Targeted Veterinary Drug Screening in Food Matrices Using EMR—Lipid QuEChERS Kit and an Agilent 6470A Triple Quadrupole LC/MS System

Application Note

Authors

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Abstract

Veterinary drugs are used to treat animal diseases and improve animal growth. Due to bioaccumulation of these drugs and their metabolites in adipose tissue and subsequent entry into the food chain, strict regulations for maximum residue levels are in place. The combination of an Agilent EMR—Lipid QuEChERS sample preparation kit and an Agilent 6470A LC/MS system provides a superior solution for the screening and quantitation of 105 veterinary drugs in beef, beef liver, pork, and salmon.

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Introduction

Governmental Agencies, such as FDA-USDA, WHO, and the United Nation Food and Agriculture Organization, have set maximum residue levels (MRLs) for veterinary drugs in different food matrices to limit the use of veterinary drugs in food producing animals [1,2]. Sensitive screening and quantitation of veterinary drugs at MRL is essential for adherence to those regulations.

The Agilent Bond Elut Enhanced Matrix Removal (EMR—Lipid) QuEChERS kit is specifically designed and formulated to remove fatty acid components from food matrices. It has been demonstrated that the extensive removal of fatty materials does not negatively impact analyte recovery in complex matrices, thus improving detection sensitivity [3,4]. Sample preparation with an EMR—Lipid QuEChERS kit enables a fast, robust, and effective analysis of fatty samples.

The stringent requirements for targeted veterinary drug screening require a sensitive and selective instrument capable of delivering robust results. The Agilent 6470A Tandem Quadrupole system (LC/TQ), features innovative technologies such as quadrupole prefilters, tapered and curved hexapole collision cell, and a high-energy dynode detector (HED). These improvements enhance ion transmission efficiencies for precursor ions, eliminate cross-talk while promoting rapid collision-cell evacuation, and allow for better sensitivities. These lead to enhanced linear dynamic ranges (LDRs) and limits of quantitation (LOQs).

Methods and Experiments

Beef, beef liver, organic pork, and salmon were purchased from local grocery stores, and were prepared per EMR—Lipid Protocols as previously described [3]. One-hundred five veterinary drugs (Table 4) sourced from Ultra Scientific (N. Kingstown, RI, USA) were spiked into the matrices at nine levels ranging 0.1–100 ng/g (Table 1). Agilent 1290 Infinity UHPLC conditions and parameters for the 6470A LC/TQ equipped with an Agilent Jet Stream ESI source are listed in Tables 2 and 3.

Analytes were detected with dynamic MRM acquisition with two optimized MRM transitions per compound. Fast polarity switching was fully used to detect all compounds in a single run. Data analysis was carried out with Agilent MassHunter Quantitative Analysis software (version B.07).

Table 1. Spike Levels

Level	Concentration
1	0.1 ng/g
2	0.5 ng/g
3	1 ng/g
4	2 ng/g
5	5 ng/g
6	10 ng/g
7	20 ng/g
8	50 ng/g
9	100 ng/g

Table 2. Agilent 1290 Infinity HPLC Conditions

Parameter	Value	
Column	Agilent PoroShell 120, EC-C18, 2.1 × 150 mm, 2.7 µm (p/n 693775-902)	
Temperatures	Column: 40 °C Sampler: 6 °C	
Injection volume	2 µL	
Mobile phase	A) Water, 0.5 mM NH_4F + 0.1 % formic acid B) ACN + 0.1 % formic acid	
Flow rate	0.4 mL/min	
Gradient	Time (min)	%B
	0	2
	0.5	2
	3	35
	8	45
	15	98
	18	98
	18.1	2
Stop time	19 minutes	
Post time	2 minutes	

Table 3. Agilent Jet Stream Source and Agilent 6470A Triple Quadrupole Parameters

