TECHNICAL NOTE 65666

Rapid quantitation of veterinary drugs in meat extracts using a VeriSpray PaperSpray source coupled to a TSQ Altis triple quadrupole mass spectrometer

Authors: Katherine Walker[1], Laura E. Burns[2], Dwayne E. Schrunk[2], Venkat Uppalapati[1], Ed George[1], Neloni Wijeratne[1]

[1]Thermo Fisher Scientific, San Jose, CA
[2]Iowa State University, Veterinary Diagnostic Laboratory, Ames, IA

Keywords: PaperSpray, VeriSpray, TSQ Altis, veterinary drugs, food safety

Goal

The goal of this technical note is to show how PaperSpray technology is used to rapidly screen for and quantitate commonly used veterinary drugs in salmon and bovine muscle extracts for food safety applications.

Introduction

Veterinary drugs are frequently administered to production animals in order to ensure animal health and well-being throughout the lifetime of the animal. Veterinary drugs can be inappropriately administered, which results in adverse effects to both human and animal health. Global agencies provide regulatory information regarding acceptable residue levels of veterinary drugs in various animal tissue types available for human consumption.[1-2] It is important to develop quick and efficient analytical methods to screen for veterinary drug residues in animal tissues that meet these regulatory requirements. Analysis of veterinary drug residues in animal tissue matrices is challenging because of the complexity and diversity of chemical structures in the various drug classes. LC-MS/MS methods are frequently used to screen for veterinary drugs in animal tissues because they provide the selectivity and sensitivity needed to identify and quantify veterinary drugs at the given maximum residue limits (MRLs) set by the regulatory agencies.[3-4] While ambient ionization techniques generally provide a cost-effective platform for the analysis of veterinary drugs, LC-MS/MS instrument analysis time can increase the cost of analysis and delay the reporting of results.

PaperSpray-MS is a rapid, low-cost technique for screening and quantifying analytes in dried matrix spots such as a biological or food matrix. Little to no sample preparation is required; sample-to-sample run times are 2 min or less; and small quantities of solvents are used. Figure 1 shows the PaperSpray-MS workflow from dried sample spot to mass spectrometer. First, solvent is applied directly onto the dried sample spot to rewet it and extract analytes. Next, a spray solvent is dispensed onto the paper. Finally, a high voltage is applied to the paper to facilitate spray and ion formation. The new Thermo Scientific™ VeriSpray™ PaperSpray ion source system uses PaperSpray technology to make food safety workflows faster and more efficient by combining ease-of-use and increased automation with the speed that PaperSpray technology provides. The VeriSpray system consists of the VeriSpray ion source and the VeriSpray plate loader (Figure 2a). The VeriSpray plate loader holds up to 10 VeriSpray sample plates (Figure 2b). Each VeriSpray sample plate contains 24 single-use paper strips (12 on each side, A and B, Figure 2c). The magazine can be run in a fully automated fashion.

By using PaperSpray technology, veterinary drugs can be rapidly screened directly from meat extracts. There are multiple classes of veterinary drugs; in this work we analyze two classes: veterinary dyes in salmon extract and sulfonamides in bovine muscle extract.

