Application Note

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Quantitative Analysis of 210 Veterinary Drugs in Organ Meat Using the Agilent 6470 Triple Quadrupole LC/MS

Authors

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Abstract

This application note demonstrates the applicability of the Agilent Comprehensive Veterinary Drug dMRM Solution for the screening of 210 target residues in organ meat. The described method features a single-cartridge sample preparation protocol and fast LC separation for comprehensive target analysis, covering more than 28 different chemical classes. The method performance was evaluated using matrix-matched samples, and recovery analyzed using matrix-spiked samples. This method provided sensitive detection and quantitative analysis in dMRM mode for quick MRL-based screening.

Introduction

Laboratories that perform routine veterinary drug residue analysis are often challenged by the regulatory agencies to screen large numbers of targets from complex animal origin matrices. This is traditionally achieved by running multiple, single-class analyses, which require significant time and resource commitments. This application note addresses these demands by combining one sample preparation protocol covering multiclass targets, a single fast LC separation to shorten analysis time, quick method customization possibilities, comprehensive/target-specific MRM data acquisition, and efficient software to simplify quantitative analysis. The sample preparation protocol covers sample extraction and purification for >28 analyte-class targets. Additionally, a fast LC gradient approach helped to identify the targets in significantly less time. The multiwash feature within the Agilent 1290 Infinity II LC system prevented heavy matrix components found in organ meats from fouling the detectors. The Agilent 6470 triple quadrupole LC/MS system allowed trace level detection and confident quantitation of 210 veterinary drug target residues.

Experimental

Standards, reagents, matrix-matched calibration levels, and matrix-spiked (pre-extraction) QC samples were prepared per the workflow guide in the Agilent Comprehensive Veterinary Drug dMRM Solution (G5368AA).[1] In this study, an approximately 2.0 ±0.1 g portion of homogenized chicken kidney and liver sample was used. The sample preparation was performed per the procedure defined in the workflow guide.[1]

To characterize method sensitivity, linearity, accuracy, and precision, 10 levels of matrix-matched calibration levels from 0.1 to 100 µg/kg (ppb) were used. A dilution factor of 10x introduced during sample preparation was applied while preparing matrix-matched calibration levels by spiking standards to the respective blank matrix. Target recovery was demonstrated using three levels of QC samples: 1 µg/kg for low-range QC (LQC), 10 µg/kg for mid-range QC (MQC), and 25 µg/kg for high-range QC (HQC). To calculate recovery repeatability, four technical preparations for LQC and MQC were prepared.

The method included in the Comprehensive Veterinary Drug dMRM Solution (designed for the 1290 Infinity II LC system and 6470 triple quadrupole LC/MS) was directly used for acquisition. An Agilent InfinityLab Poroshell 120 EC-C18 column (part number 695575-302) was used for chromatographic separation. Please refer to the workflow guide[1] included with the Comprehensive Veterinary Drug dMRM Solution for more information.

Results and discussion

Sensitivity and linearity

The overall elution profile from chicken kidney and liver was similar to other animal origin matrices such as muscle, seafood, milk, and egg (Figure 1).[2-4] The limit of detection (LOD) results of all 210 targets from kidney and liver matrices are summarized in Figure 2, and the sensitivity of over 95% of analytes was ≤5 µg/kg. The LOD calculation in liver matrix for four targets (2,4,6-triamino-pyrimidine-5-carbonitrile, doxycycline, nicarbazine, and moxidectin) was affected by high endogenous presence.