Parameter	Value
Drying gas temperature	250 °C
Drying gas flow	10 L/min
Sheath gas temperature	350 °C
Sheath gas flow	11 L/min
Nebulizer pressure	35 psi
Capillary voltage	3,500 V(+), 3,500 V(–)
Nozzle voltage	500 V(+), 1,000 V(–)
Delta EMV	200 V(+), 200 V(–)
Cycle time	500 ms

Table 4. Compound List of 105 Veterinary Drugs

17-*alpha*-19-Nortestosterone	Halofuginone	Prednisolone
17-*beta*-Estradiol	Hexestrol	Propionylpromazine
17-Methyltestosterone	HMMNI Hydroxydimetridazole (Dimetridazol-OH)	Ractopamine
Amino-Mebendazole	Hydroxy-Ipronidazole	Rifaximin
Betamethasone	Hydroxymetronidazole	Robenidine
Boldenone (Dehydrotestosterone)	Ibuprofen	Ronidazole
Brombuterol	Ipronidazole	Salbutamol (Albuterol)
Carprofen	Isopyrin	Salinomycin
Chlorbrombuterol (Bromoclenbuterol)	Isoxsuprine (Isolait)	Spiramycin I
Chlormadinone acetate	Josamycin	Stanozolol
Chlormadinone	Ketoprofen	Sulfachloropyridazine
Chlorpromazine	Levamisole	Sulfadiazine (Silvadene)
Cimaterol	Lincomycin	Sulfadimethoxine
Cimbuterol	Mabuterol	Sulfadimidine (Sulfamethazine)
Clenbuterol	Maduramycin	Sulfadoxine
Clenbuterolhydroxymethyl	Mapenterol (Methylmabuterol)	Sulfamerazine
Clencyclohexerol	Marbofloxacin	Sulfamethoxazole
Clenpenterol	Mebendazole	Sulfamethoxypyridazine (Midicel)
Clenproperol	Mebendazole-hydroxy	Sulfanilamide
Clopidol	Medroxyprogesterone	Sulfathiazole
DCL Diclazuril	Mefenamic acid	Terbutaline
Decoquinate	Megestrol acetate	Testosterone
Dexamethasone	Melengestrol acetate	Thiabendazole
Diclofenac	Meloxicam	Tilmicosin
Dienestrol	Methylprednisolone	Tolfenamic acid
Diethylstilbestrol	Metronidazole	Toltrazuril
Dimetridazole	Monensin	Trenbolone
Dinitolmide	Nandrolone	Triclabendazole
Erythromycin	Narasin	Triclabendazole sulfoxide
Ethynyl estradiol	Nicarbazin	Trimethoprim
Febantel	Oxfendazole	Tulobuterol
Fenbendazole	Oxibendazole	Tylosin
Fenoterol (Th 1165a)	Oxolinic acid	Zearalanone (Zanone)
Flumequine	Phenylbutazone	Zeranol
Flunixin	Praziquantel	Zilpaterol

Results and Discussion

Increased method performance

The EMR—Lipid QuEChERS sample preparation kit helps remove most of the lipids in the food matrices, thus providing cleaner matrices for better analytical sensitivity. Figure 1 shows the response of 105 analytes at 1.0 ng/g in beef and pork, the two most complex matrices in the study.

