Application Note

Food Testing and Agriculture

Agilent

Trusted Answers

Analysis of Multiresidue Pesticides in Salmon Using Agilent Captiva EMR—Lipid with GC/MS/MS

Authors

Pimpernelli J. dos Santos, Sónia M. V. S. Cardoso, Osmar D. Prestes, Martha B. Adaime, and Renato Zanella
Universidade Federal de Santa Maria, Departamento de Química, Laboratório de Análises de Resíduos de Pesticidas (LARP), Santa Maria-RS, Brasil, 97105-900

Mariana Baptistão
Agilent Technologies do Brasil, Barueri-SP, Brasil, 06455-000

Abstract

This application note describes an analytical method for determining multiresidue pesticides in salmon. The sample preparation method is based on liquid extraction followed by Agilent Captiva EMR—Lipid cleanup and analysis with Agilent Intuvo 9000 GC and 7010B Triple Quadrupole mass spectrometry (GC/MS/MS). Captiva EMR—Lipid cleanup provides efficient removal of major interferences, such as lipids, from salmon. A total of 38 pesticides were determined in a 20-minute run using an Agilent HP-5ms Ultra Inert column, presenting good linearity (R^2 ≥0.990) in a concentration range from 0.5 to 25 µg/kg for all the compounds in salmon. Overall recoveries ranged from 83% to 125% with RSD <25%.

Introduction

A considerable rise in demand for aquaculture products has created an increased need for monitoring pesticide residues to ensure a safe food supply. The complex composition of fish samples (high protein and fat content), makes sample preparation a challenge to ensure the quality of analytical results. An adequate sample preparation requires satisfactory and consistent extraction for target analytes and efficient matrix removal.

Tracking a broad scope of pesticides is necessary to determine whether the residual levels of these pesticides comply with regulated maximum residue limits (MRLs). Salmon is a good source of omega-3, which contains approximately 20% of proteins and 10% of lipids in its centesimal composition. According to the Food and Agriculture Organization (FAO) of the United Nations, salmon is the ninth most cultivated fish globally and the use of some pesticides is allowed in its cultivation due to the possible presence of parasites.[1] However, the presence of these compounds, even in trace amounts in the food supply, can cause disturbances in the environment.

The aim of this study was to develop an efficient and simple GC/MS/MS based analytical method for the detection and quantification of 38 pesticides residues in salmon samples. The method was based on solid-liquid extraction followed by Captiva EMR—Lipid cleanup and water residue removal. The GC/MS/MS method was based on dynamic multiple reactions monitoring (dMRM) with a high efficiency source (HES) and a 30 m HP-5ms Ultra Inert column.

Experimental

Chemicals and reagents

- Pesticides standards (high purity ≥95%), Dr. Ehrenstorfer (Germany) and Sigma-Aldrich (USA)
- Acetonitrile (ACN), methanol (MeOH), and ethyl acetate (EtOAc), HPLC grade, J.T. Baker (USA)
- Reagent-grade isooctane, Mallinckrodt (Ireland)
- Polypropylene tubes (15 mL and 50 mL), Sarstedt (Germany)
- Eppendorf microtubes (2 mL), Axygen Scientific (EUA)

Solutions and standards

Individual pesticide stock solutions (1000 mg/L) were prepared in adequate solvent (ACN, MeOH, or toluene) and stored at ≤−5 °C. The mixture solution (10 mg/L) was prepared in ACN and stored at ≤−5 °C.

The 80:20 ACN/EtOAc extraction solvent and 16:64:20 ACN/EtOAc/water elution solution were prepared and stored at room temperature.

