

## 盐析辅助液液萃取-超高效液相色谱串联质谱法测定山羊角中 53种禁用兽药残留

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**摘要:** 建立了UHPLC-MS/MS测定山羊角中53种禁用兽药残留的检测方法, 包括16种镇静剂类、14种 $\beta$ -受体激动剂类、氯霉素、4种氟喹诺酮类、7种硝基咪唑类、3种喹噁啉类化合物、8种其他类化合物。采用盐析辅助液液萃取样品, Zorbax Eclipse Plus C18色谱柱(1.8  $\mu\text{m}$ , 3.0 mm  $\times$  150 mm, Agilent), 以0.1%甲酸溶液-乙腈为流动相, 梯度洗脱, 在电喷雾离子化正负离子模式下, 以多反应监测方式(MRM)检测。53种待测物在各自浓度范围内均成良好线性关系, 相关系数均在0.99以上; 三水平平均加样回收率为70.4%~118.7%, 相对标准偏差(RSD)为0.69%~12.07%, 检出限为0.1~50  $\mu\text{g}\cdot\text{kg}^{-1}$ , 定量限为0.2~100  $\mu\text{g}\cdot\text{kg}^{-1}$ 。本方法已应用于实际样品的测定。

**关键词:** 山羊角; 禁用兽药残留; 盐析辅助液液萃取; 超高效液相色谱串联三重四极杆质谱

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## Determination of 53 prohibited veterinary drug residues in goat horn by salting-out assisted liquid-liquid extraction combined with UHPLC-MS/MS

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**Abstract:** A detection method using UHPLC-MS/MS was established for the determination of 53 prohibited veterinary drug residues in goat horns, including 16 tranquilizers, 14  $\beta$ -agonists, chloramphenicol, 4 fluoroquinolones, 7 nitroimidazoles, 3 quinoxalines, and 8 other compounds. Samples were extracted using salting-out assisted liquid-liquid extraction (SALLE). A Zorbax Eclipse Plus C18 column (1.8  $\mu\text{m}$ , 3.0 mm  $\times$  150 mm, Agilent) was used with 0.1% formic acid solution-acetonitrile as the mobile phase for gradient elution. Detection was performed in positive and negative electrospray ionization modes using multiple reaction monitoring (MRM). All 53 analytes showed good linearity within their respective concentration ranges, with correlation coefficients above 0.99. The average recoveries at three spiked levels ranged from 70.4% to 118.7%, with relative standard deviations (RSDs) ranging from 0.69% to 12.07%. The limits of detection (LODs) ranged from 0.1 to 50  $\mu\text{g}\cdot\text{kg}^{-1}$ , and the limits of quantitation (LOQs) ranged from 0.2 to 100  $\mu\text{g}\cdot\text{kg}^{-1}$ . This method has been applied to the determination of real samples.

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**Key words:** goat horn; prohibited veterinary drug residue; salting-out assisted liquid-liquid extraction; UHPLC-MS/MS

兽药是用于预防、治疗或诊断动物疾病或有目的地调节动物生理功能的药物<sup>[1]</sup>,被广泛用于畜牧业和农业,以实现增产增效。在动物使用兽药或食用混有兽药的饲料后,兽药大多可通过尿液和粪便排出体外,少数会以原型结构或代谢产物的形式贮存于动物细胞、组织、器官<sup>[2,3]</sup>。目前,兽药残留问题引起了许多国家和组织的关注,我国<sup>[4]</sup>及美国<sup>[5]</sup>、日本<sup>[6]</sup>、欧盟<sup>[7]</sup>、国际食品法典委员会(CAC)<sup>[8]</sup>等已制订了兽药最大残留限量。

