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Rapid Quantitation of Veterinary Dyes in Salmon Extracts Using PaperSpray Coupled with a TSQ Altis MS

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ABSTRACT

Purpose: To develop a method to screen common antibacterial veterinary dyes used in fish farming using a Thermo Scientific TSQ mass spectrometer coupled to the Thermo Scientific™ VeriSpray™ PaperSpray Ion Source and Plate Loader.

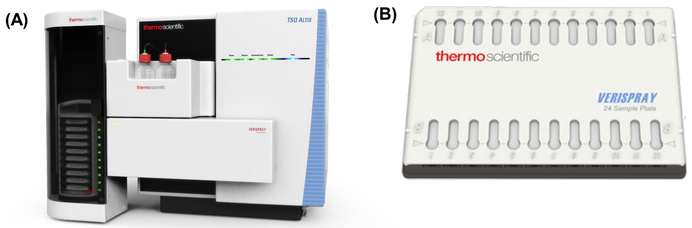
Methods: Salmon extracts were prepared from both fresh and frozen salmon using a modified Quick Easy Cheap Effective Rugged and Safe (QuEChERS) preparation protocol. Twelve vet dyes were spiked into salmon extract at calibration levels ranging from 0.2-100 ppb along with three internal standards at 50 ppb each. The spiked salmon extract was deposited onto VeriSpray sample plates, dried, and then analyzed using a Thermo Scientific VeriSpray PaperSpray ion source coupled to a Thermo Scientific™ TSQ Altis™ MS.

Results: Data were obtained rapidly, with sample-to-sample analysis times of 2 minutes or less. The lower limits of quantification (LLOQ) for 12 vet dyes ranged from 0.2-2 ppb. The results were reproducible in both frozen salmon and fresh salmon. Little to no ion suppression was observed for samples in salmon extract when compared to samples in acetonitrile. PaperSpray is a sensitive, direct technique for the analysis of dyes in salmon matrix and is a valuable technology for high-throughput screening and quantitation of analytes for food safety.

INTRODUCTION

Veterinary drugs are frequently administered to production animals in order to ensure animal health and well-being throughout the lifetime of the animal. Global agencies provide regulatory information regarding acceptable residue levels of veterinary drugs in various animal tissue types available for human consumption. It is important to develop quick and efficient analytical methods to screen for vet drug residues in animal tissues that meet the quantitation limits of these regulatory agencies. 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. While LC/MS-MS is a sensitive technique, it requires extensive sample handling and preparation. Time-consuming extraction and instrument analysis time can increase the cost of analysis and delay the reporting of results.

Figure 1. (A) VeriSpray ion source and plate loader with loaded magazine mounted to TSQ Altis mass spectrometer (B) VeriSpray sample plate.



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 turnaround times are 2 minutes or less, and small quantities of solvents are used. The VeriSpray system consists of the VeriSpray ion source and the VeriSpray plate loader (Figure 1A). Each VeriSpray sample plate contains 24 paper strips (12 on each side, A and B, Figure 1B). The magazine with up to ten plates can run 240 samples in a fully automated fashion.

Using PaperSpray, 12 vet dyes are rapidly screened directly from salmon extract with minimal sample preparation.

MATERIALS AND METHODS

Sample Preparation

- A mix of 21 drugs in the benzodiazepine, opiate, cocaine, stimulant and sedative classes were spiked into human whole blood or methanol (final concentrations 5-400 ng/mL) with corresponding internal standards (final concentration 130 ng/mL).
- Samples were put on a blood shaker for 20-30 minutes.
- The precipitated blood sample was prepared from spiked blood: 100 uL of spiked blood was mixed with 300 uL precipitation solution (2:1 methanol: 0.2M $ZnSO_4$). Vortex and store in fridge for 10 mins. Centrifuge 10 min 12000 rpm. Transfer out 200 uL supernatant.
- For each condition (in methanol, in whole blood, in precipitated blood), five replicates of each calibrator level, a matrix blank, and a matrix blank with internal standard were spotted on VeriSpray sample plates (spotting volume = 8 µL.
- Sample plates were oven-dried at 45 °C for 5 mins and 25 mins for precipitated blood and whole blood, respectively. Samples in methanol dried in 5 mins at r.t..

