Application Note

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Simultaneous screening of multiclass food-borne stimulant-related drug residues in beef using UHPLC-MS/MS

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Abstract

This Application Note describes a method for the simultaneous determination of four major classes of stimulant-related drug residues in beef by ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS). This method is based on a recently published work[1]. The beef sample was initially extracted using acidified acetonitrile, followed by filtration using a lipid filtration cartridge to remove lipid interference. The sample was then subjected to desiccation by magnesium sulfate and enrichment by nitrogen evaporation. The resultant residue was redissolved and centrifuged, followed by UHPLC-MS/MS analysis with external matrix-matched standards calibration for quantitation. The method showed excellent linearity, with linear regression coefficients of ≥0.995 for all compounds in the concentration range of 0.10–50 µg/L. The limits of detection and limits of quantitation were in the ranges of 0.00060–0.090 µg/kg and 0.0020–0.30 µg/kg, respectively. At the spiking levels of 0.40, 1.0, and 2.0 µg/kg, the recoveries for all compounds ranged 57.3–117 %, with most recovery values within 70–120 %, and all RSDs (n = 5) within 3.07–15.6 %. This method is simple, rapid, sensitive, and reliable, and can be applied to quantitatively screen the studied four classes of stimulant-related drug residues in beef.

Introduction

To promote muscle development or increased water retention in edible animals, forbidden or regulated stimulant substances may illegally be added to the animal feed. This is done to gain economic profit, and leads to stimulant drug residues in tissues of the edible animals. Athletes eating food contaminated with these substances may lead to anti-doping testing positive[2]. According to the regulation by the WADA organization, the Certification and Accreditation Administration of China (CNCA) regulated four classes of illegal stimulant-related substances including β-antagonists, steroids, glucocorticoid, and zeranol in Olympic food before the Beijing Olympic games in 2008[3].

These stimulant-related substances not only have an impact on athletes[2], but also have adverse health risks to the general public such as associating with hormone-dependent cancers[4], leading to acute toxicity[5], increasing risk on male infertility[6], and so forth. China MOA B-235 specifies that the maximum tolerance level for betamethasone and dexamethasone is 0.75 µg/kg in animal tissues. The residues for clenbuterol, salbutamol, testosterone-17-propionate, zeranol, and trenbolone cannot be detected in food of animal origin, and hydrocortisone can only be used externally, although there is no Maximum Residue Limit (MRL) specified[7]. The European Union set the MRL for prednisolone and clenbuterol residues in bovine tissue at 4 µg/kg and 0.1 µg/kg, respectively. The MRL for dexamethasone and betamethasone in bovine and porcine tissues is 0.75 µg/kg[8]. To meet the requirement for supervision, a highly sensitive and reliable method needs to be developed.

Some of the methods for the determination of these stimulant-related drug residues include ELISA, HPLC, GC/MS, and LC-MS/MS. The LC-MS/MS method has the following advantages:

- Simultaneous monitoring multiple classes of compounds with high selectivity and sensitivity
- Does not require any derivation
- Generates the least false positive results

The LC-MS/MS method has been widely adapted in veterinary drug residue analysis[1, 9-11]. Its reliability depends highly on the sample extraction and cleanup procedure. For the determination of stimulant-related drug residues, the currently available cleanup methods include the solid phase extraction (SPE) method and the QuEChERS method. The SPE method often involves two hyphenated columns to remove interferences effectively[9-10]. Unfortunately, this method has the disadvantage of being laborious, time-consuming, and uses a large amount of environmentally unfriendly solvents. The QuEChERS method has been used widely in residue analysis. This method requires attention to minimize the quantitation bias induced by the interference effects, particularly for some complicated matrices[10-11].

Alternatively, one commercialized lipid-removal filtration cartridge, which is filled with a specific chemical sorbent, has the capacity to selectively absorb the lipid, protein, and surfactants from plasma by combining with vacuum filtration. This method has been applied to determine the drug concentration in blood effectively[12]. To achieve a sensitive and reliable determination of food-borne stimulant-related drug residues in beef, this Application Note develops a method based on cleanup using the lipid-removal filtration cartridge followed by UHPLC-MS/MS analysis.

