Application Note

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Comparison of Different Sample Matrix Cleanup Techniques for Multiresidue Pesticide Determination in Bovine Meat Extracts

Using Intuvo 9000 GC/MS/MS

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Abstract

This application note describes an analytical method for multiresidue pesticide determination in bovine, based on a solid-liquid extraction and cleanup by sorbent cartridges for the interference removal step. Three different matrix cleanup techniques (Agilent Bond Elut C18, Bond Elut NH2, and Captiva EMR–Lipid) were evaluated to compare matrix removal and pesticides recovery. The analysis was performed by GC/MS/MS using the Agilent Intuvo 9000 GC and the Agilent 7010B triple quadrupole GC/MS. The acetonitrile (ACN) extraction followed with the Captiva EMR–Lipid cleanup demonstrated efficient meat matrix removal, such as lipids and proteins, and acceptable pesticides recovery. Overall recoveries of 56 pesticide residues ranged from 62 to 119% with RSD ≤16%.

Introduction

Meat is an important type of food in the human diet and is consumed worldwide. However, despite its benefits, the presence of contaminant residues, such as pesticides[1,2,10], in meat has raised ever more food safety concerns.[2,3,4,5] The awareness of the toxic effects of pesticides on human health has induced the development of a series of legal limits as strategies to ensure consumer protection, for example the maximum residue limit (MRL) set by EU for hexachlorocyclohexane and acetochlor in bovine muscle is 0.01 mg/kg. On the other side, the MRL established for hexachlorobenzene is 0.005 mg/kg.[1,6,7]

Considering the high lipid and protein contents in meat, an efficient matrix cleanup procedure is important for reliable and consistent sample analysis. The use of organic solvents (such as ACN) for extraction in animal-origin food plays a crucial role, acting as an additional cleaning procedure and providing a suitable extract before the cleanup step. The use of cartridge cleanup products, such as C18, amine (NH_2) and, more recently, the Agilent Captiva Enhanced Matrix Removal–Lipid (EMR–Lipid)[5,8,9,10] enables better detection and quantification of pesticide residues in complex matrices, including meat.

Captiva EMR–Lipid provides high efficiency and selective lipid removal with the combination of size exclusion and hydrophobic interactions that selectively trap the long, unbranched, aliphatic chains on lipid-like molecules. The unique pass through functionality of Captiva EMR–Lipid simplifies the sample preparation workflow.[9] The highly selective interaction mechanism also significantly reduces the unwanted interactions with "bulk" molecules, minimally impacting target recoveries.

The aim of this study was to compare sample cleanup using three products (Bond Elut C18, Bond Elut NH2, and Captiva EMR–Lipid) for pesticide analysis in bovine meat using GC/MS/MS. The sample preparation method was based on solid-liquid extraction followed by cartridge pass through cleanup. The GC/MS/MS method was based on dynamic multiple reaction monitoring (dMRM) with a high efficiency source (HES) and an Agilent J&W HP-5MS Ultra Inert column.

Experimental

Chemicals and reagents

- Pesticide standards (high purity ≥95%) were from Dr. Ehrenstorfer (Germany) and Sigma-Aldrich (USA).
- HPLC-grade ACN was from J.T. Baker (USA).

Solutions and standards

Individual pesticide stock solutions (1,000 mg/L) were prepared in ACN, MeOH, or toluene and stored at ≤–5 °C. The combined spiking solution (10 mg/L) was prepared in ACN and stored at ≤–5 °C.

