Analysis of Multiclass Veterinary Drugs in Baby Food by Ultra Fast Chromatography with High Performance Triple Quadrupole Mass Spectrometry

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Introduction

The quantification of different multi-class veterinary drug residues (albendazole, chlorotetracycline, danoflozacin, doxycycline, enrofloxacin, erythromycin, fenbendazole, ivermectin, oxfendazole, oxolinic acid, oxytetracycline, sarafloxacin, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaquinoxaline, tetracycline, thiabendazole, tilmicosin, trimethoprim, and tylosin) in baby food usually involves sample preparation with either solid phase extraction or liquid-liquid extraction, which requires substantial time in both sample preparation and analytical run time. A new method, utilizing ultra fast chromatography, a high performance triple quadrupole mass spectrometer and a quick analysis software is described in this poster. The advantages to this approach are that very little sample cleanup is necessary prior to injection and LC/MS run times are short.

Methods

Sample Preparation

A simple “dilute and shoot” method, adjusted from the original method described by Mol et al. (2008)[1], was used. Samples of baby food (milk and pork) were extracted the following way: 26.99g of pork was ground and diluted with 100mL of buffer (90%,10%, 2% Acetonitrile, Water, Formic Acid (v/v)). The sample was shaken vigorously and put into a sonication bath for two hours. The sonciation bath warmed up the sample, causing the meat particles to turn white. After sonication, the mixture was centrifuged for 10 minutes at 10,000rpm, and the supernatant was then pipetted into 50mL centrifuge tube. The supernatant was filtered through a 0.4µm nylon filter to remove any particles before being transferred to HPLC vials for injection. Milk preparation was extracted with same buffer as mentioned above and filtered through a 0.4µm filter before being transferred to HPLC vials for injection. A calibration solution was made by spiking the multiclass vet drugs into both the neat solution and the matrices mentioned above with a calibration curve range from 10ppm -0.5ppt depending on the compound starting solution. A portion of the matrices was tested for possible contamination of veterinary drugs.

Liquid Chromatography Conditions

Thermo Scientific™ Dionex™ UltiMate 3000 HPLC Stack: Pump: HPG 3400RS, Column Heater: TCC3000, Autosampler: OAS-3X00TXRS

Column: Thermo Scientific™ Accucore™ C18 column (50 x 2.1mm, 2.6µ)

Mobile phase: A: 0.1% Formic Acid in Water, B:0.1% Formic Acid in Methanol

Column Temperature: 45 ºC Injection volume: 5uL

HPLC Gradient:

Time	Flow Rate (ml/min)	%A	%B
0.0	0.6	100	0
2.0	0.6	100	0
2.1	0.6	60	40
9.0	0.6	35	65
9.5	0.6	0	100
12.0	0.6	0	100
12.1	0.6	100	0

Mass Spectrometry Conditions:

Thermo Scientific™ TSQ Quantiva™ MS

Spray Voltage: 3kV

Aux Gas: 10 Capillary Temperature: 350 ºC

Sheath Gas: 55 HESI III Temperature: 450 ºC

Sweep Gas: 2 Cycle Time: 0.5

CID Gas: 1.5 Q1, Q3 Resolution (FWHM): 0.7

Software: Thermo Scientific™ TraceFinder™ software

Data Analysis

To enable rapid data review and analysis, a new software was used. TraceFinder software has a new simplified interface for data review (Figure 1, Analysis View). The flagging of samples and compounds helps analysts quickly determine what is a positive hit or even why there is an issue with the sample or compound.

TABLE 1. Transitions that were used for this method. One transition was used for quantitation and one was used for confirmation.