值得注意的是,部分高安全风险品种,已被证实具有致癌、致畸、致突变等危害<sup>[9-11]</sup>,已在食品动物养殖过程中禁用。农业农村部第250号公告规定了食品动物中21种(类)禁止使用的药品及其化合物;第2292号公告规定在食品动物中停止使用洛美沙星、培氟沙星、氧氟沙星、诺氟沙星4种兽药;第2428号公告停止硫酸黏菌素用于动物促生长;第2583号公告禁止非泼罗尼及相关制剂用于食品动物;第2638号公告规定在食品动物中停止使用喹乙醇、氨苯胂酸、洛克沙胂3种兽药。另外,农业农村部第176号和第1519号公告对饲料和动物饮用水中禁止使用的兽药作出了明确规定;GB 31650-2019中规定了氯丙嗪等9种允许作治疗用,但不得在动物性食品中检出的兽药。

已有文献针对食品中禁用兽药残留,按类别开发了各种检测方法,如镇静剂<sup>[12,13]</sup>、 $\beta$ -受体激动剂<sup>[14,15]</sup>、喹诺酮类<sup>[16,17]</sup>、硝基咪唑类<sup>[18]</sup>、喹噁啉类<sup>[19,20]</sup>;亦有文献报道了食品中多类别禁用兽药的高通量检测方法<sup>[21-24]</sup>。

与动物源性食品类似,动物源性中药也存在兽药残留的安全问题,但尚未得到重视。本课题组前期分别对鸡内金<sup>[25]</sup>、熊胆粉<sup>[26]</sup>的兽药残留进行检测,发现这些中药存在兽药残留安全风险,特别是禁用兽药检出率较高。因此,动物源性中药中禁用兽药的监管和检

测是亟待解决的问题。

与动物源性食品不同的是,动物源性中药多为干基,水分、脂肪含量较低。本文的研究对象为传统中药山羊角,主要来源于家养山羊(*Capra hircus* Linnaeus)的角,最早记载于《神农本草经》<sup>[27]</sup>,主要成分为蛋白质类<sup>[28]</sup>、肽类、核苷类<sup>[29]</sup>、无机元素类等。根据现代药理学研究,山羊角具有促凝血、镇静、解热等药理活性<sup>[30]</sup>。山羊在中国大量养殖,2018年山羊现存数量达1.3575亿只<sup>[31]</sup>,山羊的养殖不可避免地需要使用兽药来调节生长发育、预防和治疗疾病。

基于山羊角的基质成分特点,本文采用盐析辅助液液萃取前处理,结合UHPLC/MS/MS方法检测53种禁用兽药,包括16种镇静剂类、14种 $\beta$ -受体激动剂类、氯霉素、4种氟喹诺酮类、7种硝基咪唑类、3种喹噁啉类及8种其他类化合物。

## 材料与方法

**仪器与试剂** 1290/6495型超高效液相色谱串联三重四极杆质谱仪(美国Agilent公司);Zorbax Eclipse Plus C18色谱柱(1.8  $\mu$ m, 3.0 mm  $\times$  150 mm, Agilent)。待测物对照品均购于天津阿尔塔科技有限公司,内标对照品均购于曼哈格(上海)生物科技有限公司,批号、纯度等信息见表1。乙腈、甲醇为质谱级试剂,购于北京百灵威科技有限公司;甲酸为质谱级试剂,购于美国赛默飞世尔科技有限公司;甲酸铵为色谱级试剂,购于西格玛奥德里奇(上海)贸易有限公司;NaCl、Na<sub>2</sub>SO<sub>4</sub>均为分析纯,购于国药集团化学试剂有限公司。27批山羊角样品中,样品1~19由上海凯宝药业股份有限公司提供,产地为内蒙古;样品20~27由上海康桥中药饮片有限公司提供,产地分别为山东和浙江。