Equipment and consumables

- 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 (Merck Millipore, France)
- Agilent Bond Elut C18 cartridges, 3 mL, 500 mg (part number 12102028)
- Agilent Bond Elut NH2 cartridges, 3 mL, 500 mg (part number 12102041)
- Agilent Captiva EMR–Lipid cartridges, 3 mL, 300 mg (part number 5190-1003)
- Agilent Bond Elut EMR–Lipid polish pouch, anhydrous $MgSO_4$ (part number 5982-0102)
- Agilent 12-port rack for Vac Elut 12 manifold (part number 5982-9115)
- Agilent Captiva Econofilter syringe filter, 13 mm, 0.22 µm, nylon (part number 5190-5269)
- Agilent inlet septa, bleed and temperature optimized (BTO), nonstick, 11 mm (part number 5183-4757)
- Agilent vial, 2 mL, clear, screw, certified (part number 5182-0714)
- Agilent screw caps, PTFE/red silicone septa, certified (part number 5182-0717)
- Agilent ALS syringe, fixed needle, 10 µL, PTFE-tip plunger (part number 5183-4730)
- Agilent Ultra Inert inlet liner, splitless, single taper, glass wool (part number 5190-3167)
- Agilent J&W HP-5ms Ultra Inert Intuvo GC column module, 30 m × 0.25 mm, 0.25 µm (part number 19091S-433UI-INT)
- Agilent Guard Chip, Intuvo, split/splitless (part number G4587-60565)
- Agilent Gas Clean carrier gas filter kit; includes bracket, connection unit, and carrier gas filter for water, oxygen, and organic removal (part number CP17975)
- Pipettes with variable volume (Eppendorf, USA).
- T 25 digital ULTRA TURRAX homogenizer (IKA, Germany)
- Polypropylene tubes, 15 and 50 mL (Sarstedt, Germany)
- Eppendorf microtubes, 2 mL (Axygen Scientific, EUA).

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

Instrument conditions

The GC/MS/MS instrument conditions were established based on the targeted compounds. Table 1 shows the GC/MS/MS method conditions. Table 2 shows the target acquisition conditions.

Table 1. Agilent Intuvo 9000 GC and Agilent 7010B triple quadrupole GC/MS conditions.

Parameter	Setting
Carrier Gas	Helium at 1.2 mL/min
Injection Volume	1 µL
Injection mode	Splitless
Oven Program	60 °C (1 min), 170 °C at 40 °C/min, 310 °C at 10 °C/min, Hold 3 min
Injector Temperature	280 °C
Guard Chip Temperature	Initially 85 °C, Track oven mode
Bus Temperature	280 °C
Transfer Line	300 °C
Ionization Source	Electron impact (HES)
Source Temperature	300 °C
MS1/MS2 Temperature	150 °C
Acquisition Mode	dMRM
Collision Gas	Nitrogen at 1.5 mL/min

Table 2. Pesticide MRM transitions and collision energy.

Compound	RT (min)	Quantifier (*m/z*)	CE (V)	Qualifier (*m/z*)	CE (V)
Dichlorvos	4.65	109.0 → 79.0	5	184.9 → 93.0	10
E-Mevinphos	5.50	127.0 → 109.0	10	127.0 → 94.9	15
Z-Mevinphos	5.50	127.0 → 109.0	10	127.0 → 94.9	15
Ethoprophos	6.79	157.9 → 114.0	5	157.9 → 97.0	15
Chlorpropham	6.87	127.0 → 65.1	25	153 → 125.1	10
Trifluralin	7.00	306.1 → 264.0	5	264.0 → 206.0	5
Cadusafos	7.16	158.8 → 131	5	158.8 → 97.0	15
Phorate	7.23	121.0 → 65.0	10	128.9 → 65.0	15
α-HCH	7.35	180.9 → 145.0	15	216.9 → 181.0	5
Atrazine	7.59	214.9 → 58.1	10	214.9 → 200.2	5
β-HCH	7.73	181.0 → 145.0	15	218.9 → 183.1	5
Lindane	7.82	181.0 → 145.0	15	218.9 → 183.1	5
Terbufos	7.83	152.9 → 97.0	5	230.9 → 129.0	20
Pyrimethanil	7.96	198.0 → 183.1	15	198.0 → 158.1	20
Disulfoton	8.08	88.0 → 60.0	5	142.0 → 109.0	5
Etrimfos	8.28	292.0 → 181.0	5	181.0 → 153.0	10
Pirimicarb	8.37	238.0 → 166.2	10	166.0 → 55.1	20
Chlorpyrifos-methyl	8.75	124.9 → 47.0	15	78.9 → 47.0	10
Parathion-methyl	8.75	125.0 → 47.0	10	125.0 → 79.0	5
Prometryn	8.99	241.0 → 184	10	226.0 → 184.0	10
Fenitrothion	9.17	125.1 → 47.0	15	125.1 → 79.0	5
Pirimiphos-methyl	9.17	232.2 → 151.0	5	290.0 → 125.0	20
Malathion	9.31	126.9 → 99.0	5	157.8 → 125.0	5
Metolachlor	9.46	162.2 → 133.2	15	238.0 → 162.2	10
Fenpropimorph	9,47	128.1 → 70.1	10	128.1 → 110.1	5
Triadimefon	9.57	208.0 → 181.1	5	128.0 → 65.0	20
Tetraconazole	9.62	170.9 → 136.0	10	152.9 → 97.0	5
Cyprodinil	9.94	225.2 → 224.3	10	224.2 → 208.2	20
Penconazole	10.08	248.0 → 192.1	15	248.0 → 157.1	25
Chlorfenvinphos	10.19	266.9 → 159	20	294.9 → 266.9	5
Quinalphos	10.26	146,0 → 118.0	10	146,0 → 91.0	30
Procymidone	10.36	96.0 → 67.1	10	96.0 → 53.1	15
Methidathion	10.51	144.9 → 85.0	5	144.9 → 58.1	15
α-Endosulfan	10.75	194.9 → 159.0	5	194.9 → 160.0	5
Flutriafol	10.81	123.1 → 95.0	15	123.1 → 75.1	25
Picoxystrobin	10.81	145.0 → 102.1	25	145.0 → 115.1	15
Hexaconazole	10.94	175.0 → 111.0	20	175.0 → 147.0	10
Myclobutanil	11.22	179.0 → 152.1	10	150.0 → 123.0	15
Flusilazole	11.27	233.0 → 165.1	15	314.7 → 232.9	10
Bupirimate	11.30	272.9 → 193.1	5	272.9 → 108.0	15
Fluazifop-P-butyl	11.47	281.9 → 91.0	15	281.9 → 238.0	15
β-Endosulfan	11.73	206.9 → 172.0	15	194.9 → 158.9	10
Ethion	11.90	152.9 → 96.9	10	124.9 → 96.9	0
Propiconazole I	12.36	172.9 → 145.0	15	172.9 → 109.0	30
Kresoxim-methyl	12.42	116.0 → 89.0	15	116.0 → 63.0	30
Trifloxystrobin	12.53	172.0 → 145.0	15	116.0 → 89.0	15