Experimental

Materials and reagents

Methanol and acetonitrile were LC/MS grade, and purchased from Merck. Formic acid, ammonium acetate, and acetic acid were all LC grade, ordered from Dima Technologies. Milli-Q water was used throughout the experiment. All other chemicals used in the experiment were analytical grade, and purchased from a local vendor. The Captiva nondrip lipid cartridge (p/n A5300635) and the QuEChERS extraction salt packages (p/n 5982-0032) were from Agilent Technologies. For comparison, the Agilent QuEChERS dispersing cleanup kit (p/n 5982-4956CH) was also used. The 18 chemical standards were purchased from Dr. Ehrenstorfer GmbH (Germany).These standards included:

- **β-Antagonists:** clenbuterol, penbutolol, ractopamine, salbutamol (albuterol), and terbutaline
- **Steroids:** 17-methyltestosterone, metandienone, progesterone, testosterone-17-propionate, and trenbolone
- **Corticosteroids:** beclomethasone, betamethasone, dexamethasone, fludrocortisone acetate, hydrocortisone, prednisolone, and prednisone
- Zeranol

Figure 1 presents the chemical structures of these compounds.

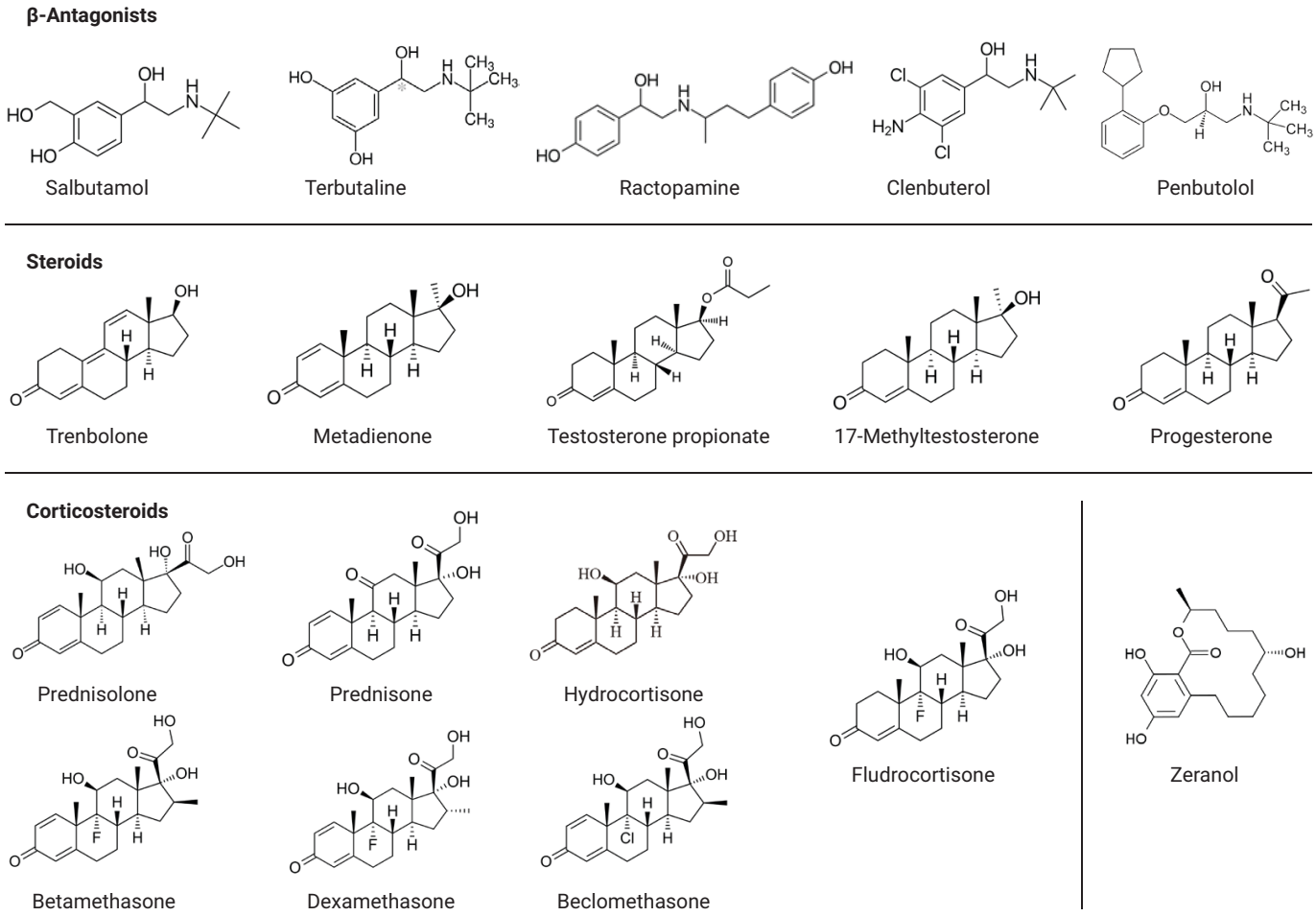


Figure 1. Chemical structures for the four classes of stimulant-related substances studied.