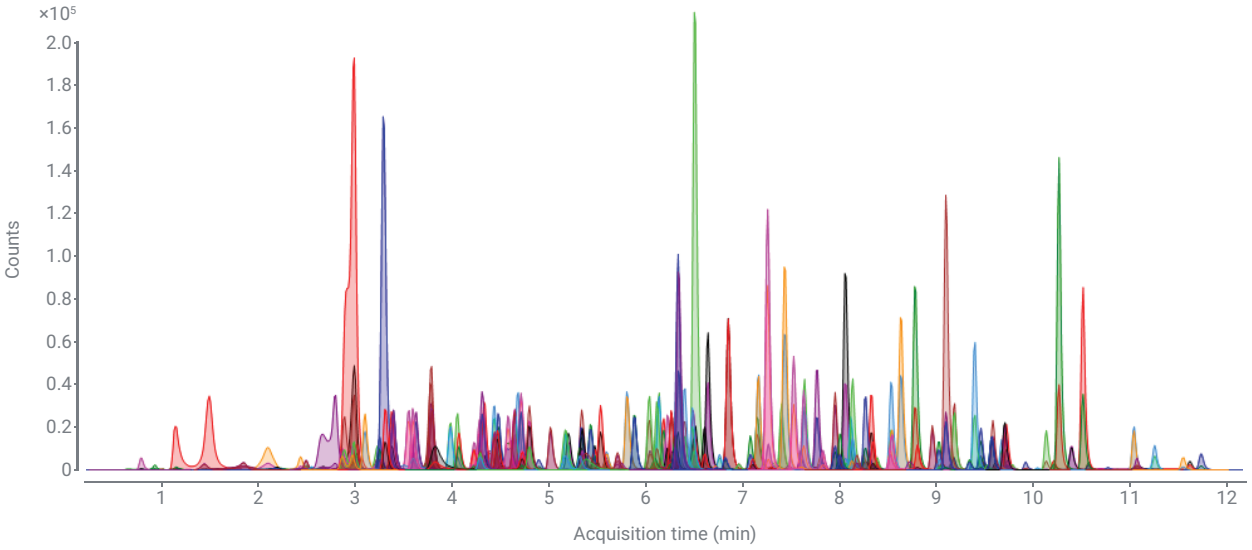

Figure 1. Representative MRM chromatogram of 210 veterinary drug targets postextraction spiked at 2.5 µg/L in the chicken kidney blank matrix.

Linear matrix-matched calibration curves for all targets were plotted from the limit of quantitation (LOQ) to the highest matrix-matched calibration level of 100 µg/kg. The linear regression was used with the ignored origin and 1/x weight. All 210 targets met the calibration curve linearity criteria of R^2 >0.99 in kidney matrix, whereas 206 targets met the linearity criteria in the liver.

Method precision and accuracy

Precision and accuracy were determined using triplicate injections of matrix-matched calibration levels; the results are summarized in Table 1. Precision and accuracy results of all targets met the criteria in kidney and liver, except moxidectin (for response %RSD and accuracy%) and nicarbazine (for accuracy%) in liver matrix.

Recovery and repeatability

The average recoveries were calculated from duplicate injections of technical preparations of each QC level. Recovery was calculated using target response in matrix-spiked QCs, and measured response using matrix-matched calibration curve equations. Intrabatch recovery repeatability was measured as %RSD of recovery values calculated using technical preparations of QC levels (n = 4). Based on the MRL values of a target, an appropriate level of QC sample was used to evaluate method recovery and intrabatch repeatability. The target recovery and repeatability results from kidney and liver matrices are summarized in Figures 3 and 4, respectively.

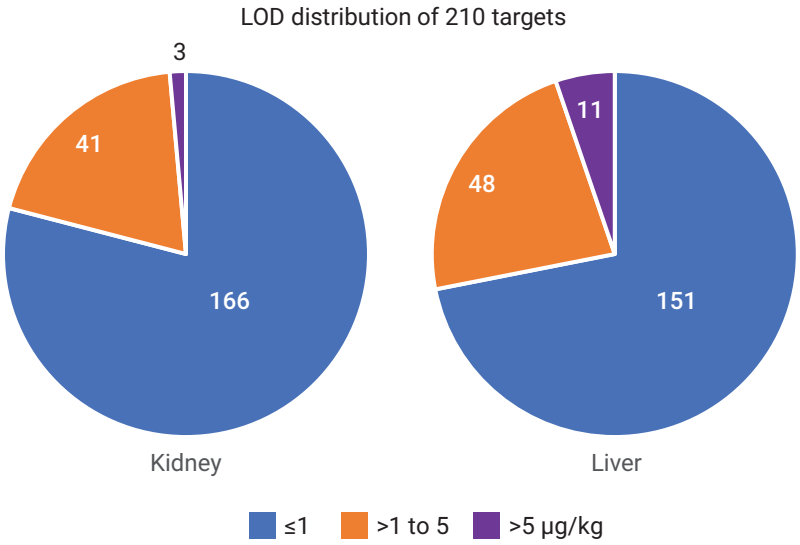

Figure 2. LOD (µg/kg) distribution of 210 targets from chicken kidney and chicken liver matrices using the Agilent 6470 triple quadrupole LC/MS.

Table 1. Summary of precision and accuracy results calculated at 25 µg/kg from kidney and liver matrices.