Sample preparation

The extraction was performed as shown in Figure 1. The bovine meat samples were grounded with a meat grinder, homogenized and stored in a freezer at ≤−10 °C. Before analysis, the samples were thawed completely at ambient temperature. Then, 5 g of bovine meat samples were weighed into 50 mL polypropylene tubes, spiked with standards as necessary. Samples were vortexed for 1 minute. An aliquot of 5 mL ACN was added for simultaneous protein precipitation and extraction of analytes. The sample mixture was further homogenized using the ULTRA TURRAX homogenizer at 10,000 rpm for 20 seconds. The tubes were centrifuged at 6,000 rpm for 8 minutes at 5 °C and the supernatants were collected. An aliquot of 4 mL sample extract was then used for subsequent pass through cleanup using a Bond Elut C18 cartridge, a Bond Elut NH2 cartridge, or a Captiva EMR–Lipid cartridge. For Captiva EMR–Lipid cleanup, the crude extract was mixed with water to generate a mixture of organic/aqueous (80/20, v/v). For Bond Elut C18 and Bond Elut NH2 cleanup, the crude extract was directly transferred to the cartridges for cleanup. The eluates from Bond Elut C18 and Bond Elut NH2 were collected and directly injected to GC/MS/MS. The eluate from Captiva EMR–Lipid was dried with anhydrous $MgSO_4$ for water removal before GC/MS/MS analysis.

Three spiking levels (10, 20, and 50 µg/kg) of meat samples were evaluated for recovery and RSD (%) in replicates of four. Analyte identification and quantification were determined based on retention times and MRM transitions.

Compound	RT (min)	Quantifier (*m/z*)	CE (V)	Qualifier (*m/z*)	CE (V)
Propiconazole II	12.50	172.9 → 145.0	15	172.9 → 109.0	30
Tebuconazole	12.71	125.0 → 89.0	15	125,0 → 99.0	20
Nuarimol	12.74	203.0 → 107.0	10	139.0 → 111.0	15
Epoxiconazole	13.01	192.0 → 138.1	10	165.0 → 138.0	10
Tebufenpyrad	13.55	275.9 → 171.1	10	332.9 → 171.0	15
Fenamidone	13.58	238.0 → 237.2	10	268.0 → 180.2	20
Metconazole	13.66	125.0 → 89.0	20	125.0 → 99.0	20
Fenarimol	14.49	219.0 → 107.1	10	139.0 → 75.0	30
Fluquinconazole	15.26	108.0 → 57.0	15	340.0 → 298.0	15
Boscalid	15.98	140.0 → 112.0	10	140.0 → 76.0	25