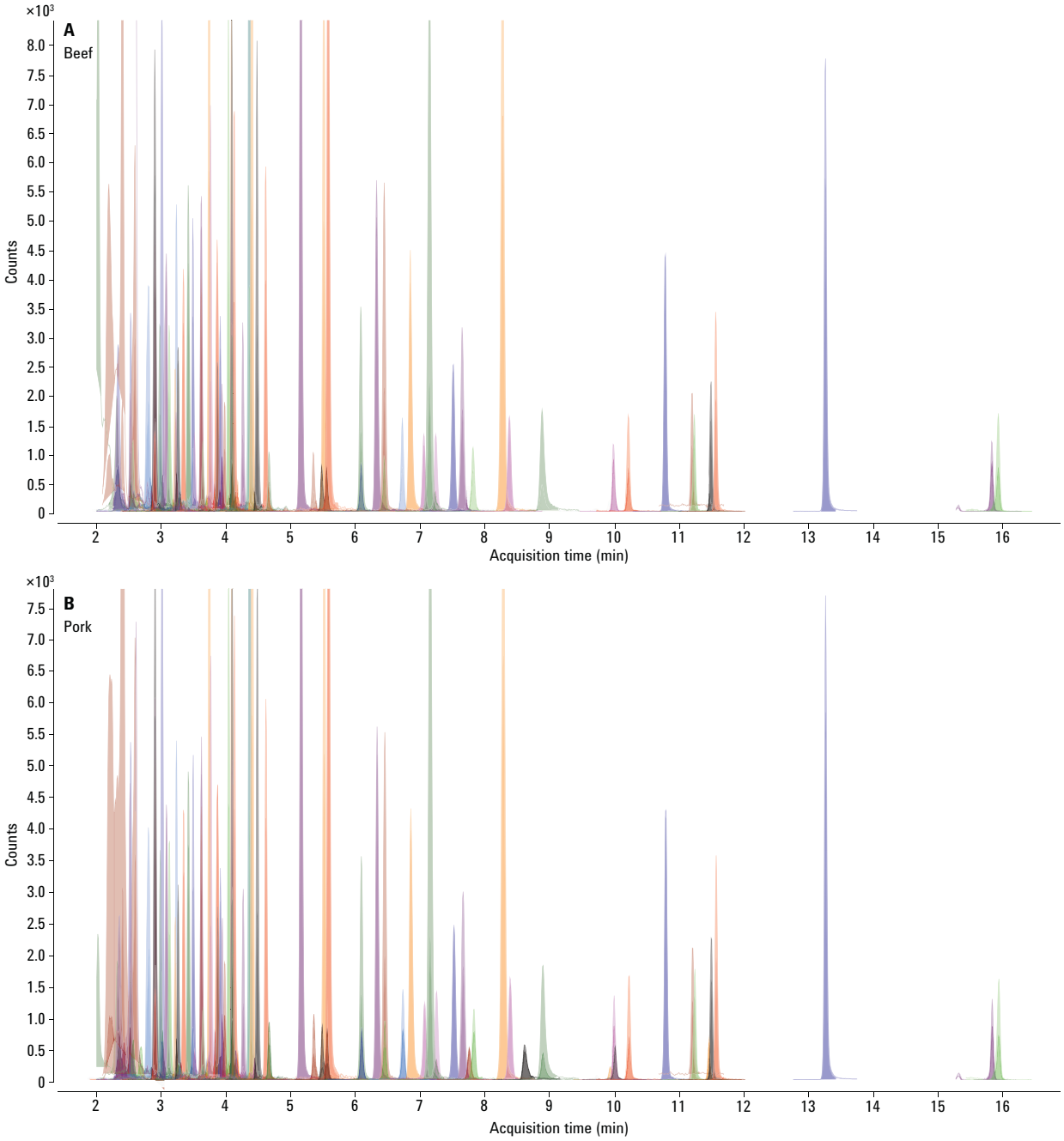

Figure 1. Excellent performance of 105 veterinary drugs in complex matrices (beef and pork). Signals overlaid in the two most complex matrices at 1.0 ng/g spike, with 2 µL injection.

Veterinary drugs screen and accuracy

Most of the veterinary drugs were detected at concentrations as low as 0.1 ng/g in the four matrices. Figure 2 shows the number of compounds that could be quantified with accuracy between 80–120 % for at least four out of six replicates. The signal-to-noise ratio (S/N) of the analyte chromatograms was well above 10, but, as expected, the quantitation accuracy at the lower spike level was affected by the contribution from the matrix baseline to cause the accuracy outside the 80–120 %.

Precision

The %RSD calculation was based on six replicate injections of 105 analytes from 0.1 ng/g to 20 ng/g depending on the matrices at the lowest limit of quantitation (LLOQ). The results shown in Figure 3 clearly demonstrate the precise quantitative capability of the 6470A Triple Quadrupole.