Equipment and material

- Centrifuges NT 825 (Novatecnica, São Paulo, Brazil) and SL 703 (Solab, São Paulo, Brazil)
- Vortex shaker QL-901 (Microtechnology, São Paulo, Brazil)
- Analytical precision balances UX-420H and AUW 220D (Shimadzu, Kyoto, Japan)
- Ultrapure water (18 MΩ cm), Milli-Q system (France)
- Captiva EMR—Lipid cartridges, 3 mL, 300 mg (p/n 5190-1003)
- Manifold Vac Elut 12 position (p/n 5982-9115)
- Ceramic homogenizers (p/n 5982-9313)
- Syringe filter, 13 mm, 0.22 µm, nylon (p/n 5190-5269)
- Inlet septa, bleed and temperature optimized (BTO), nonstick 11 mm (p/n 5183-4757)
- Vial 2 mL, clear, screw, certified (p/n 5182-0714)
- Screw caps, septa PTFE/red silicone, certified (p/n 5182-0717)
- ALS syringe, fixed needle, 10 µL, PTFE-tip plunger (p/n 5183-4730)
- Ultra Inert liner, splitless, single taper, glass wool (p/n 5190-3167)
- Planar capillary column HP-5ms UI, 30 m × 0.25 mm, 0.25 µm (p/n 19091S-433UI-INT)
- Intuvo SSL Guard Chip (p/n G4587-60565)
- Gas Clean filter kit - includes bracket, connection unit, and carrier gas filter for water, oxygen, and organic removal (p/n CP17975)
- Pipettes with variable volume from Eppendorf (USA)

The analysis was performed using an Agilent Intuvo 9000 GC with the 7010B triple quadrupole GC/MS. The GC system was equipped with an electronic pneumatic control (EPC) and a 7693 autosampler. Agilent MassHunter workstation software was used for data acquisition and analysis.

Instrument conditions

The GC/MS/MS instrument conditions were established based on the evaluated compounds. Table 1 lists the final conditions of the GC/MS/MS operation.

Table 1. Intuvo 9000C and 7010B GC/MS/MS conditions.

Parameter	Value
Carrier Gas	Helium 1.2 mL/min
Injection Volume	2 µL (pulsed splitless mode)
Injection Pulse Pressure	50 psi
Oven Program	60 °C (1 minute), 170 °C by 40 °C/min, 310 °C by 10 °C/min, and hold 3 minutes
Injector Temperature	280 °C
Guard Chip Temperature	Initially 85 °C Track oven mode
Bus Temperature	280 °C
Transferline	290 °C
Ionization Source	Electron impact (HES)
Source Temperature	300 °C
MS1/MS2 Temperature	150 °C
Acquisition Mode	Dynamic MRM (dMRM)
Collision Gas	Nitrogen at 1.5 mL/min

Table 2. Pesticides list, mass transitions for MRM and collision energy.