PaperSpray Conditions

Rewetting (10 µL) and spraying (110 µL) solvents were both 90:10:0.1 acetonitrile: water: acetic acid. The paper tip to MS inlet distance was set to 4.5 mm.

Mass Spectrometer Conditions

The analysis of drugs of abuse was carried out on a Thermo Scientific™ TSQ Altis™ MS connected with the Thermo Scientific VeriSpray system. Table 1 and 2 show the MS source parameters and optimized MS transitions, respectively. Figure 2 shows the steps that the VeriSpray ion source performs to introduce sample to the MS.

MATERIALS AND METHODS CONT.

Table 1. (A) TSQ Altis MS source conditions for vet drug analysis (B) time dependent applied spray voltage setting.

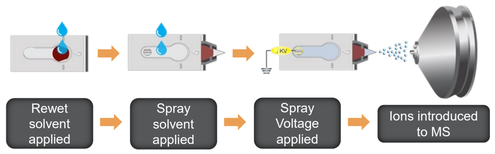
(A)

Ion Source Parameter	Value
Spray Voltage	Time Dependent
Ion Transfer Tube Temp	350 °C
Q1 Resolution	0.7
Q3 Resolution	1.2
CID Gas	1.5 mTorr

(B)

Time (min)	Voltage (V)
0	0
0.1	4000
0.95	0

Figure 2. PaperSpray-MS workflow from dried sample spot to mass spectrometer.



Data Analysis

Thermo Scientific™ TraceFinder™ 4.1 was used for processing the 1-minute chronograms and determining the area-under-the-curve (AUC).

Table 2. Optimized SRM transitions for vet dyes. Only target quantitation ion is shown; all analytes had 1-2 additional confirming ions. The internal standard is specified for quantitation of each analyte.

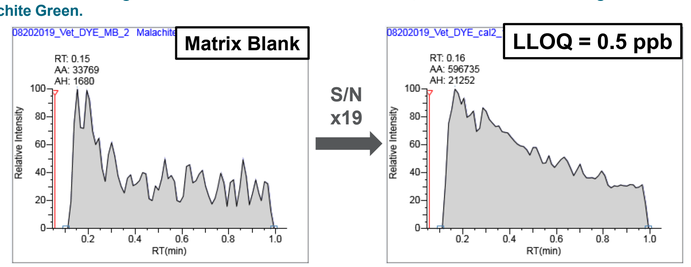
Compound	Precursor (m/z)	Product (m/z)	Collision Energy (V)	RF Lens (V)	Internal Standard
Methylene Blue	284.122	268.083	36.01	75	Crystal Violet-d6
New methylene blue	312.153	283.083	30.17	99	Crystal Violet-d6
Nile blue A	318.16	274.054	35.86	102	Crystal Violet-d6
Malachite green	329.201	313.155	36.99	112	Malachite Green-d5
Leucomalachite green	331.217	239.155	31.65	85	Leucomalachite Green-d5
Crystal violet	372.243	356.137	39.99	115	Crystal Violet-d6
Leucocrystal violet	374.259	359.238	22.81	91	Leucomalachite Green-d5
Brilliant Green	385.264	341.167	39	125	Crystal Violet-d6
Rhodamine-6g	443.233	415.167	33.69	119	Crystal Violet-d6
Ethyl violet	456.337	412.238	43.89	132	Crystal Violet-d6
Victoria blue b	470.259	349.17	37.64	183	Crystal Violet-d6
Victoria blue bo	478.322	434.259	49.65	175	Crystal Violet-d6
Malachite Green-d5	334.23	213.1	42	112	N/A
Leucomalachite Green-d5	336.25	239.1	32	85	N/A
Crystal Violet-d6	378.25	362.2	40	115	N/A

RESULTS

Results were obtained rapidly, with sample-to-sample analysis times of 2 minutes or less. Most of the vet drugs did not have a matched labeled internal standard; internal standards for each analyte were chosen to match shape of its chronogram. Chronograms comparing the matrix blank to the LLOQ for malachite green are shown in Figure 3.