Standard solution preparations

A 10.0 mg amount of each chemical standard was accurately weighed out, dissolved in methanol, and transferred to each 10-mL volumetric flask. The volume of each compound was then brought up to 10 mL using methanol, resulting a standard stock solution at 1.0 g/L for each compound. Then, 100 µL of each stock standard solution for the 18 compounds were transferred into one 10-mL volumetric flask, and the resultant solution was mixed thoroughly through vortexing. Methanol was then added to the flask to bring the total volume to 10 mL, resulting in a standard mixture solution of 10 mg/L. This standard mixture solution was further diluted using a solution of methanol:water (v:v = 7:3) to generate a series of standard calibration solutions. The matrix blank residue obtained through the extraction and cleanup process was dissolved using the solution of methanol:water (v:v = 7:3). This solution then acted as a solvent for the preparation of matrix-matched calibration solutions.

Detailed LC/MS conditions

Table 1. Instrument conditions.

LC Conditions	
Instrument	Agilent 1290 Infinity II LC with built-in degasser
Autosampler	Agilent 1290 Infinity II autosampler with temperature control
Column compartment	Agilent 1290 Infinity II thermostatted column compartment
Column	Agilent ZORBAX Phenyl-Hexyl, 2.1 × 150 mm, 1.8 µm
Column temperature	40 °C
Mobile phase	A) 5.0 mM NH_4Ac/0.01 % HAc in H_2O B) Methanol:acetonitrile (v/v 7:3)
Flow rate	0.4 mL/min
Injection volume	3.0 µL
Post time	3.0 min
Gradient elution profile	0–3 minutes: 5 %B to 15 %B, 3–3.5 minutes: 15 %B to 40 %B, 3.5–13 minutes: maintain at 40 %B, 13–15 minutes: 40 %B to 55 %B, 15–18 minutes: 55 %B to 95 %B, 18–20 minutes: maintain at 95 %B
MS/MS Conditions	
Instrument	Agilent 6490 triple quadrupole LC/MS with Agilent Jet Stream Electrospray ionization source
Ionization mode	Positive/Negative
Drying gas temperature	200 °C (±)
Drying gas flow rate	14 L/min (±)
Nebulizer gas pressure	35 psi (±)
Sheath gas temperature	350 °C (±)
Sheath gas flow rate	11 L/min (±)
Capillary voltage	3,500 V (+)/3,000 V (–)
Nozzle voltage	500 V (+)/1,500 V (–)
Fragmentor voltage	380 V
High pressure RF	150 V (+)/90 V (–)
Low pressure RF	60 V (±)
Scanning mode	Multiple reaction monitoring

Sample extraction and cleanup

Beef sample was cut and homogenized, then (5.0 ±0.1) g was transferred to a 50-mL centrifuge tube. Ten milliliters of acidified acetonitrile (1.0 % acetic acid in acetonitrile) were then added to the centrifuge tube. The tube was immediately vortexed for 2 minutes. A salt package containing 4.0 g of sodium sulfate and 1.0 g of sodium chloride was then added to the tube. The tube was again immediately vortexed for 2 minutes, then centrifuged at 5,000 rpm for 5 minutes. The supernatant (2.25 mL) was transferred to a Captiva nondrip lipid cartridge (p/n A5300635) containing 0.75 mL of Milli-Q water. The solution in the filtration cartridge was then pipetted five times to ensure thorough mixing. The cartridge was further subjected to vacuum filtration until dryness, and the filtrate was collected into a collection tube (5-mL centrifuge tube). Magnesium sulfate (0.30 g) was added to the collection tube, and the tube was mixed immediately by vortexing, then centrifuged at 5,000 rpm for 5 minutes. Then, 1.0 mL of supernatant was transferred to a clean 5-mL centrifuge tube, and dried under nitrogen at 40 °C. The residue was then dissolved using 0.35 mL methanol, followed with dilution by adding 0.15 mL of water, making the final injection solvent as methanol:water (v:v) = 7:3. The resultant sample solution was centrifuged at 14,000 rpm for 5 minutes, and the supernatant was transferred to a vial insert in a 2-mL vial for LC-MS/MS analysis.

Results and discussion

Optimization of UHPLC-MS/MS conditions

The MS/MS acquisition parameter for each compound was first selected from the Agilent veterinary drug MRM database. Those drugs not in the MRM database were optimized using Agilent Masshunter Optimizer software to obtain the MRM transition parameters. These parameters included one precursor ion, two fragment ions, and the collision energies under which the highest intensity can be achieved for each fragment ion. The ion pair of the MRM transition that provides the higher intensity was selected for quantitation, and the remaining one was used for compound confirmation. Table 2 shows the final parameters for the 18 compounds.