**Table 1** The information of 53 analysts and internal standards

Compound	CAS number	Chemical formula	Dissolved solvent	Lot. number	Purity	M.W.
$\beta$ -Agonist						
Clorprenaline	3811-25-4	C <sub>11</sub> H <sub>16</sub> ClNO	Methanol	S177845	0.828	213.1
Tulobuterol	41570-61-0	C <sub>12</sub> H <sub>18</sub> ClNO	Methanol	S139505	0.999	227.1
Ractopamine	97825-25-7	C <sub>18</sub> H <sub>23</sub> NO <sub>3</sub>	Methanol	S177845	0.989	301.2
Bambuterol	81732-65-2	C <sub>18</sub> H <sub>29</sub> N <sub>3</sub> O <sub>5</sub>	Methanol	S177845	0.993	367.2
Phenylethanolamine A	1346746-81-3	C <sub>19</sub> H <sub>24</sub> N <sub>2</sub> O <sub>4</sub>	Methanol	S148439	0.998	344.2
Mabuterol	56341-08-3	C <sub>13</sub> H <sub>18</sub> ClF <sub>3</sub> N <sub>2</sub> O	Methanol	S177845	0.999	310.1
Salbutamol	18559-94-9	C <sub>13</sub> H <sub>21</sub> NO <sub>3</sub>	Methanol	S177845	0.994	239.2
Zilpaterol	117827-79-9	C <sub>14</sub> H <sub>19</sub> N <sub>3</sub> O <sub>2</sub>	Methanol	S177845	0.961	261.2
Cimaterol	54239-37-1	C <sub>12</sub> H <sub>17</sub> N <sub>3</sub> O	Methanol	S172505	0.980	219.1
Terbutaline	23031-25-6	C <sub>12</sub> H <sub>19</sub> NO <sub>3</sub>	Methanol	S177845	0.999	225.1
Clenbuterol	37148-27-9	C <sub>12</sub> H <sub>18</sub> Cl <sub>2</sub> N <sub>2</sub> O	Methanol	S177845	0.988	276.1