Parameter	Criteria	Total Number of Targets Meeting the Criteria	
		Kidney	Liver
Precision: Retention Time (RT) %RSD	<0.6%	210	210
Precision: Response %RSD	<20%	210	209
Accuracy%	70% to 120%	210	208

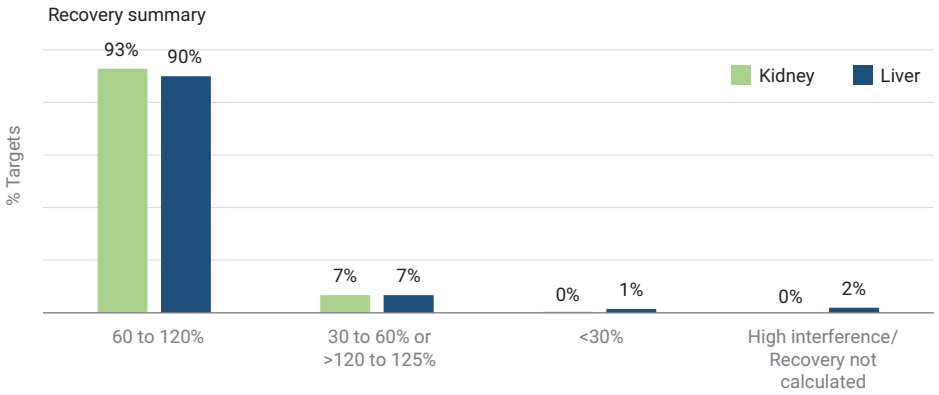

Figure 3. Target recovery results from chicken kidney and liver using respective matrix-spiked QC samples.

In kidney matrix, a total of 195 targets met the recovery criteria of 60% to 120%, whereas a total of 206 targets achieved intrabatch repeatability of RSD ≤20%.[5] Although recoveries of 14 targets (5-hydroxyflunixin, acepromazine, cefapirin, chlorpromazine, chlortetracycline, doxycycline, diminazene, erythromycin, imidocarb, maduramicin ammonium, malachite green, malathion, narasin, and propionylpromazin) were within a range of 30% to 60%, these targets still provided acceptable recovery repeatability values at RSD <20%, demonstrating consistent extraction behavior. Target dipyrone hydrate metabolite exhibited poor recovery and repeatability in kidney. The repeatability of three targets (cefalexin, dimetridazole, and tolfenamic acid) could not be calculated as there were no adequate technical replicates.

In liver matrix, a total of 189 targets met the recovery criteria of 60% to 120%, whereas a total of 195 targets achieved intrabatch repeatability %RSD ≤20%. Recoveries of 14 targets (amprolium, chlorhexidine, dapson N-acetyl, diminazene, erythromycin, imidocarb, isometamidium, malachite green, maduramicin ammonium, narasin, neo-spiramycin, salinomycin, sulfaquinoxaline, and thiamphenicol) were within a range of 30% to 60% or 120% to 125%. Recovery of three other targets (haloxon, malathion, and cefapirin) was <30%. Recovery and repeatability were not determined for the four high-interfering targets

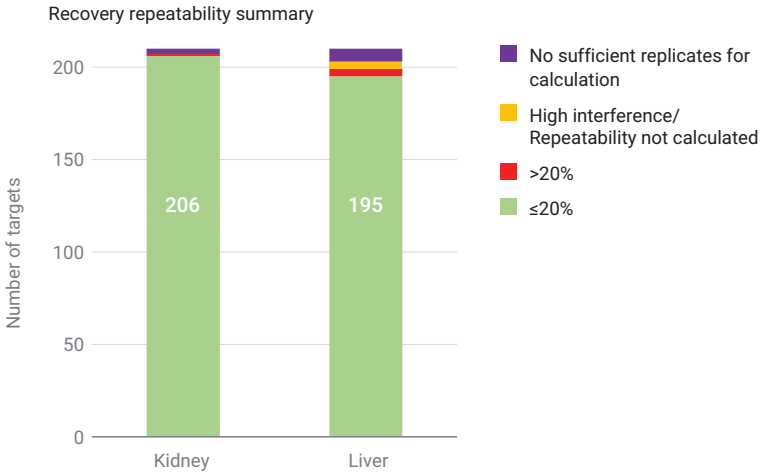

Figure 4. Intrabatch recovery repeatability results from kidney and liver using respective matrix-spiked QC samples.