Table 2. MRM parameters for detection of 18 stimulant-related compounds.

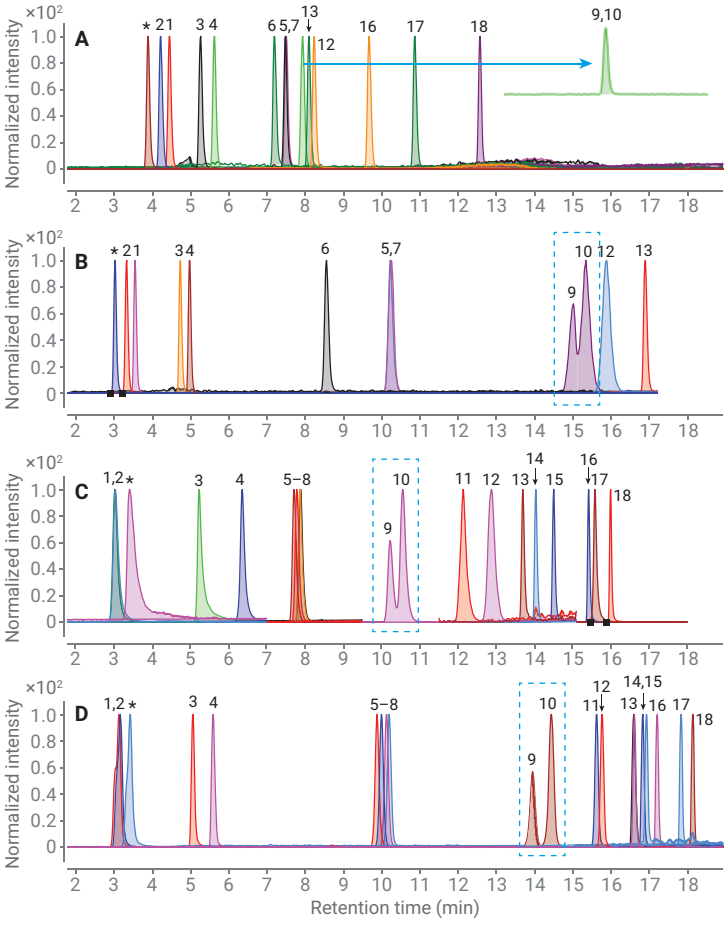
Time segment	Compound	RT (min)	Precursor ion **	Quant/Qual ion **	Collision energy (V)	Dewell time (ms)	Ionization mode
1	N/A	Flow switch to waste					
2	Salbutamol (Albuterol)	3.13	240.2	148.1*/166.1	20*/10	100	+
2	Terbutaline	3.15	226.1	152.1*/107	10*/40	100	+
3	Ractopamine	5.06	302.2	284.2*/107	10*/40	100	+
3	Clenbuterol	5.58	277.1	203*/259.1	20*/10	100	+
4	Prednisolone	9.88	361.2	147.1*/307.1	24*/8	80	+
4	Prednisone	9.99	359.2	323.2*/147.1	10*/20	80	+
4	Hydrocortisone	10.13	363.2	121.1*/309.2	20*/20	80	+
4	Fludrocortisone acetate	10.19	381.1	239.1*/181.1	27*/35	80	+
5	Betamethasone	13.95	393.2	373.2*/355.2	10*/10	100	+
5	Dexamethasone	14.44	393.2	355.2*/373.2	10*/10	100	+
5	Beclomethasone	15.63	409.2	391.2*/279.2	10*/20	120	+
5	Trenbolone	15.76	271.2	253.2*/199.1	20*/20	110	+
6	Zeranol	16.61	321.1	277.1*/303.0	23*/23	100	–
6	Penbutolol	16.59	292.2	236.2*/201.1	20*/20	10	+
6	Metandienone	16.83	301.2	149*/121	15*/29	30	+
6	17-Methyltestosterone	17.21	303.2	97.1*/109.1	30*/32	30	+
7	Progesterone	17.83	315.2	109.1*/97.1	20*/20	150	+
7	Testosterone-17-propionate	18.14	345.2	109.1*/97.1	40*/20	150	+