Compounds	MRM transitions				RT (min)
	Quantifier (*m/z*)	CE (V)	Qualifier (*m/z*)	CE (V)	
Acrinathrin	228.9 → 92.8	10	207.8 → 181.1	10	14.762
Alachlor	188.1 → 160.1	10	188.1 → 132.1	20	9.224
Atrazine	214.9 → 200.2	5	214.9 → 58.1	10	8.069
Cadusafos	158.8 → 131.0	5	158.8 → 97.0	15	7.183
Chlordane-*cis*	372.8 → 300.9	10	372.8 → 265.9	25	11.099
Chlorfenapyr	328.0 → 247.0	20	247.1 → 227.1	20	11914
Chlorfenvinphos	294.9 → 266.9	5	266.9 → 159.0	20	10.438
Chlorpyrifos	313.8 → 257.8	15	198.9 → 171.0	15	9.881
Chlorpyrifos-methyl	285.9 → 93.0	25	124.9 → 47.0	15	9.120
Cyhalothrin (Lambda)	208.1 → 181.1	10	181.1 → 152.1	30	14.657
Epoxiconazole	192.0 → 138.1	10	192.0 → 111.0	25	12.919
Ethoprophos	157.9 → 114.0	5	157.9 → 97.0	15	7.183
Etrimfos	292.1 → 181.1	5	181.1 → 153.1	10	8.560
Fenitrothion	277.0 → 260.1	5	125.1 → 79.0	5	9.118
Fenpropimorph	128.1 → 110.1	5	128.1 → 86.1	10	9.990
Fenthion	278.0 → 109.0	15	124.9 → 79.0	5	9.974
Fipronil	366.8 → 212.8	25	350.8 → 254.8	15	10.488
Fluazifop-*p*-butyl	382.9 → 282.0	10	281.9 → 238.0	15	12.049
Fluquinconazole	340.0 → 298.0	15	340.0 → 107.8	40	15.798
HCH-alpha	218.9 → 183.0	5	216.9 → 181.0	5	7.766
HCH-beta	218.9 → 183.1	5	216.9 → 181.1	5	7.866
Hexachlorobenzene	283.9 → 248.8	15	283.8 → 213.9	30	7.834
Indoxacarb	202.9 → 134.0	15	202.9 → 106.0	25	17.900
Malathion	172.9 → 99.0	15	157.8 → 125.0	5	9.734
Methidathion	144.9 → 85.0	5	144.9 → 58.1	15	11.033
Metolachlor	240.0 → 162.2	10	238.0 → 162.2	10	9.866
Pendimethalin	251.8 → 162.2	10	251.8 → 161.1	15	10.449
Pirimicarb	238.0 → 166.2	10	166.0 → 96.0	15	8.699
Pirimiphos-methyl	290.0 → 125.0	20	232.9 → 151.0	5	9.533
Pyrazophos	232.0 → 204.1	10	221.0 → 193.1	10	15.031
Pyrimethanil	198.0 → 183.1	15	198.0 → 158.1	20	8.451
Quinalphos	157.0 → 129.1	15	146.0 → 118.0	10	10.757
Spiromesifen	273.0 → 255.1	5	272.0 → 254.2	5	13.611
Terbufos	230.9 → 175.0	10	230.9 → 129.0	20	8.256
Tetraconazole	336.0 → 217.9	20	336.0 → 203.8	30	10.049
Trifloxystrobin	172.0 → 145.1	15	116.0 → 89.0	15	12.830
Trifluralin	306.1 → 264.0	5	264.0 → 206.0	5	7.269
Vinclozolin	212.0 → 172.1	15	197.9 → 145.0	15	9.158

Sample preparation

The sample preparation method was established based on a previously reported method used for determination of polycyclic aromatic hydrocarbons (PAHs) in salmon.[2] The salmon samples were purchased from a local grocery store in Santa Maria, Rio Grande do Sul, Brazil. The samples were chopped, homogenized, and stored in a freezer at ≤–10 °C. The method was carried out in three major sections:

1. Solid-liquid extraction
2. Captiva EMR—Lipid cleanup
3. Water removal

The homogenized samples were weighed at 2.5 g into 50 mL centrifuge (polypropylene) tubes, spiked as necessary, vortexed for one minute, and equilibrated for 15 to 20 minutes. The complete sample procedure is described in Figure 1.