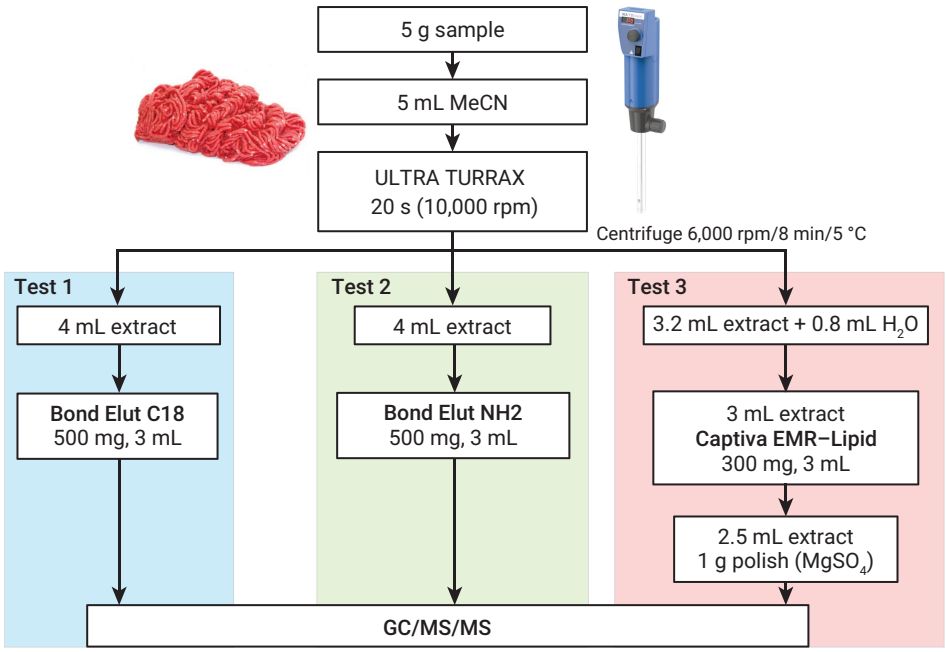

Figure 1. Bovine meat sample preparation procedure using solid–liquid extraction and three different pass through cleanups using Agilent Bond Elut C18, Agilent Bond Elut NH2 and Agilent Captiva EMR–Lipid cartridges, respectively.

Results and discussion

Matrix extract cleanup evaluation by GC/MS full scan

For the evaluation of different sample extraction overall cleanliness, the GC/MS full scan (FS) chromatograms were collected for comparison. Figure 2 shows the FS chromatograms of bovine meat samples prepared by three types of cartridge cleanup. The chromatogram comparison shows that the Bond Elut C18 cleanup provides the best sample cleanup with the lowest GC/MS FS background. In contrast, the results from the Bond Elut NH2 cleanup, with help of the NIST library, show the presence of cholesterol even after the cleanup, which is evidence of lipid presence after the cleanup step. It is important to reinforce that this specific peak does not show up in the sample chromatograms when using either Bond Elut C18 or Captiva EMR–Lipid.

Target recovery and reproducibility

Overall recoveries for 56 pesticide residues ranged from 62 to 119% with RSD ≤16%. All compounds presented acceptable recoveries (70 to 120%) using Captiva EMR–Lipid and good reproducibility (RSDs <20%) at 50 mg/kg spiking level.

Targets recovery and reproducibility were evaluated and compared at the spiking level of 10 µg/kg in bovine meat. Figure 3 shows the recovery comparison results for the challenging compounds.