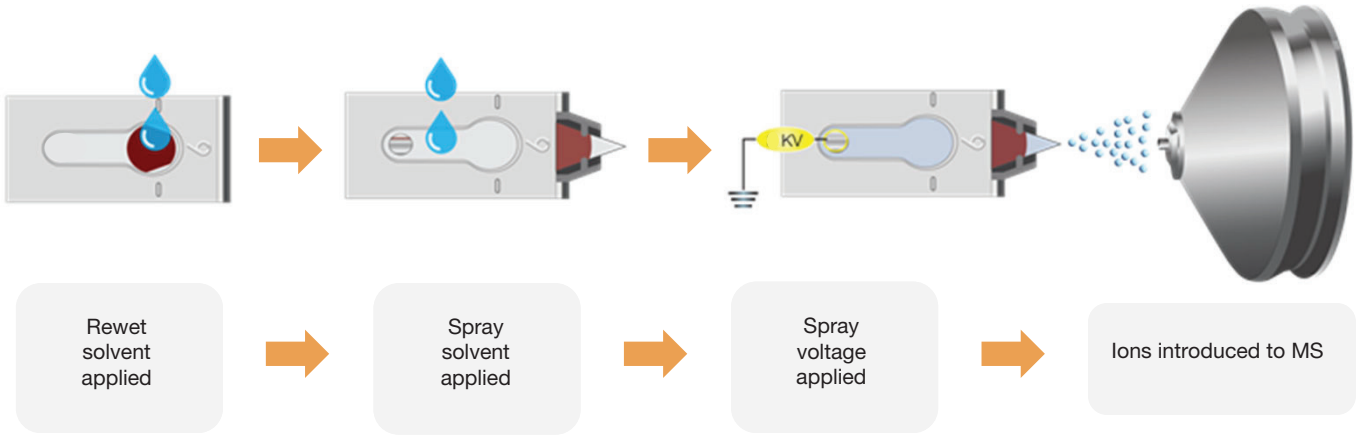

Figure 1. PaperSpray-MS workflow from dried sample spot to mass spectrometer

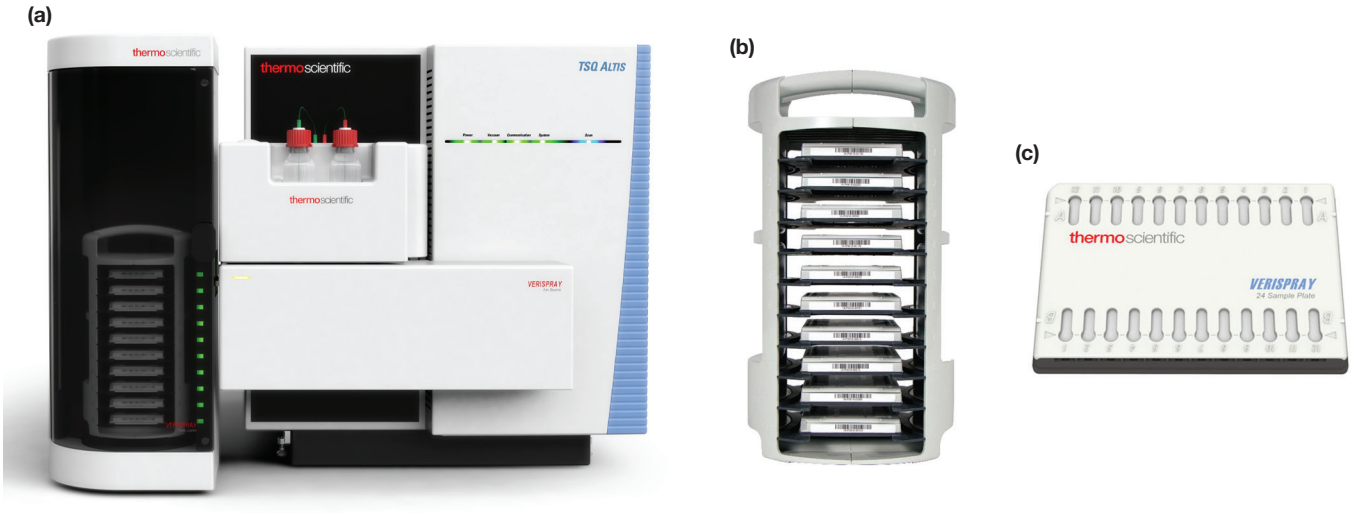

Figure 2. (a) VeriSpray ion source and plate loader, (b) magazine, and (c) VeriSpray sample plate

Experimental

Reagents and supplies

- Buffer: 0.2 M ammonium oxalate monohydrate/0.1 M disodium EDTA dihydrate
- 5 g Sodium sulfate, Slim Line Pouch, 50 pk (P/N 60105-368-SP)
- 500 mg CEC18, Slim Line Pouch, 50 pk (P/N 60105-367-SP)
- Corning™ Falcon™ tubes (50 mL), 50 pk (P/N 60106-425)
- 0.45 µm PTFE filters, 17 mm, 100 pk (P/N F2513-3)
- 10 mL Luer-lock syringe, 100 pk (P/N S7515-10)
- Water, Optima™ LC/MS grade (P/N W6-1)
- Acetonitrile, Optima™ LC/MS grade (P/N A955-1)
- Acetic acid, Optima™ LC/MS grade (P/N A1131AMP)
- Veterinary drugs and dyes available from Ultra Scientific, North Kingstown, RI
- Internal standards available from Sigma-Aldrich, St. Louis, MO

Sample preparation

Salmon extract and bovine muscle extract were prepared using a modified Quick Easy Cheap Effective Rugged and Safe (QuEChERS) preparation protocol that was optimized to be easy for laboratories to implement.[5] This QuEChERS extraction protocol reduced matrix co-extractives, which resulted in enhanced sensitivity. Salmon extracts were prepared from both fresh and frozen fillets, and the steps of the extract preparation are described in Figure 3. The PaperSpray results were identical for both matrices.

- Twelve veterinary dyes—brilliant green, crystal violet, ethyl violet, leucocrystal violet, leucomalachite green, malachite green, methylene blue, new methylene blue, nile blue A, rhodamine-6G, victoria blue B, and victoria blue BO—were spiked into salmon extract at calibration levels ranging from 0.2 to 100 ppb.
- Three internal standards—crystal violet-D6, malachite green-D5, and leucomalachite green-D5—were also spiked into the salmon matrix at 50 ppb.
- Fourteen sulfonamides in two sets—set 1: sulfadoxine, sulfamerazine, sulfamoxole, sulfamonomethoxine, sulfaguanidine, sulfachlorpyridazine, sulfapyridine, sulfadiazine, sulfaquinoxaline, and sulfamethizole and set 2: sulfadimethoxine, sulfisoxazole, sulfamethazine, sulfamethoxypyridazine, and sulfamethazine—were spiked into bovine muscle extract at calibration levels ranging from 5 to 500 ppb.
- One internal standard—sulfathiazole-D4—was also spiked into the bovine muscle matrix at 80 ppb.