Continued

Compound	CAS number	Chemical formula	Dissolved solvent	Lot. number	Purity	M.W.
Cimbuterol	54239-39-3	C <sub>13</sub> H <sub>19</sub> N <sub>3</sub> O	Methanol	S096141	0.999	233.2
Brombuterol	41937-02-4	C <sub>12</sub> H <sub>16</sub> Br <sub>2</sub> N <sub>2</sub> O	Methanol	S134561	0.994	364.0
Formoterol	73573-87-2	C <sub>19</sub> H <sub>24</sub> N <sub>2</sub> O <sub>4</sub>	Methanol	S146953	0.999	344.2
Amphenicol						
Chloramphenicol	56-75-7	C <sub>11</sub> H <sub>12</sub> Cl <sub>2</sub> N <sub>2</sub> O <sub>5</sub>	Methanol	S145561	0.998	322.0
Tranquilizer						
Chlorpromazine	50-53-3	C <sub>17</sub> H <sub>19</sub> ClN <sub>2</sub> S	Methanol	S167751	0.980	318.1
Methaqualone	72-44-6	C <sub>16</sub> H <sub>14</sub> N <sub>2</sub> O	Methanol	ALT028069	0.999	250.1
Xylazine	7361-61-7	C <sub>12</sub> H <sub>16</sub> N <sub>2</sub> S	Methanol	S159793	0.990	220.1
Estazolam	29975-16-4	C <sub>16</sub> H <sub>11</sub> ClN <sub>4</sub>	Methanol	S119943	0.999	294.1
Midazolam	59467-70-8	C <sub>18</sub> H <sub>13</sub> ClFN <sub>3</sub>	Methanol	S119943	0.994	325.8
Triazolam	28911-01-5	C <sub>17</sub> H <sub>12</sub> Cl <sub>2</sub> N <sub>4</sub>	Methanol	S119943	0.992	342.0
Alprazolam	28981-97-7	C <sub>17</sub> H <sub>13</sub> ClN <sub>4</sub>	Methanol	S119943	0.999	308.1
Diazepam	439-14-5	C <sub>16</sub> H <sub>13</sub> ClN <sub>2</sub> O	Methanol	S119943	0.999	284.1
Nitrazepam	146-22-5	C <sub>15</sub> H <sub>11</sub> N <sub>3</sub> O <sub>3</sub>	Methanol	S119943	0.999	281.1
Oxazepam	604-75-1	C <sub>15</sub> H <sub>11</sub> ClN <sub>2</sub> O <sub>2</sub>	Methanol	S119943	0.996	286.1
Clonazepam	1622-61-3	C <sub>15</sub> H <sub>10</sub> ClN <sub>3</sub> O <sub>3</sub>	Methanol	S119943	0.999	315.0
Lorazepam	846-49-1	C <sub>15</sub> H <sub>10</sub> Cl <sub>2</sub> N <sub>2</sub> O <sub>2</sub>	Methanol	S119943	0.999	320.0
Phenobarbital	50-06-6	C <sub>12</sub> H <sub>12</sub> N <sub>2</sub> O <sub>3</sub>	Methanol	S142179	0.999	232.1
Barbital	57-44-3	C <sub>8</sub> H <sub>12</sub> N <sub>2</sub> O <sub>3</sub>	Methanol	S142179	0.999	184.1
Amobarbital	57-43-2	C <sub>11</sub> H <sub>18</sub> N <sub>2</sub> O <sub>3</sub>	Methanol	S142179	0.999	226.1
Secobarbital	76-73-3	C <sub>12</sub> H <sub>18</sub> N <sub>2</sub> O <sub>3</sub>	Methanol	S142179	0.999	238.1
Fluoroquinolone						
Norfloxacin	70458-96-7	C <sub>16</sub> H <sub>18</sub> FN <sub>3</sub> O <sub>3</sub>	Methanol	S117199	0.999	319.1
Pefloxacin	70458-92-3	C <sub>17</sub> H <sub>20</sub> FN <sub>3</sub> O <sub>3</sub>	Methanol	S117199	0.987	333.2
Lomefloxacin	98079-51-7	C <sub>17</sub> H <sub>19</sub> F <sub>2</sub> N <sub>3</sub> O <sub>3</sub>	Methanol	S117199	0.904	351.1
Ofloxacin	82419-36-1	C <sub>18</sub> H <sub>20</sub> FN <sub>3</sub> O <sub>4</sub>	Methanol	S117199	0.995	361.1
Nitroimidazole						
Dimetridazole	551-92-8	C <sub>5</sub> H <sub>7</sub> N <sub>3</sub> O <sub>2</sub>	Methanol	S169555	0.999	141.1
Dimetridazole-2-hydroxy	936-05-0	C <sub>5</sub> H <sub>7</sub> N <sub>3</sub> O <sub>3</sub>	Methanol	S169555	0.980	157.1
Metronidazole-hydroxy	4812-40-2	C <sub>6</sub> H <sub>9</sub> N <sub>3</sub> O <sub>4</sub>	Methanol	S169555	0.996	187.1
Metronidazole	443-48-1	C <sub>6</sub> H <sub>9</sub> N <sub>3</sub> O <sub>3</sub>	Methanol	S169555	0.999	171.1