2,4,6-triamino-pyrimidine-5-carbonitrile, doxycycline, nicarbazine, and moxidectin. Repeatability of seven targets (cefalexin, clenbuterol, dimetridazole, firocoxib, maduramicin ammonium, tilmicosin, and tolfenamic acid) was not calculated due to insufficient technical replicates. The method performance summary for each chemical class is listed in Table 2.

MRL-based screening

Out of 210 target analytes in this study, 91 of them have maximum residue limits (MRL) established in chicken organ meat by the AOAC.[6] Their values ranged from 0.7 to 2,000 µg/kg.[6] In this study, all 91 AOAC-listed targets in kidney and 89 targets in liver were successfully achieved. MRL was not determined for two targets (doxycycline and nicarbazine) in liver matrix due to high endogenous interference.

Matrix effect and regulatory screening

In this study, the matrix effect (ME) of 91 MRL established targets was assessed using target response from postextraction-spiked calibration levels at 2.5 µg/L in blank matrix extract, compared with corresponding neat standards.[2-4]

Out of 91 MRL-established analytes, about 92% of targets showed negligible matrix effect in both matrices (Figure 5). In kidney, four targets (cyromazine, narasin, sulfabenzamide, and sulfaguanidine) had moderate ME, whereas one target (amprolium) had significant ME. In liver, six targets (cyromazine, sulfacetamide, sulfadiazine [silvadene], sulfaguanidine, sulfisomidine, and sulfathiazole) exhibited moderate ME. One target, erythromycin, exhibited severe ME in both kidney and liver.

Table 2. Method performance summary from chicken kidney and liver matrices.

Class	Functional Use/ Chemical Class	Number of Targets	Number of MRL Established Targets (AOAC)	MRL Range (µg/kg)	Sensitivity: LOD Range (µg/kg)		Linearity: Targets meeting R^2 >0.99		Recovery: Targets Meeting Limit 60 to 120%[5]		Repeatability: Targets Meeting QC RSD <20%	
					Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver
1	Anesthetic	1			0.1	0.1	1	1	1	1	1	1
2	Anthelmintic	16	5	10–100	0.1– 5	0.1–5	16	16	16	14	16	15
3	Anthelmintic/Avermectins	3			0.25–5	0.25–2.5	3	2	3	2	3	2
4	Anthelmintic/Benzimidazoles	14	6	50–100	0.1–0.5	0.1–1	14	14	14	14	14	14
5	Anthelmintic/Nitroimidazoles	5			0.25–5	0.25–5	5	5	5	5	5	5
6	Anti-Herbivore	1			5	5	1	1	1	1	1	1
7	Anti-Inflammatory	2			0.25	0.25	2	2	2	2	2	2
8	Antibiotic	7	3	50–1,000	0.1–1.0	0.1–5	7	7	7	7	7	7
9	Antibiotic/Aminoglycosides	5	3	50–200	0.25–2.5	0.25–2.5	5	5	5	5	5	5
10	Antibiotic/Amphenicols	3	2	50–200	0.5–2.5	0.5–2.5	3	3	3	2	3	3
11	Antibiotic/Beta-lactam	16	7	10–300	0.5–10	0.5–10	16	16	15	15	15	14
12	Antibiotic/Macrolides	10	8	40–300	0.25–5	0.25–10	10	10	9	8	10	8
13	Antibiotic/Quinolones	10	8	10–400	0.1–0.5	0.1–1.0	10	10	10	10	10	10
14	Antibiotic/Sulfonamides	27	25	100	0.1–2.5	0.1–2.5	27	27	27	25	27	27
15	Antibiotic/Tetracycline	6	6	100–200	0.25–2.5	0.25–2.5	6	5	4	5	6	5
16	Antiemetic	1			0.1	0.25	1	1	0	1	1	1
17	Antimicrobial	6	3	50–500	0.1–2.5	0.1–2.5	6	6	6	4	6	6
18	Antimicrobial/Furans	1			5	5	1	1	1	1	1	1
19	Coccidiostats	14	10	10–2,000	0.1–10	0.1–10	14	13	10	8	13	11
20	Dopamine receptor	1			0.25	0.25	1	1	1	1	1	1
21	Fungicides and Dyes	3			0.1–0.5	0.1–0.5	3	3	2	2	3	3
22	Growth Promoters/Anabolic Steroids	3			0.25–0.5	0.25–0.5	3	3	3	3	3	3
23	Growth Promoters/Beta-Agonists	4			0.1–0.25	0.1–10	4	4	4	4	4	3
24	Growth Promoters/Corticosteroids	4	1	0.7	0.5–1	0.5–5	4	4	4	4	4	4
25	Hormones	9			0.25–2.5	0.25–2.5	9	9	9	9	9	9
26	Insecticide	15	3	25–500	0.1–5	0.1–5	15	14	14	13	15	13
27	NSAIDs	14			0.1–10	0.1–10	14	14	12	14	12	12
28	Quinoxalines	1			5	5	1	1	1	1	1	1
29	Tranquilizer	8	1	20	0.1–2.5	0.1–2.5	8	8	6	8	8	8
	Total	210	91	–	–	–	210	206	195	189	206	195