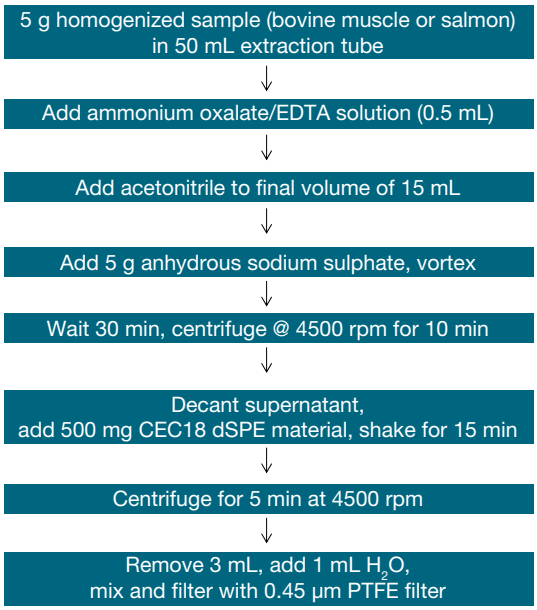

Figure 3. QuEChERS extraction procedure for bovine muscle and salmon

Chemical structures of the veterinary dyes and sulfonamides analyzed are shown in Figure 4. Five microliters of each salmon extract sample and eight microliters of each bovine muscle extract sample were spotted onto VeriSpray sample plates.

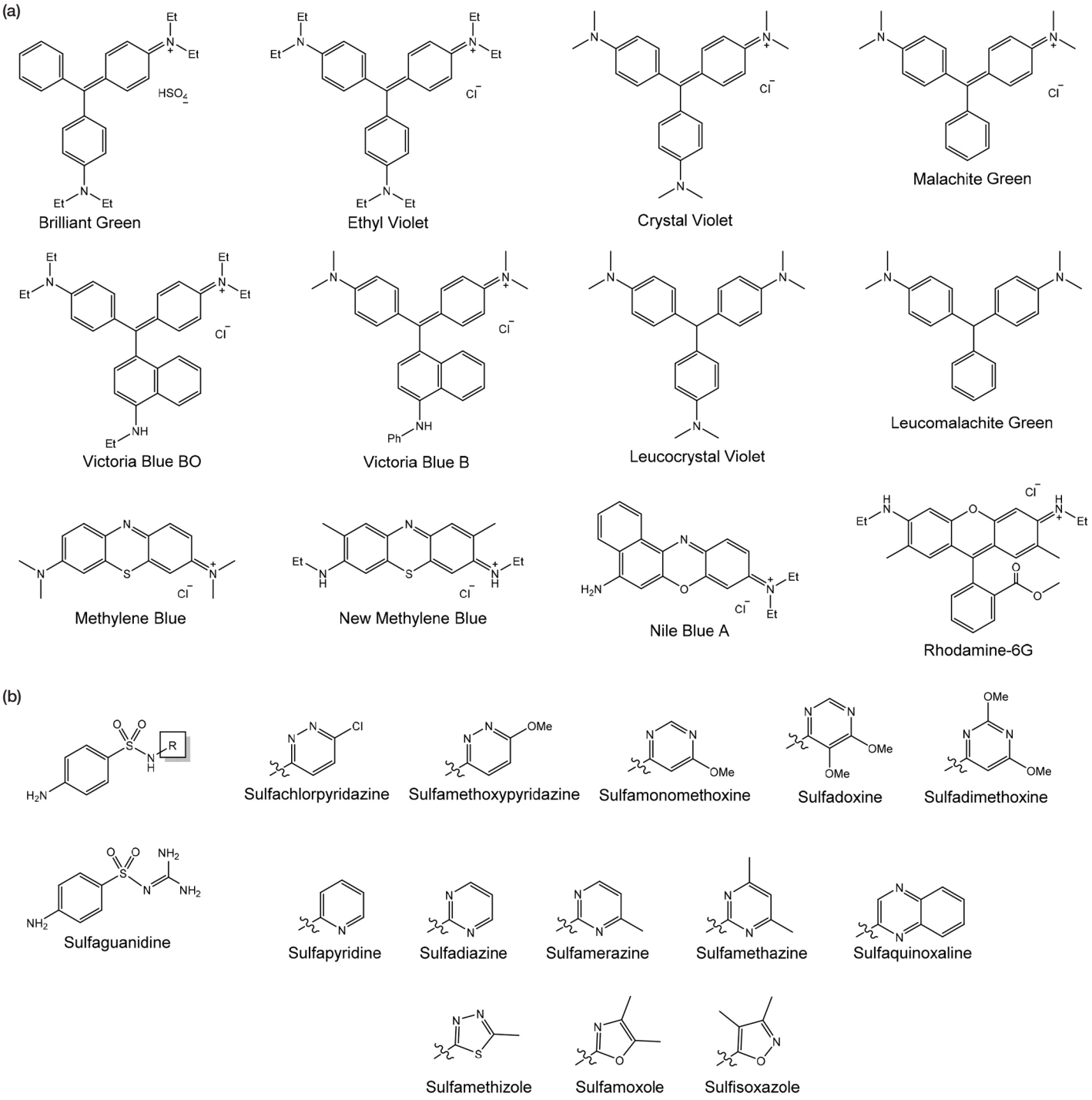

Figure 4. Chemical structures of (a) veterinary dyes and (b) sulfonamides

PaperSpray analysis

Sample plates were loaded into the VeriSpray plate loader. Thermo Scientific™ Xcalibur™ software was used to run three replicates for each of the calibration levels and the matrix blank in an automated fashion. A mixture of 90% acetonitrile, 10% water, and 0.1% acetic acid was used as the rewet and spray solvent. One 10 µL dispense was used for the rewet solvent step to extract the dried sample spot (Figure 1, first panel). Then the VeriSpray ion source pushed the paper strip forwards to the spray position (Figure 1, second panel). Eleven 10 µL dispenses, with a delay time of 1 s between the first five dispenses and a delay time of 5 s between the remaining six dispenses, were applied behind the dried sample spot for the spray solvent step. Delays were implemented to ensure that the solvent was well absorbed by the paper. This volume of spray solvent provides a steady spray for at least 1 min; no solvent is added during data acquisition.