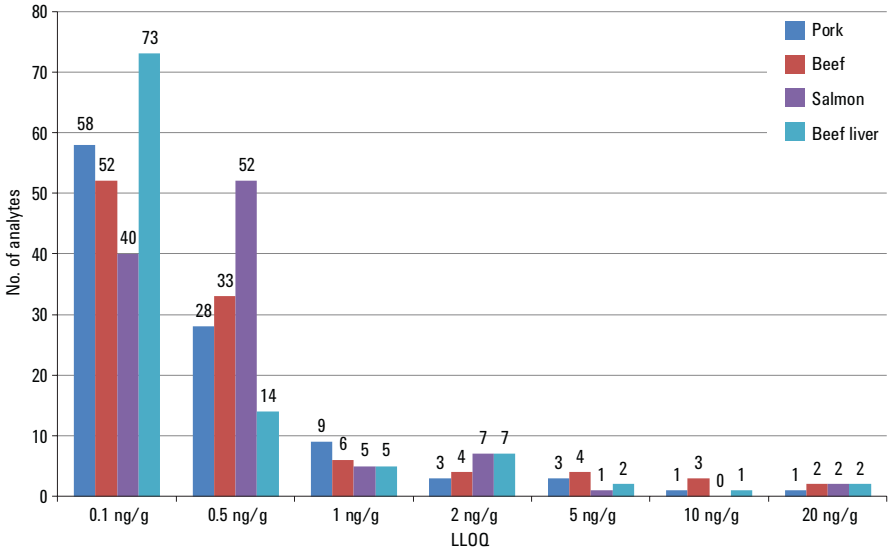

Figure 2. Number of compounds that could be quantified at the LLOQ level with accuracy of 80–120 % in four matrices.

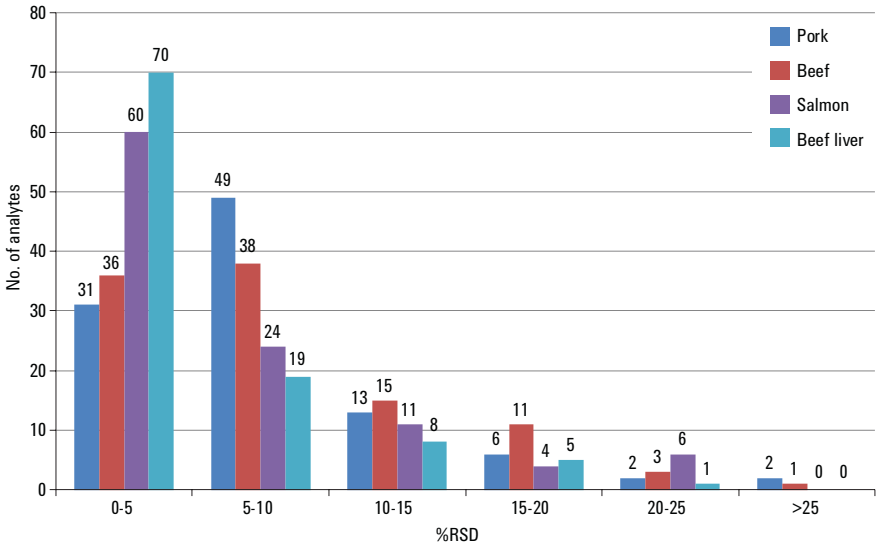

Figure 3. Measurement precision of six replicates at LLOQ levels. Most of the analytes have %RSD less than 10 %.

Calibration curves and dynamic range

The calibration curves shown in Figure 4 were generated from 0.1–100 ng/g with linear fitting and 1/x weighing. More than 96 % of the analytes gave linear responses with R^2 >0.99. Several analytes showed quadratic fitting, possibly due to material loss at lower concentration levels or partial saturation at the higher spike levels.