Compound	Precursor *(m/z)*	Product *(m/z)*	Collision Energy (V)	RF-Lens (V)
albendazole	265.93	234.107	19	94
albendazole	265.93	159.162	38	94
chlorotetracycline	479.25	462.161	18	89
chlorotetracycline	479.25	444.085	22	89
danofloxacin	358.25	340.179	22	95
danofloxacin	358.25	82.238	43	95
danofloxacin	358.25	314.214	17	95
danofloxacin	358.25	283.236	22	95
doxycycline	445.25	428.158	18	91
doxycycline	445.25	341.005	19	91
doxycycline	445.25	267.18	39	91
enrofloxacin	360.25	316.214	19	97
enrofloxacin	360.25	245.189	26	97
erythromycin	734.6	576.427	18	101
erythromycin	734.6	158.145	30	101
fenbendazole	300	268.093	20	97
fenbendazole	300	159.169	36	97
fenbendazole	300	131.162	51	97
ivermectin	897.65	183.119	55	227
ivermectin	897.65	240.157	59	227
ivermectin	897.65	139.164	55	227
oxfendazole	315.95	159.129	37	98
oxfendazole	315.95	191.156	25	98
oxfendazole	315.95	284.012	18	98
oxolinic acid	261.9	244.071	20	78
oxolinic acid	261.9	160.09	43	78
oxytetracycline	461.2	426.218	21	84
oxytetracycline	461.2	443.713	19	84
oxytetracycline	461.2	201.139	45	84
sarafloxacin	386.2	342.149	22	99
sarafloxacin	386.2	299.187	30	99
sarafloxacin	386.2	368.147	26	99
sulfachloropyridazine	284.85	92.209	31	76
sulfachloropyridazine	284.85	156.156	17	76
sulfachloropyridazine	284.85	108.219	26	76
sulfadiazine	250.9	156.111	17	72
sulfadiazine	250.9	92.205	29	72
sulfadiazine	250.9	108.151	26	72
sulfamethazine	278.95	186.156	16	91
sulfamethazine	278.95	92.215	31	91
sulfamethazine	278.95	124.254	23	91
sulfaquinoxaline	301.3	92.204	34	91
sulfaquinoxaline	301.3	108.17	28	91
sulfaquinoxaline	301.3	156.082	18	91
tetracycline	445.25	410.163	21	86
tetracycline	445.25	427.353	11	86
tetracycline	445.25	428.05	18	86
thiabendazole	201.9	131.196	36	95
thiabendazole	201.9	65.169	50	95
thiabendazole	201.9	175.096	27	95
tilmicosin	869.7	174.244	45	215
tilmicosin	869.7	88.282	58	215
tilmicosin	869.7	132.215	47	215
tilmicosin	869.7	116.314	52	215
trimethoprim	291	230.183	23	104
trimethoprim	291	123.227	25	104
trimethoprim	291	110.186	34	104
tylosin	916.65	174.133	39	159
tylosin	916.65	101.163	45	159
tylosin	916.65	116.173	54	159

Results

Detection limits will vary depending on the compound and matrix. Two calibration curves were generated separately. Analysis of the two curves will show which compounds will perform better in which matrix due to sample prep and HPLC conditions. Figure 2 shows two calibration curves between the milk and pork matrices for one of the compounds being analyzed. Figures 3 also shows another compound in both matrices at the LOQ level. Analysis of the data, once completed, is the generation of the report. TraceFinder has the ability to generate customizable reporting “on the fly” after processing of the sample (Figure 4). The limit of detection and quantitation list shown in Table 3 which these compounds meet or beat the current MRLs.

FIGURE 1. Flags on both samples and compounds give the analyst quick information about issues with samples and with compounds.

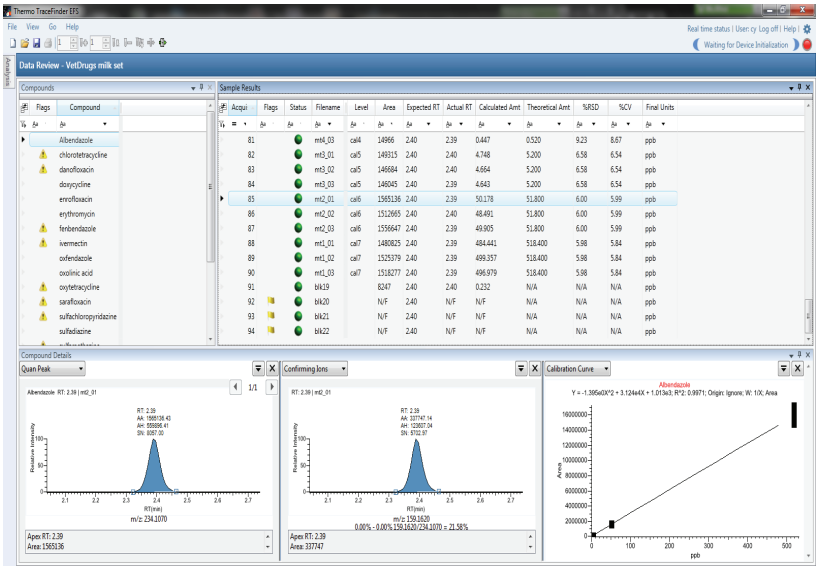

FIGURE 2. Albendazole compared in milk (left panel) and meat (right panel) matrix