Following solvent application, data were acquired using a 1 min method with a Thermo Scientific™ TSQ Altis™ triple quadrupole mass spectrometer coupled to the VeriSpray ion source. The spray voltage was applied to the metal rivet at the back to the paper strip to generate ions and a steady electrospray (Figure 1, third panel). The voltage, which was set at 4000 V, was turned on at 0.1 min and turned off at 0.9 min. The distinct on- and off-time for the voltage produces a square-shaped chronogram that is typical of PaperSpray.

Optimized transitions were monitored for compounds (Table 1) at a collision gas pressure of 2 mTorr. The ion transfer tube temperature was set to 350 °C for salmon extract samples and 400 °C for bovine muscle extract samples. The paper tip was held 4.5 mm away from the ion transfer tube. A summary of TSQ Altis MS settings is given in Table 2. Using Thermo Scientific™ TraceFinder™ software, data were analyzed, and the square-shaped chronograms were integrated to determine the area-under-the-curve (AUC).

Table 1a. Optimized SRM transitions for (a) veterinary dyes and (b) veterinary drugs. Target quantitation ion is bolded; other ions are confirming. For veterinary dyes, the internal standard is specified for quantitation of each analyte; for veterinary drugs only one internal standard was added.

Compound	Precursor (*m/z*)	Product (*m/z*)	Collision energy (V)	RF lens (V)	Internal standard
Methylene blue	284.1	252.0	53.45	75	Crystal Violet-d6
	284.1	**268.1**	**36.01**	**75**	
New methylene blue	312.2	254.1	39.15	99	Crystal Violet-d6
	312.2	268.1	35.13	99	
	312.2	**283.1**	**30.17**	**99**	
Nile blue A	318.2	245.0	54.55	102	Crystal Violet-d6
	318.2	246.1	52.73	102	
	318.2	**274.1**	**35.86**	**102**	
Malachite green	329.2	208.1	34.22	112	Malachite Green-d5
	329.2	241.1	52.91	112	
	329.2	**313.2**	**36.99**	**112**	
Leucomalachite green	331.2	223.1	53.67	85	Leucomalachite Green-d5
	331.2	**239.2**	**31.65**	**85**	
	331.2	316.2	21.37	85	
Crystal violet	372.2	235.1	54.73	115	Crystal Violet-d6
	372.2	340.2	54.51	115	
	372.2	**356.1**	**39.99**	**115**	
Leucocrystal violet	374.3	238.1	27.89	91	Leucomalachite Green-d5
	374.3	358.2	31.04	91	
	374.3	**359.2**	**22.81**	**91**	
Brilliant green	385.3	297.2	53.52	125	Crystal Violet-d6
	385.3	**341.2**	**39**	**125**	
Rhodamine 6G	443.2	341.2	48.97	119	Crystal Violet-d6
	443.2	386.2	42.49	119	
	443.2	**415.2**	**33.69**	**119**	
Ethyl violet	456.3	368.2	55	132	Crystal Violet-d6
	456.3	382.2	55	132	
	456.3	**412.2**	**43.89**	**132**	
Victoria blue B	470.3	333.1	52.08	183	Crystal Violet-d6
	470.3	349.2	37.64	183	
	470.3	454.2	44.39	183	
Victoria blue BO	478.3	390.2	55	175	Crystal Violet-d6
	478.3	**434.3**	**49.65**	**175**	
Malachite green-d5	**334.2**	**213.1**	**42**	**112**	N/A
Leucomalachite green-d5	**336.3**	**239.1**	**32**	**85**	N/A
Crystal violet-d6	**378.3**	**362.2**	**40**	**115**	N/A

Table 1b. Optimized SRM transitions for veterinary drugs. Target quantitation ion is bolded; other ions are confirming. For veterinary dyes, the internal standard is specified for quantitation of each analyte; for veterinary drugs only one internal standard was added.