* Quantitative ion

** Unit resolution was used for both precursor and product ions

With the optimized MS acquisition conditions, the standard compound mixture was subjected to separation using different columns. Initially, the Agilent Poroshell C18 column was tested. With varying mobile phase components and gradient elution profiles (conditions shown in Figure 2), it does not show any tendency for the separation of the two isomers betamethasone and dexamethasone (insert in Figure 2A). Using the same mobile phase components and the gradient elution profile, the Agilent ZORBAX Eclipse Plus C18 column exhibits some tendency for separation of both isomers. Further changing the mobile phase and the gradient elution profile, it was found that separation for the pair of isomers can be better by using methanol/acetonitrile (v/v = 7/3) as one of the binary mobile phases, but the retention time is longer (~15 minutes), and the peak widths for the isomers are wide (Figure 2B). Considering most target compounds share the similar structure of aromatic or heterocyclic rings, the phenyl-hexyl stationary phase could provide a different selectivity for separation compared to C18. Hence, the same length of Agilent ZORBAX Phenyl-Hexyl column was examined. As shown in Figure 2C, a nearly baseline separation can be achieved with shorter retention times (10–11 minutes).

Increasing the length of the Phenyl-Hexyl column facilitates further separation of the pair of isomers. Considering that zeranol needs to be analyzed under high pH to guarantee satisfactory sensitivity, the additive in water was changed from 0.1 % formic acid/5 mM ammonium acetate to 0.01 % acetic acid/5 mM ammoniun acetate. As shown in Figure 2D, baseline separation can be achieved with narrow peaks for most compounds. Therefore, the ZORBAX Phenyl-Hexyl column (2.1 × 150 mm, 1.8 µm) was selected as the analytical column for the separation. Table 1 shows the optimized mobile phase components and the gradient



Mobile phase: A) 0.1 % Formic acid/(5 mmol/L ammonium acetate in water)
B) Methanol
Flow rate: 0.3 mL/min
Gradient : 0–3 minutes, 5 to 15 %B;
3–3.5 minutes, 15 to 45 %B;
3.5–5 minutes, hold at 45 %B;
5–5.1 minutes, 45 to 65 %B;
5.1–9 minutes: 65 to 75 %B;
9–12 minutes: 75 to 95 %B

Mobile phase: A) 5 mmol/L Ammonium acetate/0.1% formic acid in water
B) 5 mmol/L ammonium acetate/0.1% formic acid in methanol/acetonitrile (7/3, v/v)
Flow rate: 0.4 mL/min
Gradient : 0–3 minutes, 5 to 15 %B;
3–3.5 minutes, 15 to 45 %B;
3.5–15 minutes, 45 to 55 %B;
15–23 minutes, 55 to 95 %B

Mobile phase: A) 2 mmol/L Ammonium acetate in water
B) Methanol/acetonitrile (7/3, v/v)
Flow rate: 0.4 mL/min
Gradient : 0–3 minutes, 5 to 15 %B;
3–3.5 minutes, 15 to 40 %B;
3.5–12 minutes, hold at 40 %B;
12–12.5 minutes, 40 to 55 %B;
12.5–16 minutes, 55 to 95 %B;
16–18 minutes, hold at 95 %B

Figure 2. The overlaid MRM chromatograms demonstrating the separation of the 18 compounds, particularly for separation of the pair isomers, betamethasone/dexamethasone, using four columns under their corresponding optimized gradient elution. A) Poroshell C18 (100 × 2.1 mm, 2.7 µm); B) ZORBAX Eclipse Plus C18 (100 × 2.1 mm, 1.8 µm); C) ZORBAX Eclipse Plus Phenyl-Hexyl (100 × 2.1 mm, 1.8 µm); D) ZORBAX Eclipse Plus Phenyl-Hexyl (100 × 2.1 mm, 1.8 µm); the mobile phases and gradient elution profile for column D are shown in the Experimental section. **Note:** the arrow in A and the dashed rectangles in B–D annotate the chromatographic peaks for the pair of isomers; the peak labeled with * was not included in the final method. The sequence of the labeling on the peaks is in the order of retention time for compounds in Table 2.

elution profile for the selected column, and Table 2 shows the resultant retention time for each compound.

Optimizing the procedures for sample extraction and cleanup

Simplifying the sample extraction and cleanup procedure is an ideal way to ensure high throughput for an analytical method and reduce cost and labor. Direct analysis of the sample extract is simple and straightforward, but often suffers serious interference issues, and hardly provides valid measurement. For example, direct analysis of an acidified acetonitrile extract of a beef sample spiked with the 18 target compounds at a level of 2.0 µg/kg demonstrated that all 18 compounds had recoveries below 60 %; among them, the recoveries for salbutamol and terbutaline are below 20 %, as the bar graphs show with procedure 1 in Figure 3 (the left bar in Figure 3 using procedure 1). This indicates that the co-extracted matrix with the target compounds has nonnegligible interference effects on the accurate measurement of all the 18 compounds.