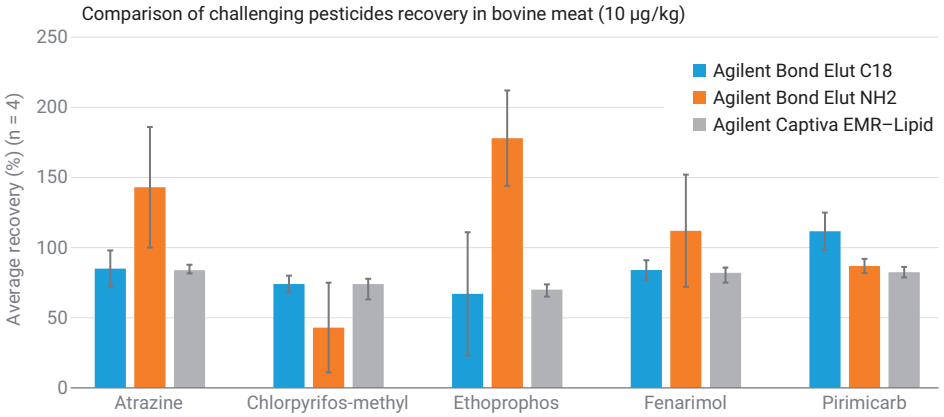

Figure 3. Recoveries of five challenging pesticides in bovine meat at 10 µg/kg spiking level.

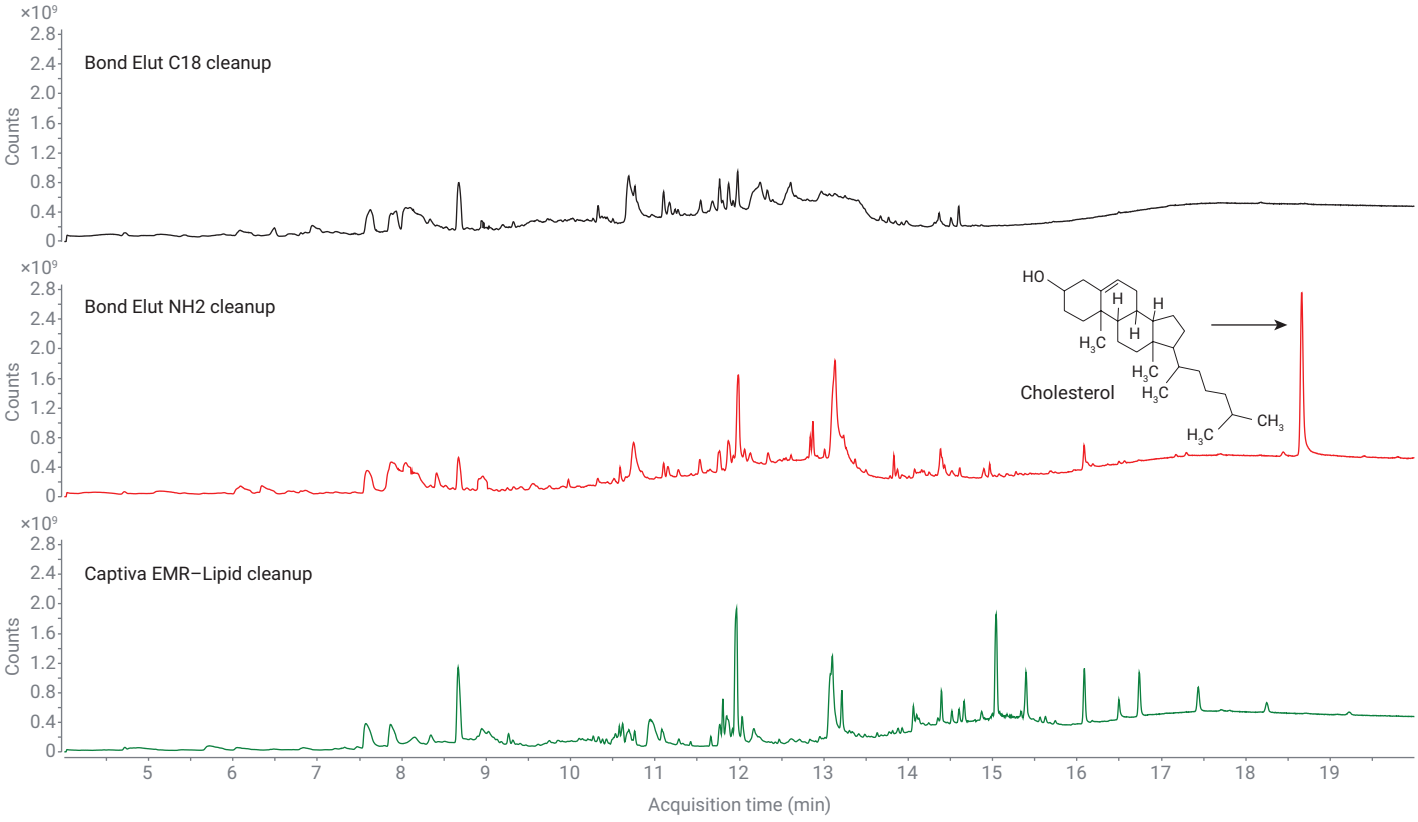

Figure 2. GC/MS FS chromatograms of bovine meat samples prepared by different cartridge cleanups.