Compound	Precursor (*m/z*)	Product (*m/z*)	Collision energy (V)	RF lens (V)
Sulfaguanidine	**215.06**	**92.071**	**24.71**	**48**
	215.06	108	22.28	48
	215.06	155.97	14.55	48
Sulfapyridine	250.064	92.071	26.38	62
	250.064	**155.97**	**16.07**	**62**
Sulfadiazine	251.06	92.071	25.77	56
	251.06	**155.97**	**15.31**	**56**
Sulfamerazine	265.075	108	25.28	65
	265.075	**155.97**	**17.28**	**65**
	265.075	172	16.41	65
Sulfamoxole	268.075	92.071	27.7	60
	268.075	**156.042**	**16.07**	**60**
Sulfamonomethoxine	281.07	92.071	28.58	66
	281.07	108.042	26.42	66
	281.07	**155.97**	**17.81**	**66**
Sulfachlorpyridazine	285.021	92.071	27.1	57
	285.021	**155.97**	**15**	**57**
Sulfaquinoxaline	301.075	92.071	28.96	62
	301.075	108.042	25.54	62
	301.075	**155.97**	**16.56**	**62**
Sulfadoxine	311.081	108.042	25.62	72
	311.081	140	26.68	72
	311.081	**156.042**	**18.07**	**72**
Sulfamethizole	**271.032**	**92.071**	**26.15**	**55**
	271.032	108.000	26.15	55
	271.032	155.97	26.15	55
Sulfamethazine	279.091	108.042	27.63	73
	279.091	124	25.05	73
	279.091	**186.042**	**17.39**	**73**
Sulfaisoxazole	268.125	252.137	35.08	249
	268.125	**224.417**	**35.2**	**249**
	268.125	196.196	53.48	249
Sulfamethoxypyridazine	281.07	92.071	28.73	65
	281.07	108	25.62	65
	281.07	**155.97**	**17.17**	**65**
Sulfadimethoxine	311.081	218	18.53	79
	311.081	**245.083**	**19.02**	**79**
Sulfathiazole-d4	**260.087**	**160.042**	**15.49**	**64**

Table 2. TSQ Altis MS parameters for the analysis of veterinary drugs and dyes and time-dependent spray voltage settings

TSQ Altis MS parameters	
Ion source parameter	**Value**
Spray voltage	Time-dependent
Positive ion	4000 V
Sweep gas	0 Arb
Ion transfer tube temperature	350/400 °C
Q1 resolution	0.7
Q3 resolution	1.2
CID gas	2 mTorr
Time-dependent spray voltage settings	
Time (min)	Voltage (V)
0	0
0.1	4000 V
0.9	0

Results and discussion

The AUC was integrated for each of the calibration standards, and calibration curves were constructed for the 12 vet veterinary and 14 sulfonamides in their respective meat extract matrices. Example calibration curves for two veterinary dyes and two sulfonamides are given in Figures 5 and 6, respectively, and example ion chronograms of the matrix blank and LOQ for those four analytes are shown in Figures 7 and 8.