The major components of beef matrix are protein and lipid. Since acidified acetonitrile extraction can precipitate most protein from beef, the matrix interference results primarily from the lipid co-extracted from the matrix. Initially, QuEChERS dispersing cleanup was tested. The extractant was subjected to cleanup using PSA and C18 as sorbents followed by centrifugation before analysis using LC-MS/MS. As shown in Figure 3 using procedure 2, 13 out 18 compounds have the recoveries within 70–120 %. However, the recoveries for the remainng compounds are out of the satisfying range, particularly for penbutolol and testosterone-17-propionate, their recoveries are 21 % and 25 %, respectively. To efficiently remove lipids, the Captiva lipid cartridge was evaluated. Analysis of the filtrate showed that recoveries for 15 out of 18 compounds ranging from 70 to 120 %, indicating that most of the matrix has been removed (the bars using procedure 3 in Figure 3). Compared to the commercial QuEChERS packages as shown in the bars using procedure 2 in Figure 3, the recoveries for most compounds are similar. However, for penbutolol, the recovery is much higher (71 %) in beef using Captiva than that using QuEChERS, with a recovery as low as 21 %. Therefore, for this group of compounds, a Captiva lipid cartridge achieved better performance than the QuEChERS method.

Nonetheless, there was one compound showing recovery below 40 % at a spiked level of 2.0 µg/kg using Captiva cartridge filtration, as shown in Figure 3 using procedure 3. Considering that the sample was diluted to 3/8 of the original spiked level after extraction and filtration, accurate determination of some compounds with low MS response became more challenging. To solve this issue, examination of the types and amounts of desiccants followed by nitrogen drying to bring the concentration levels of the compounds in the final analysis solution to the original spiked level was performed. It was found that by using 0.30 g of magnesium sulfate (the right bar in Figure 3 using procedure 4), all compounds had recoveries within 70–120 %, except two compounds had recoveries of approximately 58 %. This result suggests that filtration using the Captiva lipid cartridge followed with an

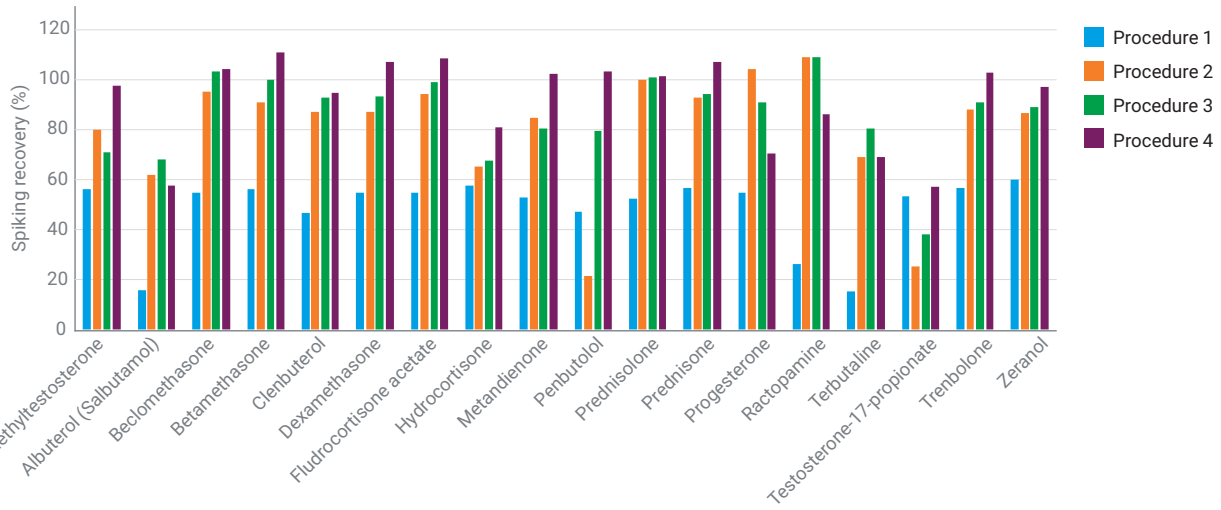


Figure 3. Comparison of the recoveries of the target compounds for spiking samples subjected to different cleanup procedures. Procedure 1: direct analysis of supernatant solution after acidified acetonitrile extraction; Procedure 2: analysis of the supernatant solution after QuEChERS dispersing SPE cleanup; Procedure 3: analysis of the filtrate collected from Captiva lipid removal filtration cartridge; Procedure 4: following Procedure 3, the obtained filtrate was further enriched before analysis. **Note:** the spiked level for each compound is 2.0 µg/kg.

optimized enrichment step is effective, and can be selected for the sample cleanup. The Experimental section shows the complete sample extraction and cleanup procedure.