Conclusion

Quantitative analysis of 210 multiclass veterinary drugs in chicken kidney and liver matrices was achieved using the Agilent Comprehensive Veterinary Drug dMRM Solution. The sample preparation protocol and methodology were shown to be efficient for target extraction, matrix cleanup, and analysis for organ meat. These results are consistent with those previously described using an identical protocol for animal muscle. The method performance using the Agilent 6470 triple quadrupole LC/MS offered sub-5 µg/kg sensitivity for most analytes. More than 90% targets were within the average recovery of 60% to 120%. The linearity, precision, accuracy, recovery, and repeatability results confirmed the method reliability for regulatory-based routine analysis of veterinary drug residues in kidney and liver.

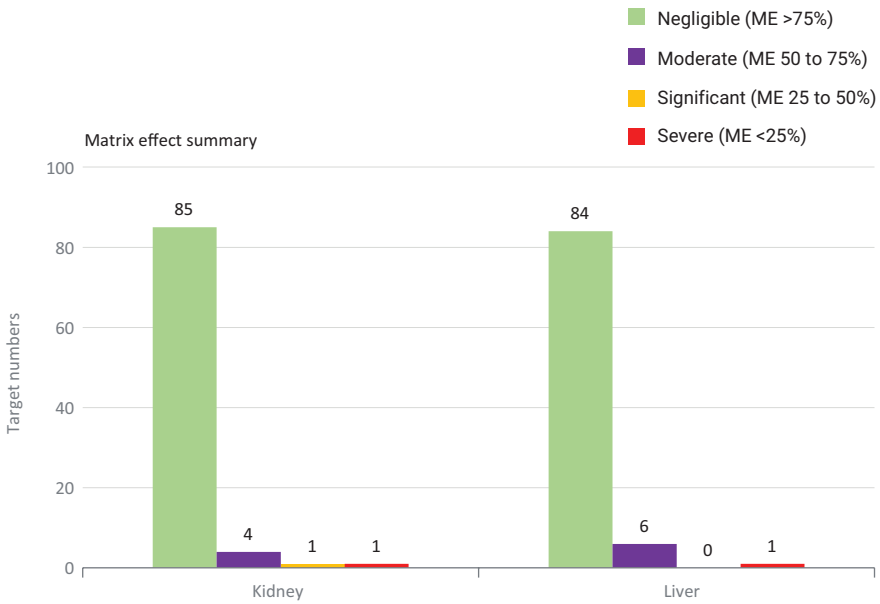

Figure 5. Matrix effect summary of 91 AOAC MRL-established veterinary drug targets from kidney and liver.

References

1. G5368AA Comprehensive Veterinary Drug dMRM Solution. *Agilent workflow guide*, D0002979.
2. An End-To-End Workflow for Quantitative Screening of Multiclass, Multiresidue Veterinary Drugs in Meat Using the Agilent 6470 Triple Quadrupole LC/MS. *Agilent Technologies application note*, publication number 5994-1932EN, **2020**.
3. Quantitative Screening of Multiresidue Veterinary Drugs in Seafood Using the Agilent 6470 Triple Quadrupole LC/MS. *Agilent Technologies application note*, publication number 5994-2832EN, **2020**.
4. Quantitative Screening of Multiresidue Veterinary Drugs in Milk and Egg Using the Agilent 6495C Triple Quadrupole LC/MS. *Agilent Technologies application note*, publication number 5994-3124EN, **2021**.
5. Guidelines for Standard Method Performance Requirements, *AOAC Official Methods of Analysis* **2016**, Appendix F.
6. AOAC guidelines on Screening and Identification Method for Regulated Veterinary Drug Residues in Food, *Version 7*; June 20, **2018**.

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