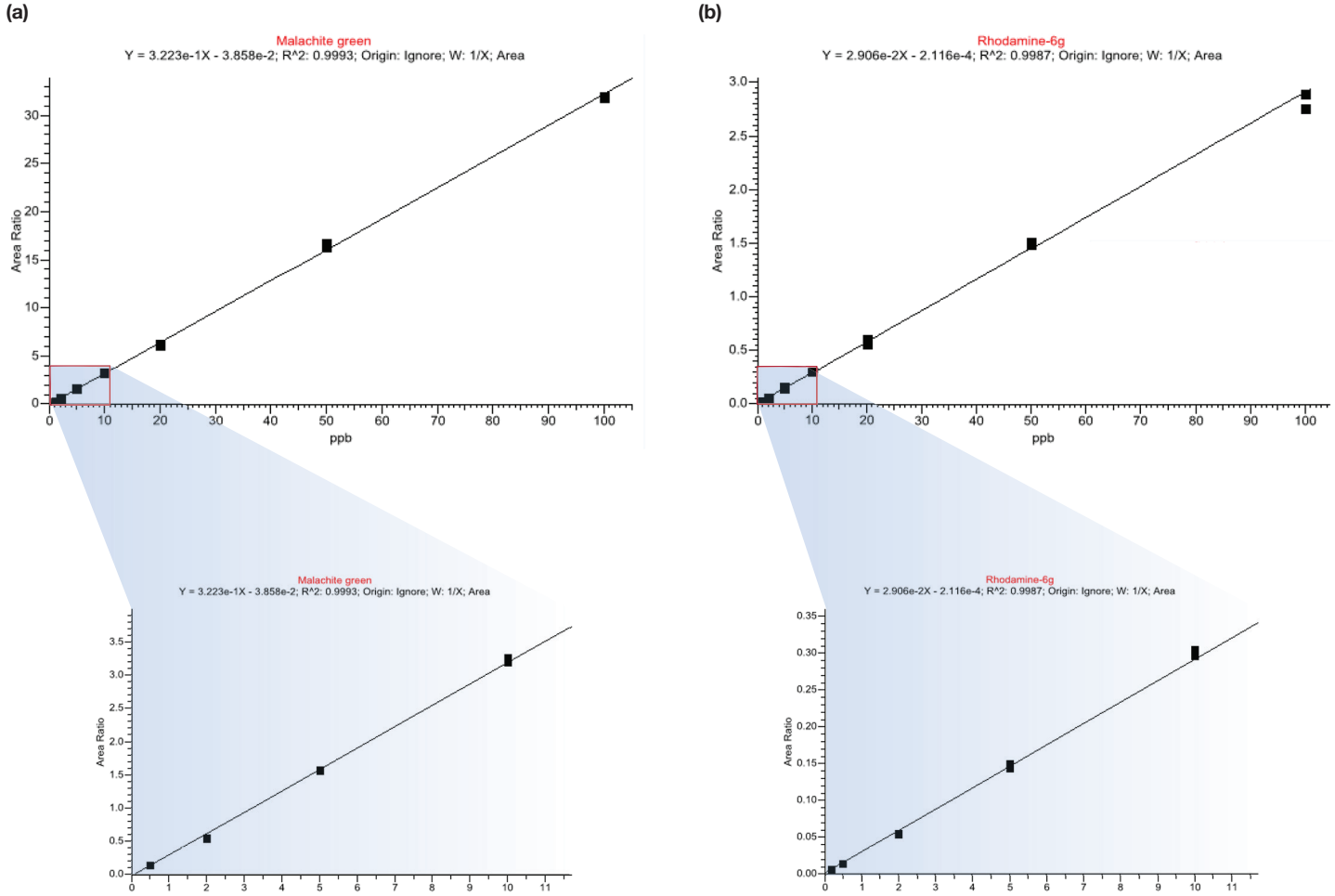

Figure 5. Example veterinary dye calibration curves of (a) malachite green from 0.5 to 100 ppb; inset: calibration levels from 0.5 to 10 ppb and (b) rhodamine-6G from 0.2 to 100 ppb; inset: calibration levels from 0.2 to 10 ppb. Three replicates were run for each calibration level.

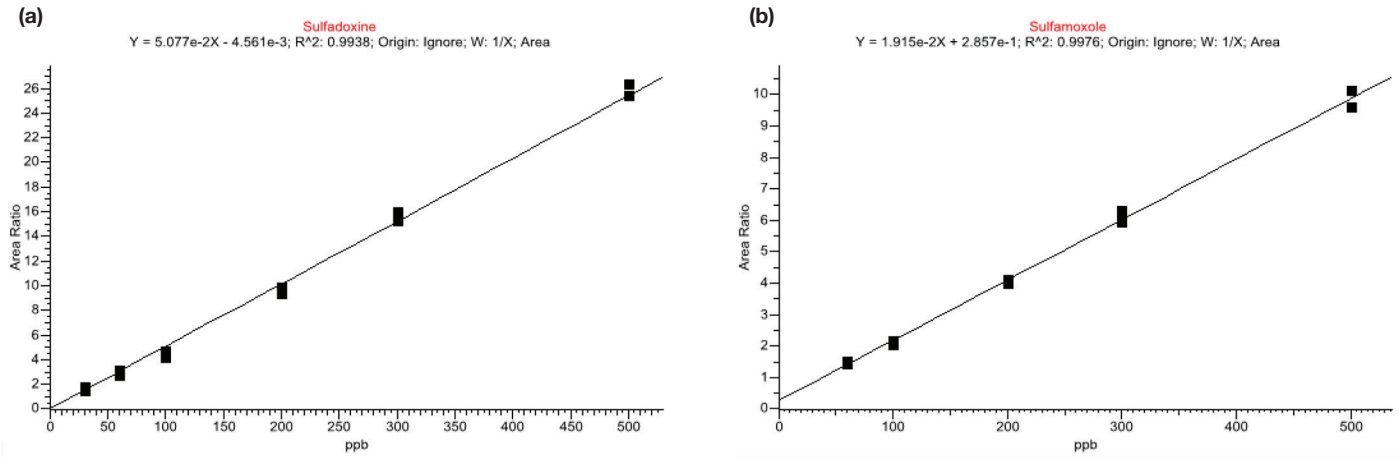

Figure 6. Example veterinary drug calibration curves of (a) sulfadoxine from 30 to 500 ppb and (b) sulfamoxole from 60 to 500 ppb. Three replicates were run for each calibration level.