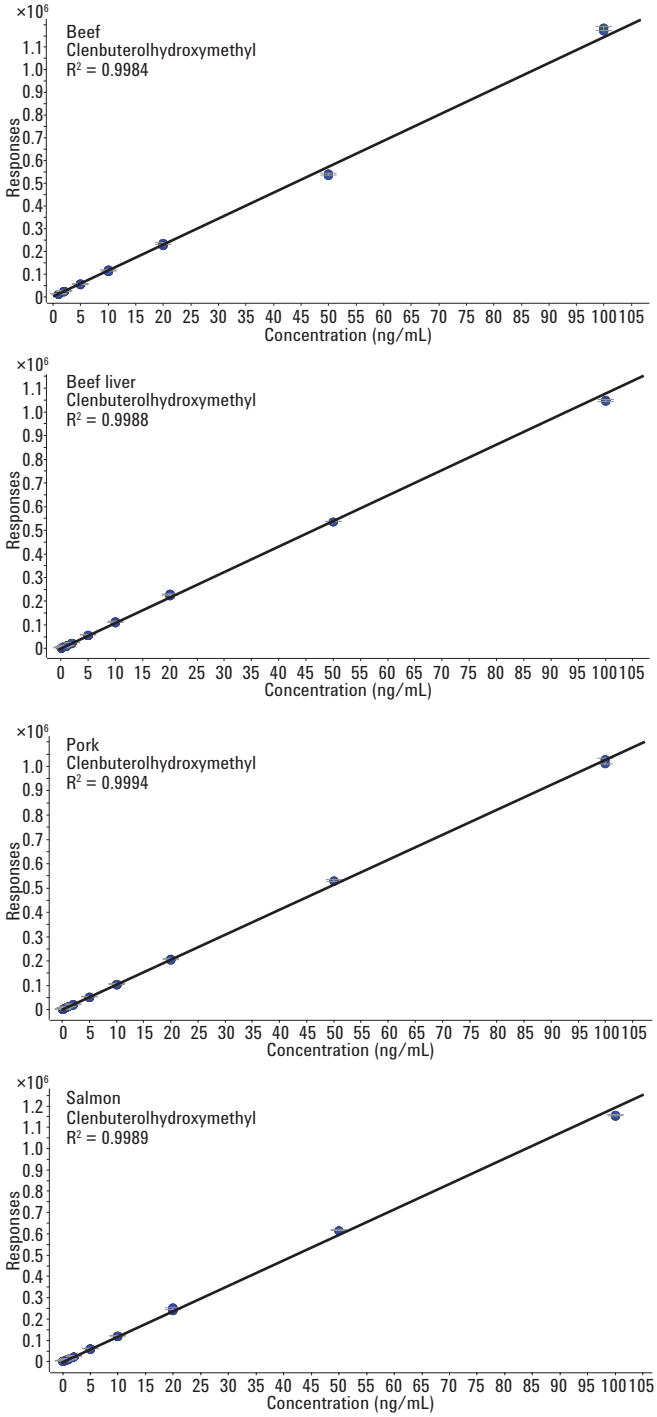

Figure 4. Calibration curves of clenbuterolhydroxymethyl in four matrices. More than 96 % of analytes gave R^2 >0.99.

Veterinary drugs detected in blank matrices

Some of the veterinary drugs were detected in products purchased from local grocery markets. Figure 5 shows examples of the analytes detected in beef liver and salmon. There are several other veterinary drugs that were detected at concentrations less than 1.0 ng/g. In ground beef, robenidine and ketoprofen were detected at 1.0 ng/g and 2.2 ng/g respectively (graphs not shown). However, in organic pork none of the veterinary drugs analyzed in this study were detected at a concentration above 0.5 ng/g.

Conclusions

Analysis and detection of veterinary drugs can be achieved in animal tissue at 1.0 ng/g with a 2.0 µL injection. A combination of an Agilent EMR—Lipid QuEChERS kit and an Agilent 6470A LC/TQ system provide enhanced removal of lipid content from the animal matrices, and sensitive detection at promulgated MRLs. The complete Agilent solution, including powerful data analysis software, facilitates method development and validation, and provides a powerful tool kit for veterinary drug screening and quantitation in animal tissues.