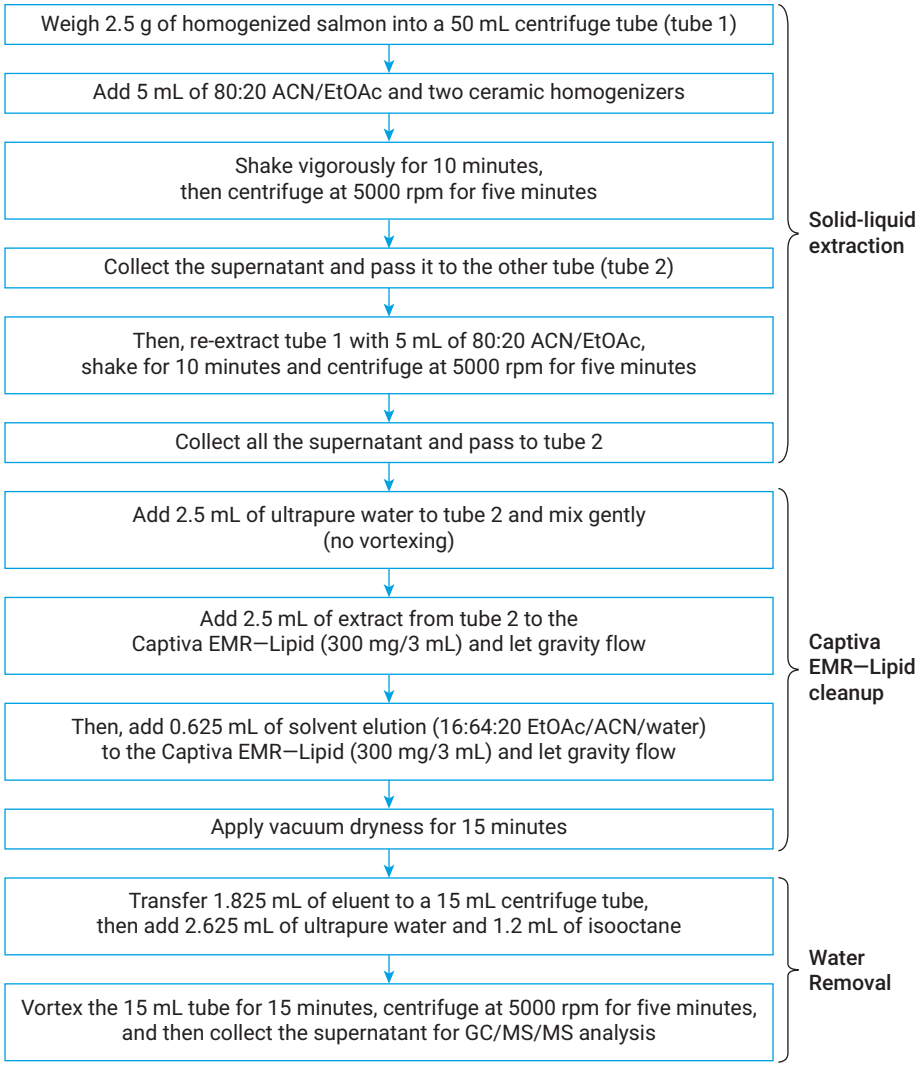

Figure 1. Procedure for sample preparation of a salmon sample based on solid-liquid extraction followed with Captiva EMR—Lipid cleanup.

Method validation

Parameters such as limit of quantification (LOQs), linearity, precision, and accuracy were evaluated. The calibration curve standards included five different points (2, 4, 25, 50, and 100 µg/kg). Three spike levels (25, 50, and 100 µg/kg) were evaluated in terms of recovery and RSD% (n = 3). Analyte identification and quantification were determined from retention times and MRM transitions.

Results and discussion

GC method

Dynamic MRM is a useful tool for multiresidue methods. By constructing automatic tables of MRM based on retention times (RTs) in a detectable retention time window (delta RT) for analytes, compounds are identified and quantified with better precision. In addition, ultra inert (UI) liners and columns were used to prevent labile pesticides, such as organophosphorus and organochlorides, from causing poor results due to the interaction with the GC flow path surface active sites. The susceptibility of highly sensitive compounds to active sites in the instrument flow path can result in poor peak shape and reproducibility, and significant loss of sensitivity.[3,4] Figure 2 shows the MRM chromatogram for all the 38 compounds in salmon blank sample spiked at 25 µg/kg level.