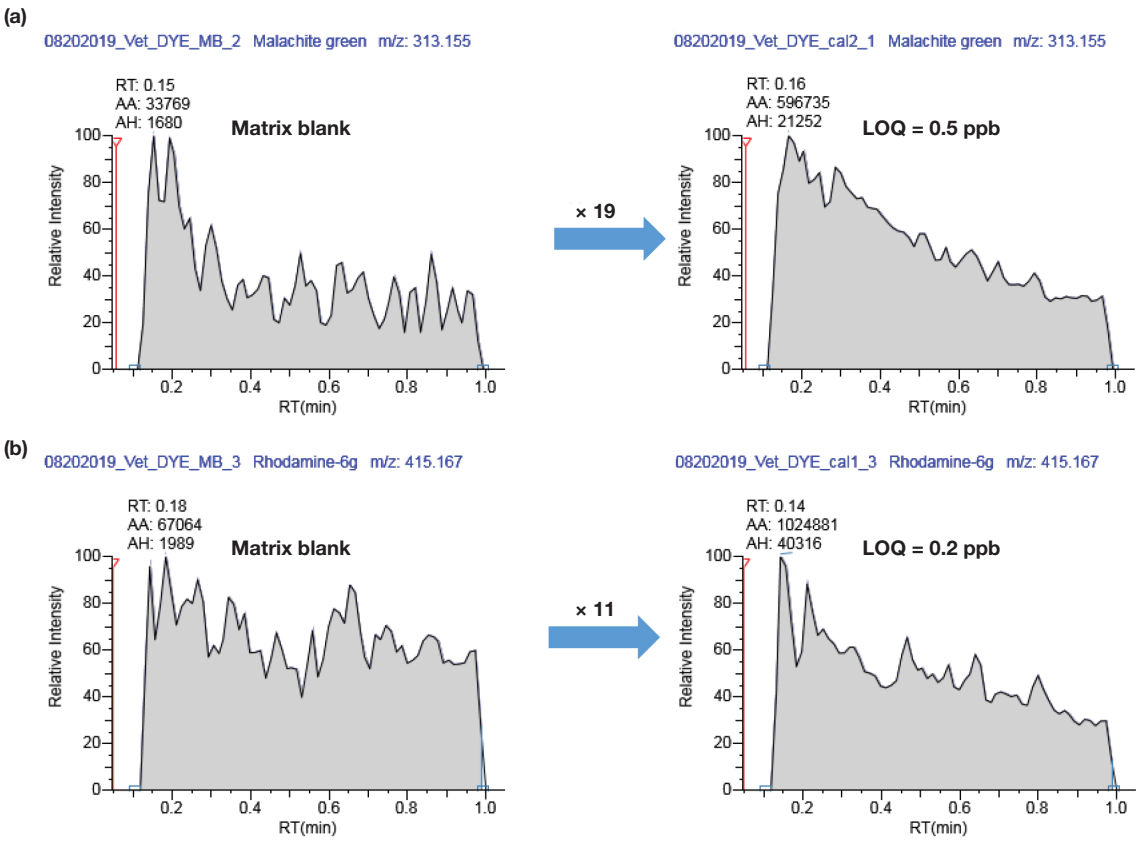

Figure 7. Example ion chronogram for the matrix blank and LOQ and their increase in signal-to-noise for (a) malachite green and (b) rhodamine 6G

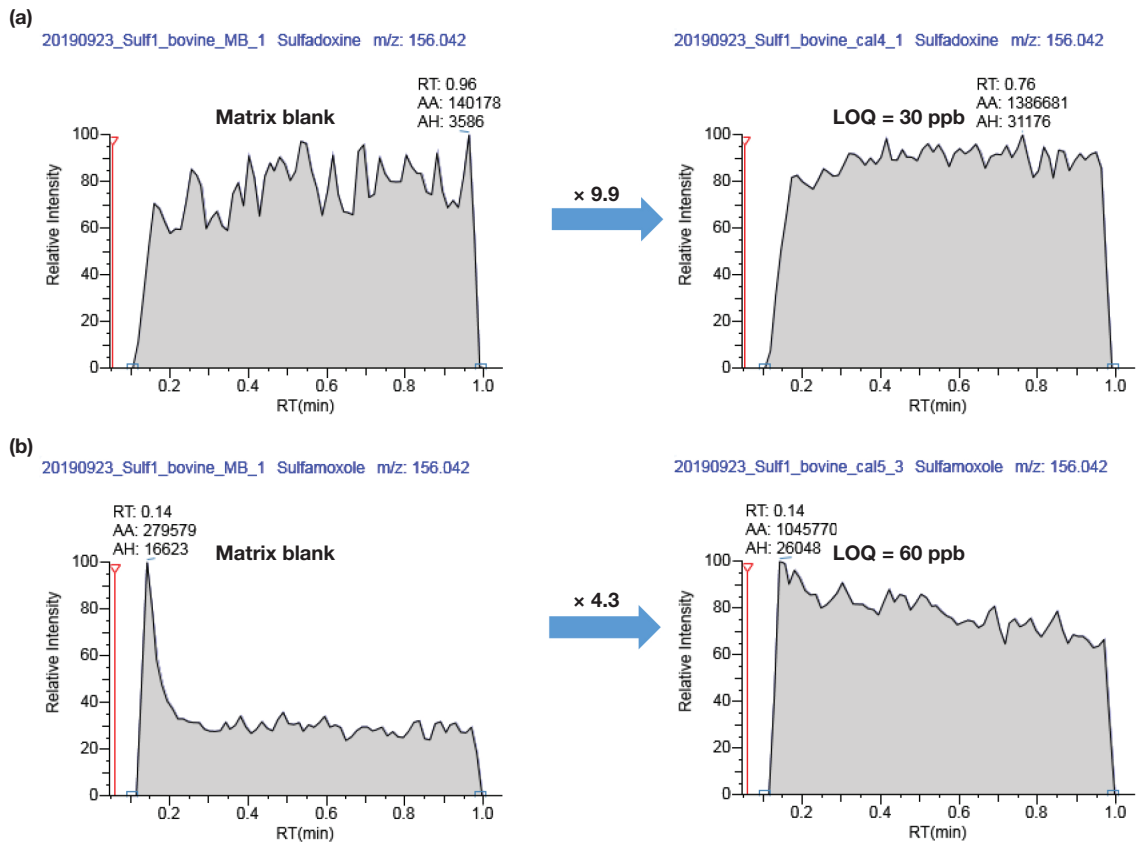

Figure 8. Example ion chronogram for the matrix blank and LOQ and their increase in signal-to-noise for (a) sulfadoxine and (b) sulfamoxole