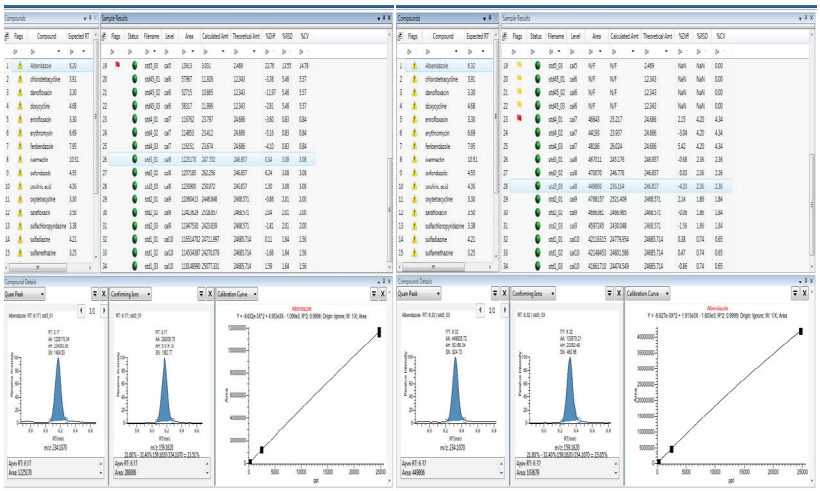

FIGURE 3. Trimethoprim in pork (top panel) and milk (lower panel) matrix.

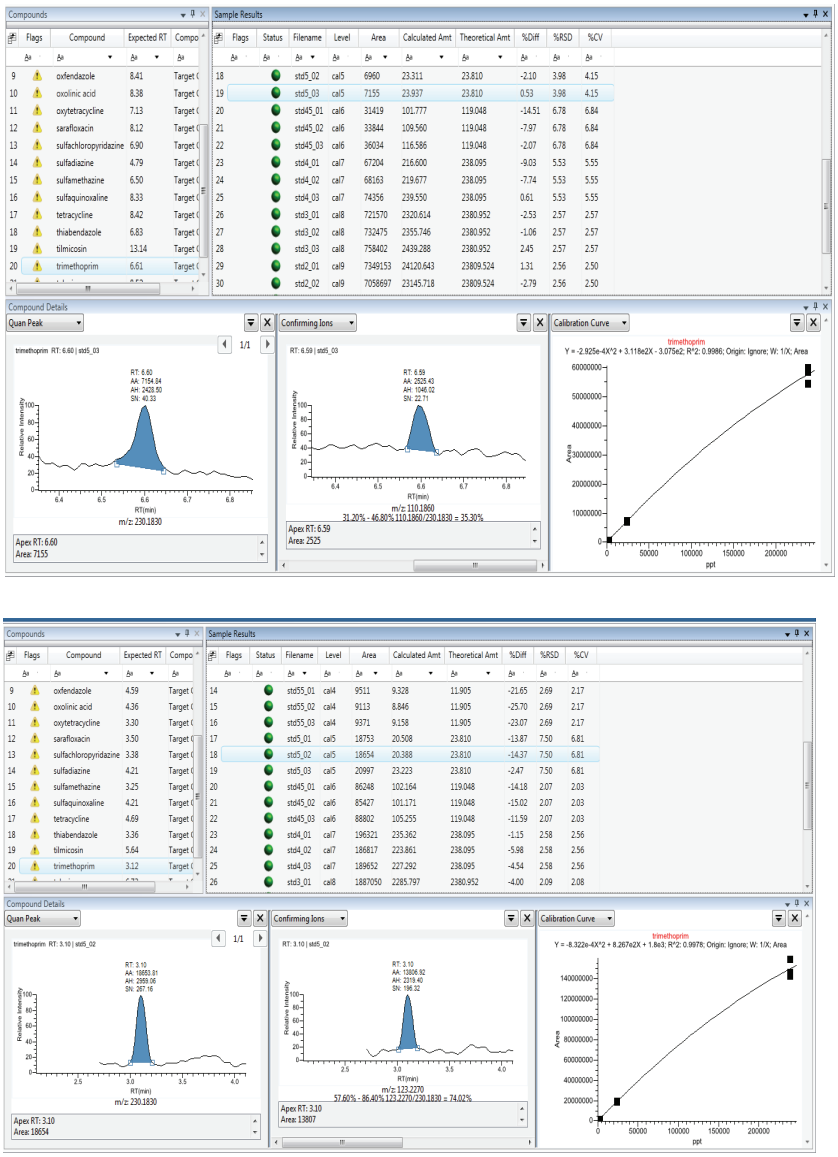

FIGURE 4. Customizable reporting in TraceFinder software helps analyst quickly make new reports "on the fly" immediately after processing.