Matrix effect evaluation

Matrix interference is a common phenomenon in LC/MS analysis. To evaluate whether the presence of matrix interferes with the method performance, two sets of calibration solutions were prepared. One was prepared using pure solvent, and the other was prepared using the blank matrix. The linear response of the target compounds prepared in both solutions was obtained. Then, the matrix effect (ME%) was evaluated using the ratio of the linear slope for the calibration curve obtained in the matrix-matched standard solution ($slope_{matrix}$) to that obtained in the solvent standard solution ($slope_{solvent}$). The higher the ratio deviates from 100 %, the stronger the matrix effect. Figure 4 demonstrates that 13 out of 18 compounds show negligible matrix effect, with the relative slope within 80–120 %. Turbutaline is the exception, showing a strong matrix enhancement effect, with a relative slope ratio reaching 140 %, while the other four compounds including trenbolone, 17-methyltestosterone, progesterone, and testerosterone-17-proprioate show a matrix suppression effect. Among the four compounds, tranbolone and 17-methyltestosterone are slightly suppressed, with relative slope ratio within 45–75 %; in comparison, progesterone and testerosterone-17-proprioate, with relative slope ratios below 25 %, indicate that the matrix suppression for both compounds can be higher than 75 %. Therefore, matrix-matched external standard calibration was selected for quantitation to minimize the measurement bias due to matrix effects. Table 3 lists the matrix-matched calibration equation for each compound. All compounds show very good linearity within the examined concentration range of 0.10–50.0 µg/L, with linear regression coefficients all above 0.995.

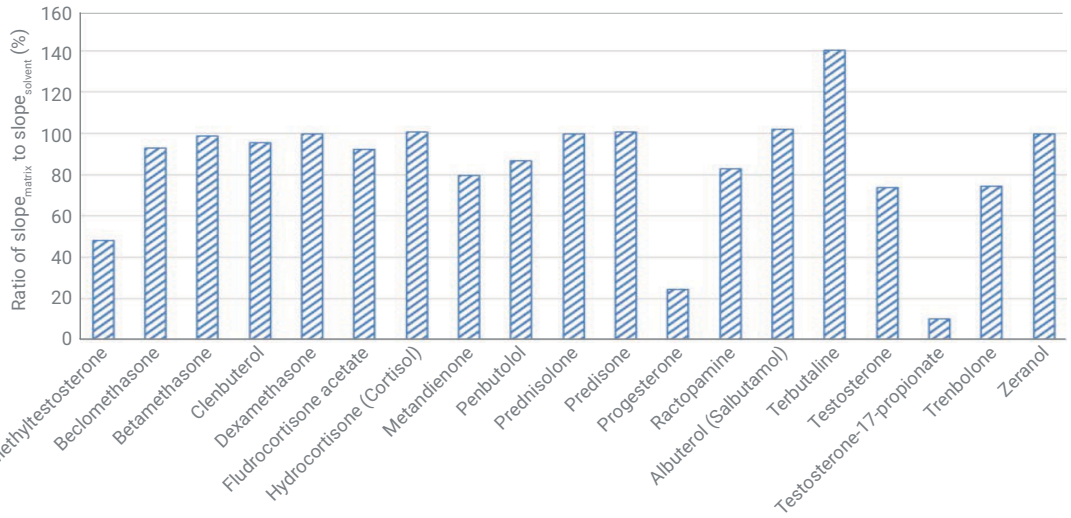


Figure 4. Ratio of the slope of the calibration curve obtained in the matrix-matched calibration standard solution to that obtained in pure solvent solution. This ratio demonstrates that matrix interference is not negligible for some compounds.

Limit of detection (LOD) and limit of quantitation (LOQ)

Using the matrix-matched standard solution for external quantitation, the LOD and LOQ were calculated using the signal-to-noise ratio (S/N) at the calibration level of 0.10–0.50 µg/L in the matrix-matched calibration standard solution. Table 3 shows that 6 out of 18 compounds have LOQs at or below 0.010 µg/L, and 7 out of 18 have LOQs within 0.010–0.10 µg/L. The remaining five compounds have LOQs within 0.10–0.30 µg/L. The LOQs are significantly lower than the currently available MRLs in food regulations[7-8].