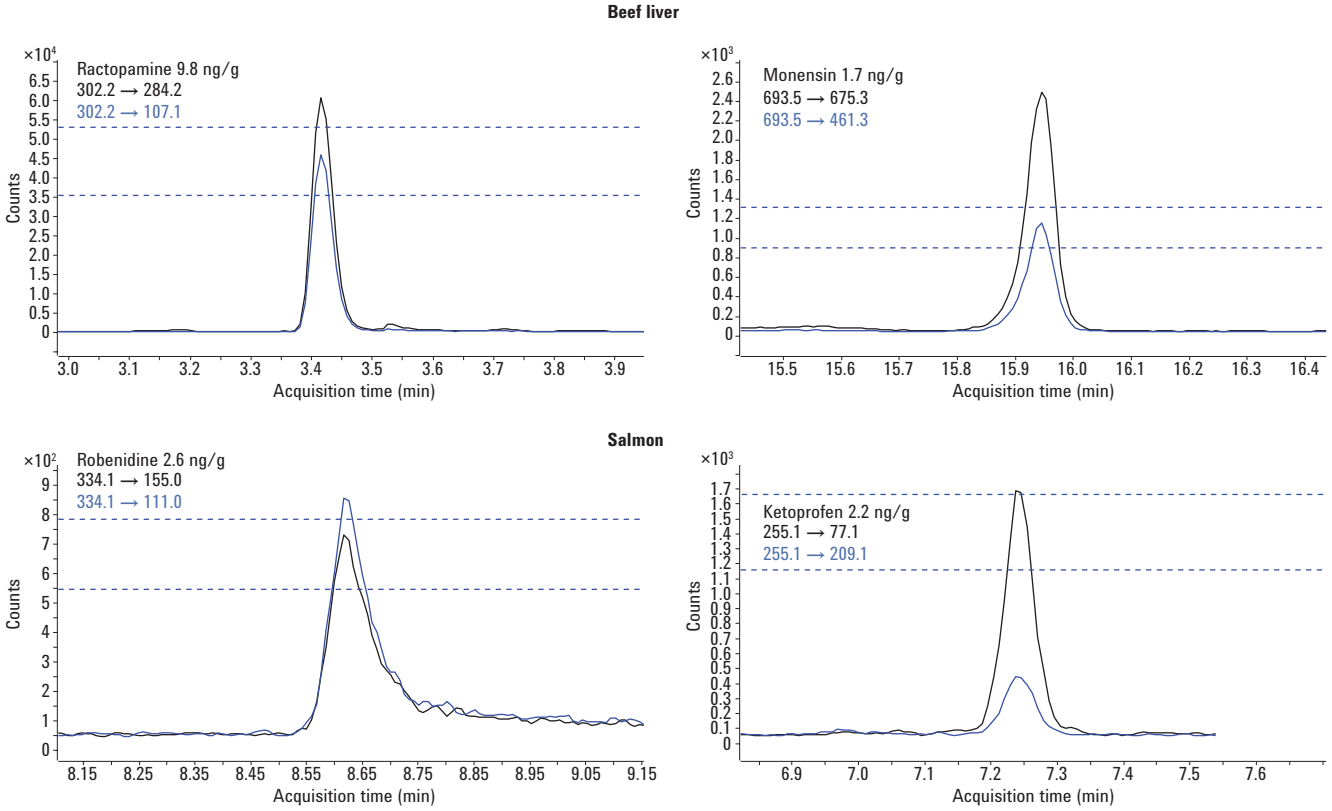

Figure 5. Chromatographs of veterinary drugs in beef liver and salmon purchased from local grocery stores. Their concentrations are calculated based on the spike-in concentration in the same matrix.

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3. L. Zhao, D. Lucas, *Agilent Technologies Application Note*, publication number 5991-6096EN.
4. Joan Stevens, *Agilent Technologies Application Note*, publication number 5991-6771EN.

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