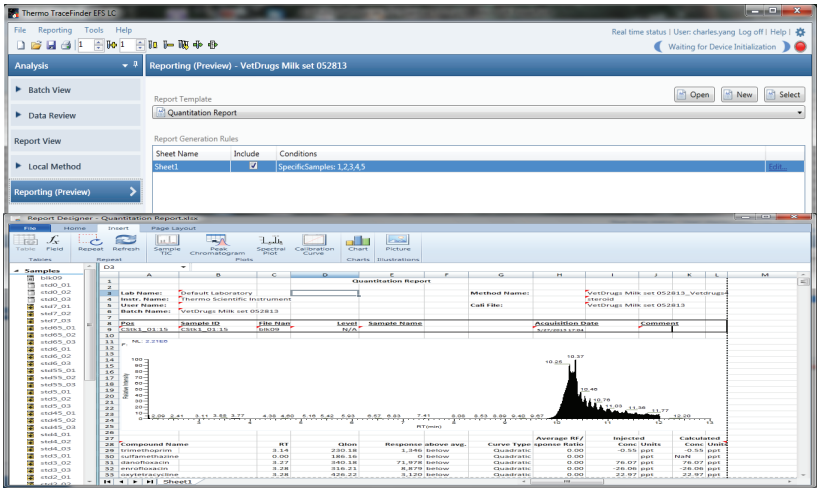

Table 3. LOD and LOQ for the compounds of interest (ppt).

Milk

Compound	Ave. Area	LOD (ppt)	%RSD	Ave. Area	LOQ (ppt)
Albendazole	36721.67	2.47	12.64	110662.67	12.34
oxfendazole	3076.67	2.44	10.86	22312.33	12.20
sulfadiazine	3637.67	23.81	17.5	18117.00	119.05
sulfamethazine	6365.00	12.62	10.62	14596.33	25.24
sulfaquinoxaline	5130.00	5.98	7.29	10404.00	11.97
thiabendazole	33981.67	24.19	3.48	80590.00	48.37
tilmicosin	3289.33	121.79	1.05	7373.33	243.57
trimethoprim	3735.00	11.91	20.91	7929.00	23.81
tylosin	4750.00	11.87	12.79	9069.33	23.74

Pork

Compound	Ave. Area	LOD (ppt)	%RSD	Ave. Area	LOQ (ppt)
Albendazole	33629.00	2.47	6.01	82256.33	12.34
chlorotetracycline	6972.00	147.62	10.07	10876.00	295.24
erythromycin	1819.33	24.05	7.23	41343.67	120.24
fenbendazole	156882.00	11.97	6.24	265338.67	23.95
oxfendazole	21823.00	12.20	3.12	41108.00	24.41
sarafloxacin	8448.33	119.64	3.21	14565.33	239.29
sulfadiazine	2465.00	23.81	5.43	15548.00	119.05
sulfamethazine	24809.33	25.24	5.41	96852.00	126.19
sulfaquinoxaline	9040.00	11.97	11.19	45163.00	59.82
thiabendazole	46751.33	24.19	15	85171.67	48.37
trimethoprim	3863.33	11.91	9.13	7221.00	23.81
tylosin	4113.00	11.87	3.71	7567.67	23.74

Conclusion

- The Limit of Detection and Limit of Quantitation determined in the exp show that with TSQ Quantiva MS we can achieve a lowered detection a small amount of sample injection.
- With the higher sensitivity of TSQ Quantiva MS, we can inject much le as proposed in this poster
- The new approach easily surpasses the current regulated MRLs.
- The method described here to analyze multiclass veterinary drugs sho
  - A simple extraction method has no issues with lower end detect
  - No need to inject larger volumes because of the sensitivity of th Quantiva MS
- No contamination of veterinary drugs was noticed in either of the matr for this experiment.
- The ability of TraceFinder software to give a user simplified views for a and reporting helps reduce the bottleneck in all routine and non-routin

References

1. Mol, H.G.J., Plaza-Bolanos, P., Zomer, P., de Rijk, T.C., Stolker, A.A.M Mulder, P.P.J. (2008). Toward a generic extraction method for simultar determination of pesticides, mycotoxins, plant toxins, and veterinary d feed and food matrixes. Analytical Chemistry, 80, 9450-9459.

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