analysis, the MS is acquiring data for 47 % of total analysis time. With **dual-channel analysis**, the MS is acquiring data for **80 %** of the total analysis time.

Dual-Channel LC-MS
TranscendTM DUO LX-2 LC

Column: Accucore C_{18} 2.1 x 100 mm, particle size 2.6 µm
Flow rate: 0.350 ml/min)
Injection volume: 2.5 µL

Triple Quadrupole
TSQ Altis™

Ion spray voltage (+): 3500 V
Ion spray voltage (-): 2500 V
Sweep gas: 1 (arbitrary units)
Ion transfer tube: 325 °C
Vaporiser temp.: 350 °C
Working mode: SRM

Figure 1. Dual-Channel LC-QqQ-MS/MS instrument.

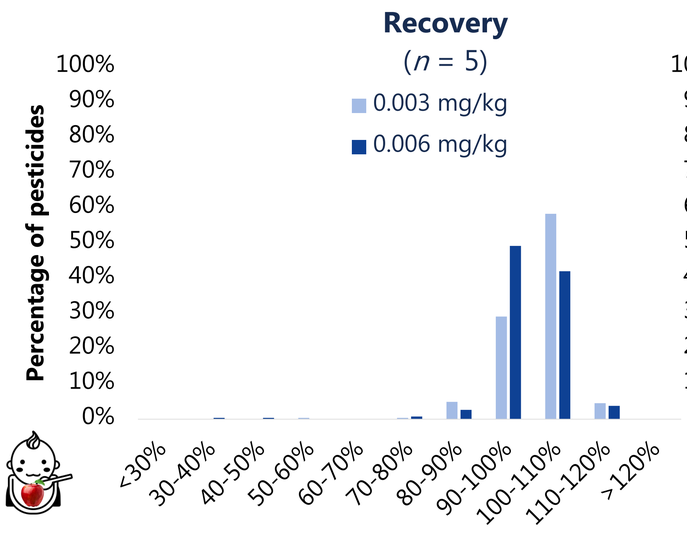
Results

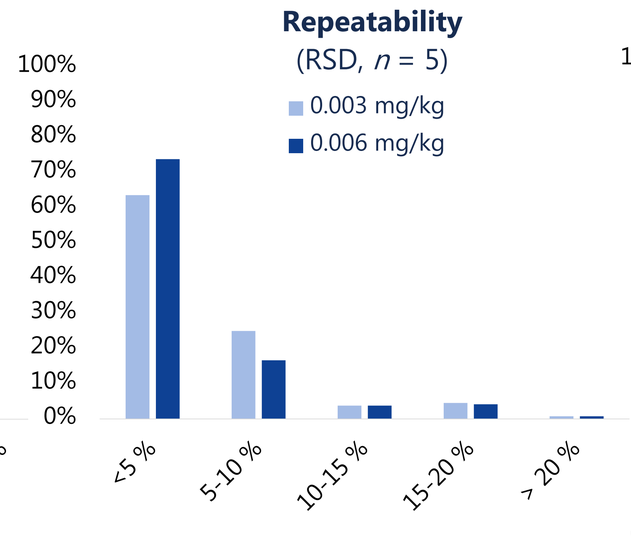
264 total pesticide residues (ESI+ and ESI-)

Recovery	<70%	70-120%	>120%
Dual-Channel 0.003 mg/kg	1	257	-
Dual-Channel 0.006 mg/kg	2	260	-

Repeatability	<5%	5-20%	>20%
Dual-Channel 0.003 mg/kg	64%	33%	1%
Dual-Channel 0.006 mg/kg	74%	25%	1%

256 pesticide residues validated at 0.003 mg/kg
260 pesticide residues validated at 0.006 mg/kg





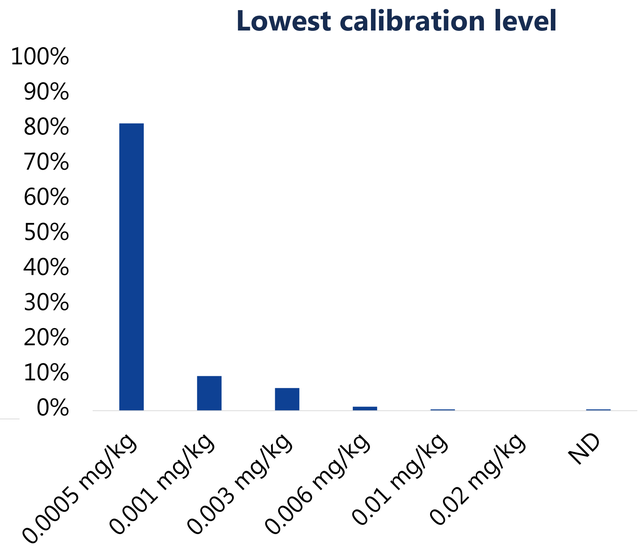


Table 1. Validation results for 26 analytes evaluated with gradient 2.
Similar results were obtained in banana/orange-baby food