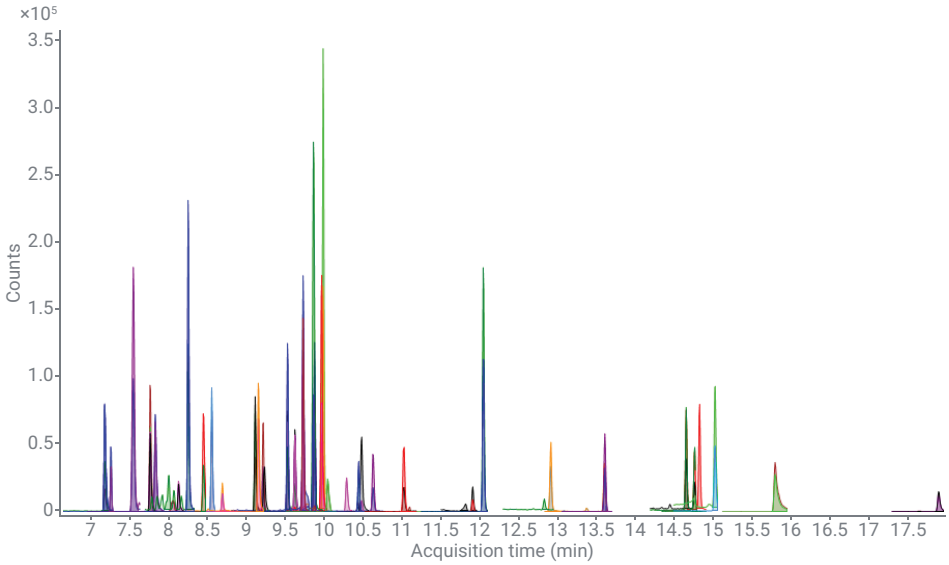

Figure 2. An MRM chromatogram in GC/MS/MS presenting separation of 38 pesticides in 20 minutes.

Validation results are listed for all 38 pesticides in Table 3. The calibration curve linearity coefficient R^2 was higher than 0.9900 for all compounds. The method limit of quantification (LOQ) was 25 µg/kg with two exceptions, cyhalothrin lambda and fenpropimorph at 50 µg/kg. The analyte recoveries ranged from 80 to 125% at 25 µg/kg level, from 93 to 119% at 50 µg/kg level, and from 83 to 107% at 100 µg/kg level. The precision in terms of relative standard deviation (RSD) was lower than 25% in all three spike levels.

Table 3. Method calibration curve linearity, recoveries with RSD (n = 3) and limit of quantification (LOQ) for all pesticides.

Compound	R^2	Rec % (RSD % with n = 3)			LOQ (µg/kg)
		25 µg/kg	50 µg/kg	100 µg/kg	
Acrinathrin	0.9922	92 (7)	101 (9)	101 (2)	25
Alachlor	0.9966	100 (5)	111 (8)	101 (9)	25
Atrazine	0.9959	113 (2)	106 (1)	92 (9)	25
Cadusafos	0.9938	107 (9)	119 (8)	105 (13)	25
Chlordane-*cis*	0.9900	95 (5)	104 (3)	83 10	25
Chlorfenapyr	0.9941	88 (1)	101 (7)	97 1	25
Chlorfenvinphos	0.9918	109 (14)	114 (2)	95 14	25
Chlorpyrifos	0.9951	83 (9)	105 (14)	96 3	25
Chlorpyrifos-methyl	0.9973	93 (2)	107 (9)	96 14	25
Cyhalothrin (Lambda)	0.9986	125 (25)	104 (6)	94 (8)	50
Epoxiconazole	0.9919	105 (1)	103 (5)	95 (9)	25
Ethoprophos	0.9934	108 (9)	119 (9)	106 (13)	25
Etrimfos	0.9967	99 (3)	110 (9)	100 (8)	25
Fenitrothion	0.9985	88 (4)	102 (12)	93 (13)	25
Fenpropimorph	0.9926	80 (25)	100 (8)	103 (15)	50
Fenthion	0.9955	95 (3)	106 (4)	95 (5)	25
Fipronil	0.9907	99 (7)	110 (5)	93 (15)	25
Fluazifop-*p*-butyl	0.9939	92 (5)	103 (7)	98 (1)	25
Fluquinconazole	0.9917	108 (12)	113 (3)	97 (14)	25
HCH-alpha	0.9901	100 (3)	116 (4)	100 (16)	25
HCH-beta	0.9930	100 (3)	116 (4)	100 (16)	25
Hexachlorobenzene	0.9917	84 (13)	93 (5)	98 (7)	25
Indoxacarb	0.9999	93 (5)	114 (4)	99 (12)	25
Malathion	0.9981	97 (7)	106 (7)	88 (3)	25
Methidathion	0.9968	97 (13)	107 (5)	92 (21)	25
Metolachlor	0.9909	98 (5)	109 (4)	96 (7)	25
Pendimethalin	0.9946	94 (0)	109 (8)	100 (3)	25
Pirimicarb	0.9937	114 (6)	112 (9)	103 (20)	25
Pirimiphos-methyl	0.9985	92 (2)	105 (4)	96 (5)	25
Pyrazophos	0.9954	91 (6)	114 (8)	97 (13)	25
Pyrimethanil	0.9924	96 (3)	104 (9)	94 (9)	25
Quinalphos	0.9909	92 (1)	106 (7)	95 (6)	25
Spiromesifen	0.9970	92 (2)	108 (6)	97 (8)	25
Terbufos	0.9960	93 (3)	108 (10)	99 (2)	25
Tetraconazole	0.9915	110 (12)	113 (2)	95 (14)	25
Trifloxystrobin	0.9978	108 (9)	109 (8)	100 (11)	25
Trifluralin	0.9920	96 (1)	113 (9)	107 (6)	25
Vinclozolin	0.9988	91 (3)	107 (7)	98 (5)	25