Captiva EMR–Lipid cleanup showed the acceptable recoveries (70 to 120%) for all the challenging compounds, with excellent reproducibility (RSDs <10%). For samples treated by Bond Elut NH2 cleanup, <70% recovery was obtained for chlorpyrifos methyl, and >120% recoveries were achieved for both atrazine and ethoprophos. In addition, four out of five challenging compounds had high RSDs for samples prepared by Bond Elut NH2 cleanup, indicating either more variations introduced during sample preparation, or more matrix impacts on these targets. For samples prepared by Bond Elut C18 cleanup, acceptable recoveries were achieved for all difficult compounds, but poor reproducibility (RSD >20%) showed up for ethoprophos.

Signal-to-noise and peak shape

To evaluate the presence of matrix effect, the signal-to-noise (S/N) values of the target pesticides were investigated. Figure 4 shows a comparison of two representative compounds, ethoprophos and atrazine, peak S/N and peak shape in the bovine meat extract at a spiking level of 10 µg/kg, prepared by three different cleanup methods.

The comparison results demonstrate that lower S/N values were shown when the Bond Elut C18 cleanup was applied. The Bond Elut NH2 cleanup provided higher S/N than the Bond Elut C18 cleanup but caused the fronting peak shape. The Captiva EMR–Lipid cleanup provided the highest S/N for both compounds, and a symmetrical peak shape. The high target S/N and excellent peak shape assure method sensitivity and integration accuracy and consistency.