Ronidazole	7681-76-7	C <sub>6</sub> H <sub>8</sub> N <sub>4</sub> O <sub>4</sub>	Methanol	S169555	0.990	200.1
Iprnidazole	14885-29-1	C <sub>7</sub> H <sub>11</sub> N <sub>3</sub> O <sub>2</sub>	Methanol	S094613	0.999	169.1
Tinidazole	19387-91-8	C <sub>8</sub> H <sub>13</sub> N <sub>3</sub> O <sub>4</sub> S	Methanol	S143801	0.999	247.1
Quinoxaline						
2-Quinoxaline-carboxylic acid (2-QCA)	879-65-2	C <sub>9</sub> H <sub>6</sub> N <sub>2</sub> O <sub>2</sub>	Methanol	S153963	0.950	174.0
3-Methyl-2-quinoxaline-carboxylic acid (MQCA)	74003-63-7	C <sub>10</sub> H <sub>8</sub> N <sub>2</sub> O <sub>2</sub>	Methanol	S160521	0.999	188.1
Carbadox	6804-07-5	C <sub>11</sub> H <sub>10</sub> N <sub>4</sub> O <sub>4</sub>	Methanol/DMSO (3:1)	S095207	0.972	262.1
Others						
Dapsone	80-08-0	C <sub>12</sub> H <sub>12</sub> N <sub>2</sub> O <sub>2</sub> S	Acetonitrile	S125593	0.994	248.1
Leucomalachite green	129-73-7	C <sub>23</sub> H <sub>26</sub> N <sub>2</sub>	Methanol	S167117	0.994	330.2
Malachite green	569-64-2	C <sub>23</sub> H <sub>25</sub> ClN <sub>2</sub>	Methanol	S167117	0.981	364.9
Clonidine	4205-90-7	C <sub>9</sub> H <sub>9</sub> Cl <sub>2</sub> N <sub>3</sub>	Methanol	S146723	0.998	229.0
Cyproheptadine	129-03-3	C <sub>21</sub> H <sub>21</sub> N	Methanol	S149739	0.998	287.4
Carbofuran	1563-66-2	C <sub>12</sub> H <sub>15</sub> NO <sub>3</sub>	Acetonitrile	2250832	0.999	221.1
Chlordimeform	6164-98-3	C <sub>10</sub> H <sub>13</sub> ClN <sub>2</sub>	Methanol	S168431	0.989	196.1
Fipronil	120068-37-3	C <sub>12</sub> H <sub>4</sub> Cl <sub>2</sub> F <sub>6</sub> N <sub>4</sub> OS	Acetonitrile	S163547	0.995	435.9
Internal standard (IS)						
Cimbuterol- <i>d</i> <sub>3</sub>	1246819-04-4	C <sub>13</sub> H <sub>10</sub> D <sub>3</sub> N <sub>3</sub> O	Methanol	G0065083	0.977	242.2
Zilpaterol- <i>d</i> <sub>7</sub>	1217818-36-4	C <sub>14</sub> H <sub>12</sub> D <sub>7</sub> N <sub>3</sub> O <sub>2</sub>	Methanol	G0088143	> 0.98	268.2
2-QCA- <i>d</i> <sub>4</sub>	2244217-89-6	C <sub>9</sub> H <sub>2</sub> D <sub>4</sub> N <sub>2</sub> O <sub>2</sub>	Methanol	H0095896	0.980	178.1
Metronidazole-hydroxy- <i>d</i> <sub>2</sub>	2196180-19-3	C <sub>6</sub> H <sub>7</sub> D <sub>2</sub> N <sub>3</sub> O <sub>4</sub>	Methanol	H3000662	0.980	189.1
Pefloxacin- <i>d</i> <sub>5</sub>	1228182-51-1	C <sub>17</sub> H <sub>15</sub> D <sub>5</sub> FN <sub>3</sub> O <sub>3</sub>	Methanol	F0058438	0.996	338.2
Dapsone- <i>d</i> <sub>8</sub>	557794-38-4	C <sub>12</sub> H <sub>4</sub> D <sub>8</sub> N <sub>2</sub> O <sub>2</sub> S	Methanol	D0054643	1.000	256.1
Carbofuran- <i>d</i> <sub>3</sub>	1007459-98-4	C <sub>12</sub> H <sub>12</sub> D <sub>3</sub> NO <sub>3</sub>	Methanol	E0066530	0.998	224.1
Fipronil- <sup>13</sup> C <sub>4</sub> <sup>15</sup> N <sub>2</sub>	-	C <sub>8</sub> <sup>13</sup> C <sub>4</sub> H <sub>4</sub> Cl <sub>2</sub> F <sub>6</sub> N <sub>2</sub> <sup>15</sup> N <sub>2</sub> OS	Methanol	A0069672	1.000	443.1
Leucomalachite green- <i>d</i> <sub>6</sub>	1173021-13-0	C <sub>23</sub> H <sub>20</sub> D <sub>6</sub> N <sub>2</sub>	Acetonitrile	G0093712	0.998	336.3
Malachite green- <i>d</i> <sub>5</sub>	-	C <sub>23</sub> H <sub>20</sub> D <sub>5</sub> ClN <sub>2</sub>	Acetonitrile	G0093712	0.596	369.9