Results were obtained rapidly, with sample-to-sample analysis times of 2 min or less. All curves had excellent linearity ($R^2 > 0.98$) over the measured concentration range. LOQs were based on the following criteria based on guidelines in reference 1: precision (%RSD) and accuracy (%Diff) at the LOQ must be ≤15% and within ±20%, respectively, and the S/N at the LOQ must be ≥4. Additionally, the LOQ and all concentrations above it had passing ion ratios, defined as within the tolerance of the average ion ratio calculated from all samples in the range of quantitation (tolerances for average ion ratios in Table 3)[1]. The ion ratios for each sample were measured as the proportion of the AUC for the target ion compared to the AUC for the confirming ions. The LOQs of the veterinary dyes ranged from 0.2 to 2 ppb, and the LOQs of the veterinary drugs ranged from 20 to 100 ppb (Table 4). Higher LOQs for sulfonamides are due to signal suppression from the bovine muscle matrix and failing ion ratios at low concentrations from the background signal of the paper. This PaperSpray method is semi-quantitative, and the LOQ for each compound can be taken as the screening target level. Follow-up quantitation by LC-MS/MS can be performed for samples that exceed a laboratory's threshold.

Table 3. Tolerances for average ion ratios. The average ion ratio is calculated from all samples in the range of quantitation.

Avg. ion ratio	Tolerance (±)
≤10%	50%
10–20%	30%
20–50%	25%
>50%	20%

Table 4. LOQs (ppb) and their linearity (R^2), precision (%RSD), accuracy (avg. %Diff), and signal-to-noise for (left) veterinary dyes in salmon extract matrix and (right) sulfa-based veterinary drugs in bovine muscle extract matrix. LOQ was calculated with three replicates for each calibration level.

Compound	LOQ in matrix (ppb)	R^2	%RSD	Avg. %Diff	S/N
Brilliant green	0.2	0.9954	3.36	15.3	42
Crystal violet	0.2	0.9989	4.80	3.7	9.0
Ethyl violet	0.2	0.9975	3.98	11.7	6.4
Leucocrystal violet	2	0.9941	4.86	8.1	5.1
Leucomalachite green	2	0.9996	0.69	1.0	11
Malachite green	2	0.9993	1.44	-9.7	76
Methylene blue	0.5	0.9922	1.00	6.7	5.6
New methylene blue	2	0.9904	3.51	-6.3	7.2
Nile blue A	2	0.9906	14.7	-10.3	12
Rhodamine 6G	0.2	0.9987	6.92	5.5	11
Victoria blue B	0.5	0.9966	4.83	15.7	6.7
Victoria blue BO	0.2	0.9984	4.47	18.0	46

Compound	LOQ in matrix (ppb)	R^2	%RSD	Avg. %Diff	S/N
Sulfadimethoxine	30	0.9901	2.96	3.8	4.1
Sulfisoxazole	20	0.9849	1.70	12.5	13.2
Sulfamethoxy-pyridazine	60	0.9854	5.47	0.6	10.4
Sulfamethazine	20	0.9878	2.29	-0.1	16.2
Sulfadoxine	30	0.9938	9.41	12.2	9.9
Sulfamerazine	30	0.9976	3.74	8.2	6.1
Sulfamoxole	60	0.9976	3.02	3.9	4.3
Sulfamonomethoxine	60	0.9946	4.17	6.8	6.3
Sulfaguanidine	100	0.9933	6.37	1.1	6.5
Sulfachlorpyridazine	60	0.9946	4.83	7.9	4.4
Sulfapyridine	30	0.9902	10.9	15.9	6.3
Sulfadiazine	100	0.9977	3.05	0.8	13.5
Sulfaquinoxaline	60	0.9927	7.33	5.3	4.4
Sulfamethizole	100	0.9959	3.73	-1.9	5.1

thermo scientific

Conclusion

Using PaperSpray technology, 12 veterinary dyes in salmon matrix extract and 14 sulfonamides in bovine muscle extract were quantified with excellent results. The linearity, accuracy, and precision meet or exceed standard analytical method requirements. The analysis time using PaperSpray-MS is 10 times faster than standard LC-MS. This method with the VeriSpray ion source allows labs to rapidly screen or semi-quantitate many samples and is valuable for high throughput food safety labs.

References

1. 2002/657/EC: European Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results.
2. CFR - Code of Federal Regulations Title 21, Food and Drugs, Chapter I—Food and Drug Administration Department of Health and Human Services Subchapter E – Animal Drugs, Feeds, and Related Products.
3. Geis-Asteggiante, L.; Lehotay, S.J.; Lightfield, A.R.; Dutko, T.; Ng, C.; Bluhm, L., Ruggedness testing and validation of a practical analytical method for >100 veterinary drug residues in bovine muscle by ultrahigh performance liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A* **2012,** *1258,* 43–54.
4. Schneider, M.J.; Lehotay, S.J.; Lightfield, A R., Validation of a streamlined multiclass, multiresidue method for determination of veterinary drug residues in bovine muscle by liquid chromatography–tandem mass spectrometry. *Analytical and Bioanalytical Chemistry* **2015,** *407* (15), 4423–4435.
5. Stubbings, G.; Bigwood, T. The development and validation of a multiclass liquid chromatography tandem mass spectrometry (LC–MS/MS) procedure for the determination of veterinary drug residues in animal tissue using a QuEChERS (QUick, Easy, CHeap, Effective, Rugged and Safe) approach. *Analytica Chimica Acta* **2009,** *637,* 68–78.

Find out more at **thermofisher.com/VeriSpray**
thermofisher.com/Altis

TN65666-EN 0420S

ThermoFisher SCIENTIFIC