Sample preparation method

Efficient matrix removal is important for multiresidue pesticide analysis to succeed in complex food matrices, as matrix interferences are usually responsible for data deterioration, shorter consumables lifetime, and more frequent system maintenance. The Captiva EMR—Lipid cleanup is a solution that can selectively remove interferences in an easy and quick way. During method development, the previous method used for PAH extraction from salmon was discovered to also be suitable for pesticide analysis in salmon.

There are two important parts of the method contributing to good analyte recovery:

1. The duplicate liquid extraction improves analyte extraction efficiency from the fatty matrix.
2. The additional elution after sample passing through the cartridge ensures more complete analyte elution.

The elution solution can vary, but should contain 10 to 20% water to prevent trapped lipids from being retreated. The mixture of 16:64:20 EtOAc/ACN/water was applied for additional elution in this method.[2]

A portion of water was added to the crude extract before sample loading onto the Captiva EMR—Lipid cartridge. This is important for the desired lipid retention on the EMR—Lipid sorbent. Generally, 20% water in the sample mixture is recommended. For GC/MS/MS detection, complete water removal is critical to ensure good chromatography and consistent analyte responses by GC/MS/MS. In this study, an isooctane solvent back extraction was used for not only water removal, but also for partial sample reconcentrating. However, for LC/MS/MS detection, water removal is not necessary. After EMR—Lipid cleanup, samples can either be run directly or with dilution by LC/MS/MS for analysis. Only when detection sensitivity cannot meet requirements, is a dry and reconstitution step necessary to concentrate the sample prior to LC/MS/MS analysis.

Figure 3 presents the recoveries of 38 pesticides at three spike levels, 25, 50, and 100 µg/kg, in salmon.