**色谱和质谱条件** Zorbax Eclipse Plus C18 色谱柱 (1.8  $\mu\text{m}$ , 3.0 mm  $\times$  150 mm, Agilent); 柱温为 40  $^{\circ}\text{C}$ , 流速为 0.5 mL $\cdot$ min $^{-1}$ , 进样量 10  $\mu\text{L}$ 。流动相: A 为 0.1% 甲酸 (含 5 mmol $\cdot$ L $^{-1}$  甲酸铵) 溶液, B 为乙腈; 梯度条件如下: 0~0.5 min, 2% B; 0.5~1.8 min, 2%~15% B; 1.8~3.5 min, 15%~20% B; 3.5~6 min, 20%~25% B; 6~7 min, 25%~30% B; 7~11 min, 30%~35% B; 11~16 min, 35%~100% B; 16~26 min, 100% B。

电喷雾离子化 (ESI) 正负离子模式; 干燥气 ( $\text{N}_2$ ) 流速 13 L $\cdot$ min $^{-1}$ , 温度 210  $^{\circ}\text{C}$ ; 雾化气 ( $\text{N}_2$ ) 0.24 MPa; 鞘气 ( $\text{N}_2$ ) 流速 12 L $\cdot$ min $^{-1}$ , 温度 380  $^{\circ}\text{C}$ ; 毛细管电压正离子为 3.5 kV, 负离子为 4.0 kV。采用多反应监测方式 (MRM) 检测, 监测离子、碰撞能量等参数见表 2。

#### 对照品溶液制备方法

标准储备溶液 将 53 种化合物和内标分别溶解在适当的溶剂中, 制备成浓度为 100  $\mu\text{g}\cdot\text{mL}^{-1}$  的溶液。