The AUC was integrated for each of the calibration standards and calibration curves for each vet drug were generated for analyte in acetonitrile, frozen salmon extract, and fresh salmon extract. All curves had excellent linearity ($R^2 > 0.98$) over the measured concentration range. The lower limit of quantification (LLOQ) was set to the lowest calibration standard analyzed that yielded < 20% accuracy, < 15% RSD, and >4 S/N for 3 replicate samples. Additionally, the LLOQ and all concentrations above it had confirmation ions with good ion ratios.

Figure 3: Ion chronograms for the matrix blank and LLOQ and the increase in signal-to-noise for Malachite Green.



RESULTS CONT.

Calibration curves (3 sets overlaid) for 3 example analytes are shown in Figure 4.

The LLOQs of the vet dyes ranged from 0.2-2 ppb in salmon extract (Table 3). There was no difference in LLOQ between frozen and fresh salmon extracts. Furthermore, the LLOQ in acetonitrile was almost identical compared to the salmon extracts which shows that the QuEChERS protocol efficiently removes matrix co-extractives that can affect sensitivity.

Figure 4. Three overlaid calibration curves of (A) Leucomalachite Green, (B) Rhodamine-6G, and (C) New Methylene Blue in frozen salmon extract. Inset: zoom from 0-10 ppb.

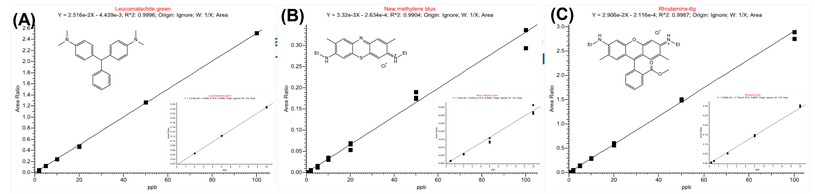


Table 3. LLOQs (ppb) for vet dyes in acetonitrile and salmon extract (fresh and frozen are identical). The linearity (R^2) of the calibration curve and precision (%RSD), accuracy (avg. %Diff), and signal to noise (S/N) at the LLOQ for vet dyes in frozen salmon extract.

Compound	LLOQ in MeCN (ppb)	LLOQ in Salmon (ppb)	R^2	%RSD	Avg. %Diff	S/N
Brilliant Green	0.2	0.2	0.9954	3.36	15.3	42
Crystal Violet	0.5	0.2	0.9989	4.80	3.7	9.0
Ethyl Violet	0.2	0.2	0.9975	3.98	11.7	6.4
Leucocrystal Violet	5	2	0.9941	4.86	8.1	5.1
Leucomalachite Green	5	2	0.9996	0.69	1.0	11
Malachite Green	2	2	0.9993	1.44	-9.7	76
Methylene Blue	0.2	0.5	0.9922	1.00	6.7	5.6
New Methylene Blue	2	2	0.9904	3.51	-6.3	7.2
Nile Blue A	2	2	0.9906	14.7	-10.3	12
Rhodamine-6G	0.5	0.2	0.9987	6.92	5.5	11
Victoria Blue B	0.5	0.5	0.9966	4.83	15.7	6.7
Victoria Blue BO	0.2	0.2	0.9984	4.47	18.0	46

CONCLUSIONS

- Using the VeriSpray ion source, 12 vet dyes in salmon matrix were quantified with excellent results and short turnaround times.
- The linearity of the calibration curves, and the accuracy and precision of the LLOQ meet or exceed standard analytical method. A matched labeled internal standard was not necessary for each analyte.
- The LLOQ of analyte in salmon extract derived from fresh and frozen salmon are the same. The LLOQ in acetonitrile compared to salmon extract was almost identical indicating that there is little to no suppression of signal due to the matrix.

REFERENCES

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3. George, E., Dang, V. Borts, D., Yang, C., and Beck, J. Multi-class Veterinary Drug Screening and Quantitation with a Comprehensive Workflow: The VetDrugs Explorer. Poster presented at ASMS 2019. https://assets.thermofisher.com/TFS-Assets/CMD/posters/po-65483-veterinary-drugs-workflow-asms2019-po65483-en.pdf

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