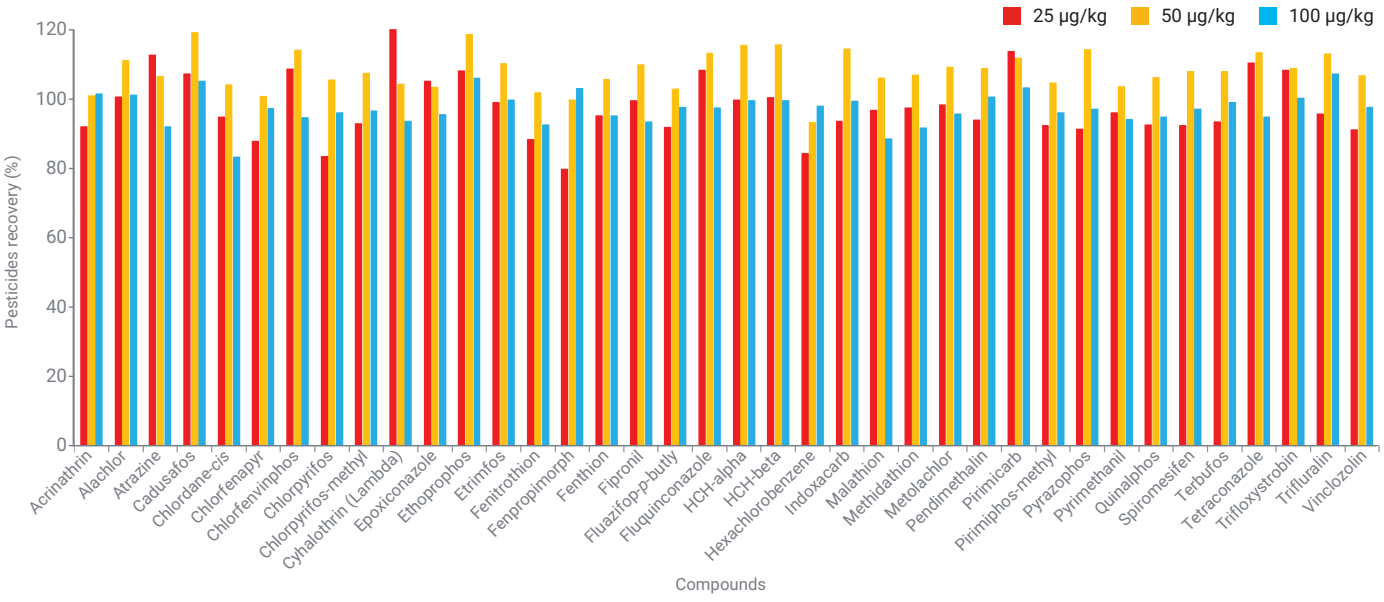

Figure 3. Recoveries from all pesticides evaluated at 25, 50, and 100 µg/kg spiked levels.

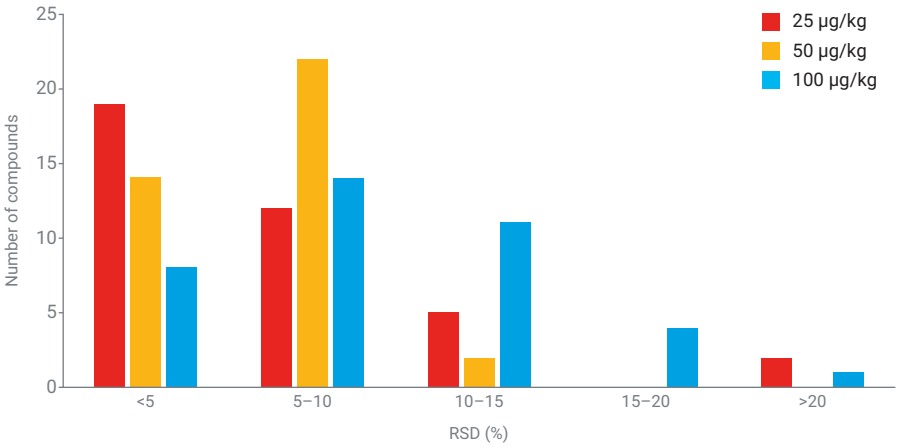

Figure 4. Method reproducibility in % RSD for pesticides analysis in salmon at each spike level, n = 3.

Conclusion

A method using Agilent Captiva EMR—Lipid cleanup and an Agilent Intuvo GC/MS/MS was developed and validated for the analysis of 38 pesticides in salmon. The sample preparation method demonstrated success and applicability for GC-amenable pesticides analysis. It is a unique method for multiclass compound extraction from fatty food matrices with efficient sample matrix cleanup, leading to a simple routine method for food testing laboratories. It is important to note that the Agilent Captiva EMR—Lipid cleanup is compatible with the LC/MS/MS technique.

References

1. Food and Agriculture Organization of the United Nations (FAO), The state of world fisheries and aquaculture, **2018**. http://www.fao.org/3/i9540en/i9540en.pdf
2. Determination of 19 Polycyclic Aromatic Hydrocarbon Compounds in Salmon and Beef, *Agilent Application Note*, publication number 5994-0553EN, **2019**.
3. Challenging Pesticide Analysis Using an Agilent J&W DB-35ms Ultra Inert GC Column, *Agilent Application Note*, publication number 5990-6595EN, **2010**.
4. Better Pesticide Analysis with Agilent J&W Ultimate Plus Tubing in an Inert Flow, *Agilent Application Note*, publication number 5991-5404EN, **2014**.

www.agilent.com/chem

Printed in the USA, February 10, 2020
5994-1717EN
DE.5752546296

Agilent
Trusted Answers