Compound	Recovery 0.003 mg/kg	Repeatability 0.003 mg/kg	Recovery 0.006 mg/kg	Repeatability 0.006 mg/kg	Lowest cal. level (mg/kg)	Highest cal. level (mg/kg)
2,4-D	97 %	11 %	109 %	6 %	0.001	0.02
Bromacil	101 %	6 %	104 %	4 %	0.0005	0.02
Dithianon	96 %	3 %	96 %	3 %	0.0005	0.02
Diuron	99 %	3 %	99 %	2 %	0.0005	0.02
Fensulfothion	99 %	3 %	100 %	3 %	0.0005	0.02
Fensulfothion-oxon-sulfone	100 %	4 %	103 %	2 %	0.0005	0.02
Fipronil	103 %	1 %	101 %	1 %	0.0005	0.02
Fipronil-desulfinyl	101 %	2 %	100 %	2 %	0.0005	0.02
Fipronil-sulfone	98 %	2 %	100 %	2 %	0.0005	0.02
Flubendiamide	104 %	1 %	97 %	2 %	0.0005	0.02
Fludioxonil	105 %	5 %	103 %	2 %	0.0005	0.02
Haloxyfop	96 %	5 %	101 %	3 %	0.003	0.02
Hexaflumuron	94 %	5 %	104 %	8 %	0.0005	0.02
Ioxynil	101 %	2 %	103 %	3 %	0.0005	0.02
Lufenuron	108 %	6 %	102 %	3 %	0.0005	0.02
MCPA	114 %	7 %	99 %	3 %	0.001	0.02
MCPB	-	-	115 %	10 %	0.006	0.02
Meptyldinocap	86 %	14 %	118 %	6 %	0.003	0.02
(*E*)-Metaflumizone	103 %	2 %	95 %	1 %	0.0005	0.02
(*Z*)-Metaflumizone	104 %	1 %	105 %	2 %	0.0005	0.02
Penthiopyrad	103 %	3 %	101 %	1 %	0.0005	0.02
Prothioconazole	106 %	3 %	90 %	5 %	0.0005	0.02
Prothioconazole-desthio	101 %	2 %	100 %	2 %	0.0005	0.02
Teflubenzuron	107 %	3 %	95 %	2 %	0.0005	0.02
TFNA	-	-	98 %	7 %	0.006	0.02
TFNG	103 %	8 %	101 %	5 %	0.003	0.02

Real sample survey

During a survey of 42 real samples, **11 pesticide residues were found** (16 positive findings) in 5 different samples. Concentrations ranged **between 0.003 mg/kg** (difenoconazole) **and 0.020 mg/kg** (imazalil). The most frequently detected compounds were **difenoconazole** (3 detections), **cyprodinil** (2 detections), **meptyldinocap** (2 detections), and **spinosad** (2 detections).

Conclusions

- **Dual-channel liquid chromatography** allows sample analysis with two different, independent mobile phases and/or columns.
- **Sample multiplexing** results in the mass spectrometer capable of data acquisition during the whole run. In this method, **80 % of analysis time is employed in data acquisition** vs. 47 % in single-channel configuration.
- **256 compounds were validated at 0.003 mg/kg** in baby food. The lowest calibration level was 0.005 mg/kg for 82 % of analytes, and ≤ 0.003 mg/kg for 98 %.

References

[1] Díaz-Galiano, F. J.; Rajski, Ł.; Ferrer, C.; Parrilla Vázquez, P.; Fernández-Alba, A. R. Cutting-edge approach using dual-channel chromatography to overcome the sensitivity issues associated with polarity switching in pesticide residues analysis. *Anal. Chim. Acta* **2021**, *1180*, 338875.
[2] Rajski, Ł.; Jesús, F.; Díaz-Galiano, F. J.; Fernández-Alba, A. R. Dual-channel chromatography a smart way to improve the analysis efficiency in liquid chromatography coupled to mass spectrometry. *J. Chrom. A* **2020**, *1633*, 461614.
[3] (a) COMMISSION DIRECTIVE 2006/125/EC on Processed Cereal-Based Foods and Baby Foods for Infants and Young Children. *Off. J. Eur. Union* **2006**, *L 339*, 1-20. (b) COMMISSION DELEGATED REGULATION (EU) 2016/127 of 25 September 2015 supplementing Regulation (EU) No 609/2013 of the European Parliament and of the Council as regards the specific compositional and information requirements for infant formula and follow-on formula and as regards requirements on information relating to infant and young child feeding. *Off. J. Eur. Union* **2016**, *L 25*, 1-29

Co-funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Health and Digital Executive Agency (HaDEA). Neither the European Union nor the granting authority can be held responsible for them.