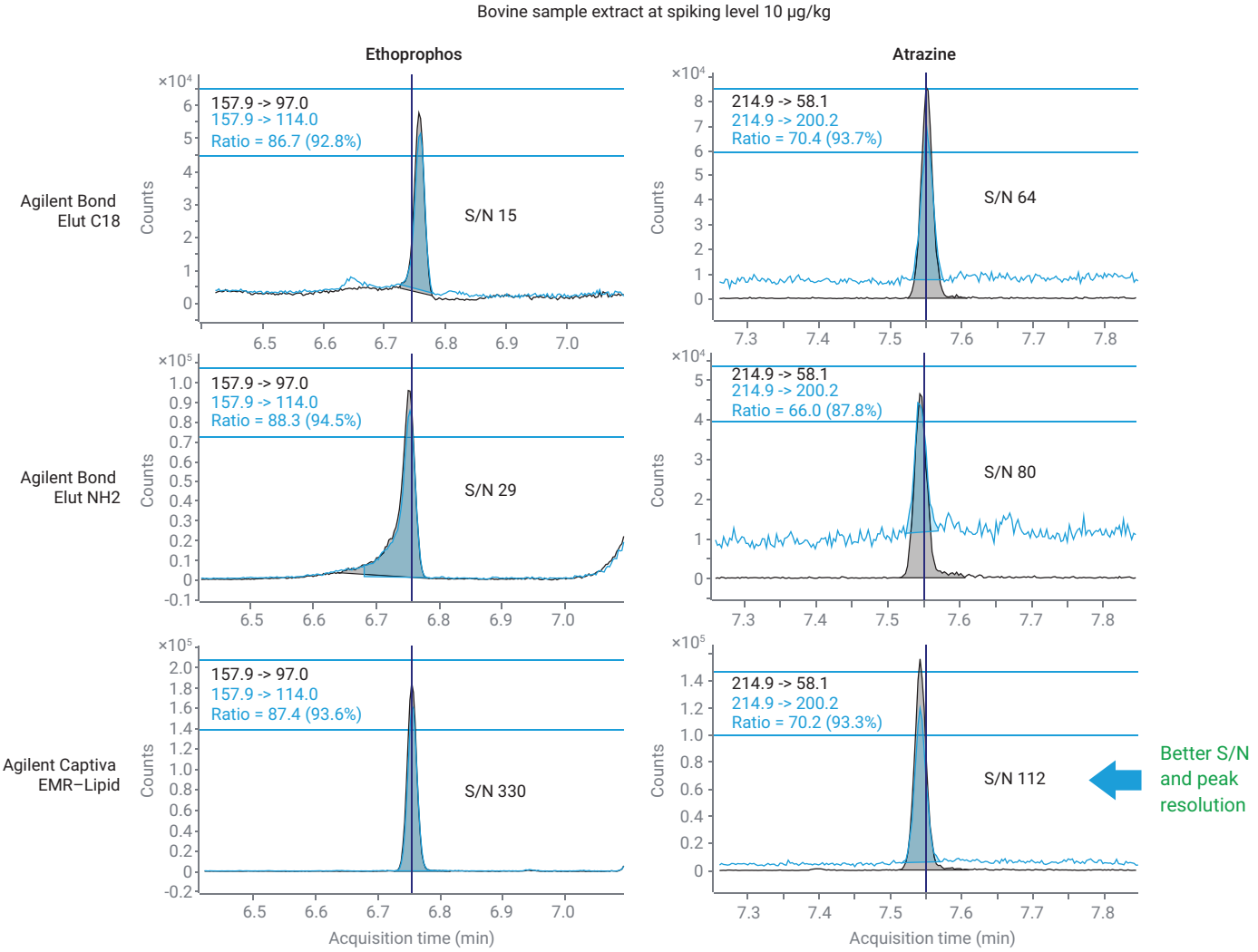

Figure 4. Representative targets ethoprophos and atrazine, S/N, and peak shape comparison.

Comparison of method limits of quantitation (LOQs) with MRLs

Table 3 shows a comparison of official MRLs by EU and Brazil (Plano Nacional de Controle de Resíduos de Contaminantes) and the method LOQs obtained for currently required pesticides in meat. Captiva EMR–Lipid cleanup demonstrated method LOQs (10 µg/kg) below the MRLs in bovine meat for most pesticides that are regulated by both EU and Brazil official methods, except epoxiconazole, which is regulated by Brazil official methods. In comparison, both Bond Elut C18 and Bond Elut NH2 cleanup showed more pesticides with method LOQs higher than the required MRLs.

Table 3. Comparison of MRLs set by EU and Brazil PNCRC legislation for bovine meat with the LOQs obtained in each tested sorbent after SLE.

Regulated Pesticide	MRL by EU	MRL by PNCRC (Brazil)	LOQ by Agilent Bond Elut C18 Cleanup	LOQ by Agilent Bond Elut NH2 Cleanup	LOQ by Agilent Captiva EMR–Lipid Cleanup
	(µg/kg in Bovine Meat)				
Atrazine	–	–	10	50	10
Boscalid	10	10	10	20	10
Chlorpropham	50	–	10	–	10
Chlorpyrifos-methyl	10	50	10	50	10
Epoxiconazole	10	2	20	50	10
Ethoprophos	10	50	20	20	10
Fenamidone	10	–	20	50	10
Fenarimol	20	20	10	50	10
Flutriafol	10	10	10	20	10
Metolachlor	10	–	50	50	10
Mevinphos (E- and Z-)	–	10	10	–	10
Myclobutanil	10	10	20	50	10
Nuarimol	10	–	20	20	10
Parathion-methyl	10	–	50	50	10
Pirimicarb	50	10	10	10	10
Pyrimethanil	100	–	50	50	10
Tebuconazole	100	50	50	50	10
Tetraconazole	50	–	10	50	10
Triadimefon	10	–	20	50	10

Conclusion

With the thorough method evaluation based on matrix removal, targets recovery and reproducibility, analyte S/N values and peak shape, and the comparison of method LOQs with regulated MRLs, the Agilent Captiva EMR–Lipid cleanup was demonstrated to be a superior cleanup method to the Agilent Bond Elut NH2 and C18 methods. The method was verified in bovine meat matrix and has a high chance of being extended to other similar meat matrices.

Acceptable recovery and reproducibility were achieved for 56 pesticides. Method LOQs meet most EU and Brazil MRLs. The efficient sample cleanup method can also be beneficial to reduce GC/MS/MS system maintenance frequency, increase column and consumable lifetimes, and deliver reliable quantification results.

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