Accuracy and precision

To test the accuracy and precision of the method, a spiking recovery test was conducted using the blank matrix. The spiking levels for this group of compounds were set at 0.40, 1.0, and 2.0 µg/kg. Five replicates were performed at each spiking level with a blank matrix as control. Table 4 shows that the average spiking recoveries ranged 57.3–113 %, with most recoveries within 70–120 %, and all RSDs (n = 5) within 3.07–15.9 %. The results indicate that the method is accurate, robust, and can meet the requirement for routine screening of these compounds in beef.

Table 3. LOD, LOQ, and matrix-matched calibration equations for the 18 stimulant-related compounds.

Compound	LOD (µg/L)	LOQ (µg/L)	Linear equation	R^2
17-Methyltestosterone	0.017	0.058	y = 38630.633850 x – 2712.183670	0.9963
Beclomethasone	0.019	0.063	y = 24440.261255 x – 1287.045836	0.9982
Betamethasone	0.0060	0.020	y = 12658.873102 x – 765.317191	0.9978
Clenbuterol	0.00060	0.0020	y = 168775.128370 x – 12445.538604	0.9984
Dexamethasone	0.0045	0.015	y = 13052.936867 x – 784.381709	0.9987
Fludrocortisone acetate	0.057	0.19	y = 4764.510997 x – 387.251224	0.9975
Hydrocortisone (Cortisol)	0.0010	0.0030	y = 12488.904021 x + 36166.820636	0.9951
Metandienone	0.023	0.078	y = 52368.606723 x – 2751.669276	0.9985
Penbutolol	0.0021	0.0070	y = 402604.216811 x – 15468.821823	0.9989
Prednisolone	0.018	0.061	y = 8134.435245 x – 647.343434	0.9978
Prednisone	0.066	0.22	y = 5669.367470 x – 542.548363	0.9984
Progesterone	0.033	0.11	y = 23658.649124 x + 20011.903129	0.9967
Ractopamine	0.0030	0.010	y = 225111.886998 x – 10996.048625	0.9978
Salbutamol	0.0015	0.0050	y = 254890.265922 x – 21764.566926	0.9982
Terbutaline	0.0012	0.0040	y = 182589.673043 x – 18301.549800	0.9969
Testosterone-17-propionate	0.090	0.30	y = 9994.984007 x – 1569.091728	0.9950
Trenbolone	0.026	0.088	y = 33847.383500 x – 2730.721444	0.9975
Zeranol	0.081	0.27	y = 2997.532529 x – 248.546671	0.9986

Table 4. Summary of the recoveries and precisions for the 18 compounds spiked in beef at three different spiking levels.

Compound	Spiking level					
	0.40 µg/kg		1.0 µg/kg		2.0 µg/kg	
	Rec.	RSD	Rec.	RSD	Rec.	RSD
17-Methyltestosterone	69.7	10.5	87.6	7.13	97.6	6.38
Beclomethasone	92.0	8.58	94.4	8.61	104	4.72
Betamethasone	97.4	6.79	98.4	9.16	111	3.09
Clenbuterol	80.8	6.06	80.8	9.93	94.4	3.87
Dexamethasone	94.5	6.89	95.5	9.17	107	3.09
Fludrocortisone acetate	96.3	7.34	97.7	7.04	109	4.33
Hydrocortisone (Cortisol)	113	10.2	73.6	12.5	80.7	15.6
Metandienone	89.8	4.93	95.6	9.34	102	5.78
Penbutolol	92.3	10.7	93.8	6.96	103	7.11
Prednisolone	91.1	7.80	91.0	8.10	101	4.31
Prednisone	95.0	8.91	95.9	9.10	107	4.35
Progesterone	105	12.8	78.0	15.9	70.3	12.9
Ractopamine	71.8	7.45	71.4	15.0	86.3	4.97
Salbutamol (Albuterol)	58.0	5.41	72.5	3.07	57.7	3.34
Terbutaline	76.0	4.35	63.6	9.15	69.1	4.93
Testosterone-17-propionate	73.4	4.35	66.8	15.4	57.3	10.7
Trenbolone	84.5	10.4	89.5	9.72	103	6.47
Zeranol	78.4	9.40	86.3	10.0	97.2	4.47

Conclusions

This Application Note describes a method based on acidified acetonitrile extraction followed with lipid cartridge filtration and enrichment procedures combined with UHPLC-MS/MS analysis. All four-class 18 stimulant-related substances show good linearity, with all linear regression coefficients above 0.995. The LOQs for these compounds were 0.30 µg/kg or below, with two-thirds of compounds showing an LOQ at or below 0.10 µg/kg. This method is easy to operate, rapid, accurate, and reliable. It can be applied for simultaneous quantitative screening of multiclass stimulant-related drug residues in beef tissues, and can potentially be extended to the analysis of these compounds in the muscle tissues of other animal origins.

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