**Table 2** Detailed MS/MS parameters of 53 analytes. \*Quantitative ion

Compound	Precursor ion ( $m/z$ )	Product ion ( $m/z$ )	Retention time/min	Fragmentor /V	Collision energy/V	Polarity	IS
<i><math>\beta</math>-Agonist</i>							
Clorprenaline	214.1	196.0*/153.9	5.97	380	8/12	+	
Tulobuterol	228.1	154.0*/119.1	7.00	380	12/32	+	
Ractopamine	302.2	284.2*/164.1	5.81	380	8/12	+	
Bambuterol	368.2	72.0*/46.1	8.29	380	57/25	+	
Phenylethanolamine A	345.2	327.2*/150.2	11.61	380	13/21	+	
Mabuterol	311.0	293.0/237.0*	8.68	380	9/17	+	
Salbutamol	240.2	166.1/148.1*	3.64	380	4/16	+	Zilpaterol- $d_7$
Zilpaterol	262.2	202.0/185.1*	3.62	380	21/25	+	Zilpaterol- $d_7$
Cimaterol	220.1	202.1*/160.1	3.80	380	4/12	+	Cimbuterol- $d_9$
Terbutaline	226.1	170.1/152.1*	3.64	380	8/12	+	Cimbuterol- $d_9$
Clenbuterol	277.1	259.1/203.0*	7.10	380	4/12	+	Cimbuterol- $d_9$
Cimbuterol	234.0	160.0*/143.0	4.35	380	13/29	+	Cimbuterol- $d_9$
Brombuterol	365.0	290.9*/212.0	8.09	380	17/33	+	Cimbuterol- $d_9$
Formoterol	345.2	149.1*/121.3	6.98	380	21/33	+	Cimbuterol- $d_9$
<i>Amphenicol</i>							
Chloramphenicol	321.0	257.0/152.1*	9.61	380	16/14	-	
<i>Tranquilizer</i>							
Chlorpromazine	319.1	86.1/58.2*	13.94	380	16/44	+	
Methaqualone	251.1	132.1/91.0*	14.16	380	37/49	+	
Xylazine	221.1	90.0*/72.0	6.94	380	40/40	+	
Estazolam	295.1	267.0*/192.0	13.55	380	25/23	+	
Midazolam	326.1	291.1*/223.2	10.83	380	29/45	+	
Triazolam	343.1	314.9/308.0*	14.01	380	25/28	+	
Alprazolam	309.1	281.3/205.2*	13.88	380	29/53	+	
Diazepam	285.1	222.1/193.1*	15.23	380	33/37	+	
Nitrazepam	282.1	207.2*/180.2	13.58	380	37/41	+	
Oxazepam	287.1	241.0*/162.9	13.51	380	25/45	+	
Clonazepam	316.0	240.9/213.8*	13.89	380	34/38	+	
Lorazepam	321.0	302.9/275.0*	13.79	380	21/25	+	
Barbital	183.2	140.1*/84.9	5.92	380	9/9	-	
Secobarbital	237.2	194.2*/84.9	13.71	380	9/9	-	
Phenobarbital	231.0	188.1*/84.9	9.51	380	9/9	-	
Amobarbital	225.2	182.2*/84.9	13.02	380	13/13	-	
<i>Fluoroquinolone</i>							
Norfloxacin	320.1	302.1*/231.1	5.32	380	25/40	+	Pefloxacin- $d_5$
Pefloxacin	334.2	316.3*/290.1	5.47	380	21/28	+	Pefloxacin- $d_5$
Lomefloxacin	352.2	334.0/265.0*	5.86	380	20/25	+	Pefloxacin- $d_5$
Ofloxacin	362.2	318.2*/261.1	5.38	380	20/40	+	Pefloxacin- $d_5$
<i>Nitroimidazole</i>							
Dimetridazole	142.1	96.1*/81.1	5.22	380	16/28	+	Metronidazole-hydroxy- $d_2$
Dimetridazole-2-hydroxy	158.1	140.0/55.2*	4.25	380	8/20	+	Metronidazole-hydroxy- $d_2$
Metronidazole-hydroxy	188.1	126.1*/123.1	3.76	380	16/8	+	Metronidazole-hydroxy- $d_2$
Metronidazole	172.1	128.0*/82.1	4.31	380	12/24	+	Metronidazole-hydroxy- $d_2$
Ronidazole	201.1	140.1*/55.2	4.69	380	8/25	+	Metronidazole-hydroxy- $d_2$
Iprnidazole	170.1	124.0*/109.0	9.62	380	16/26	+	Metronidazole-hydroxy- $d_2$
Tinidazole	248.1	121.0/29.2*	6.00	380	17/21	+	

Continued

Compound	Precursor ion ( <i>m/z</i> )	Product ion ( <i>m/z</i> )	Retention time/min	Fragmentor /V	Collision energy/V	Polarity	IS
Quinoxaline							
2-QCA	175.0	128.9/102.0*	5.22	380	16/40	+	2-QCA- <i>d</i> <sub>4</sub>
MQCA	189.1	145.0*/143.0	5.24	380	12/16	+	2-QCA- <i>d</i> <sub>4</sub>
Carbadox	263.1	231.2*/130.1	5.43	380	13/21	+	2-QCA- <i>d</i> <sub>4</sub>
Others							
Dapsone	249.1	156.0/92.0*	7.76	380	10/28	+	Dapsone- <i>d</i> <sub>8</sub>
Leucomalachite green	331.2	316.2/239.2*	17.57	380	20/28	+	Leucomalachite green- <i>d</i> <sub>6</sub>
Malachite green	329.2	313.1*/284.1	14.66	380	41/65	+	Malachite green- <i>d</i> <sub>5</sub>
Clonidine	230.0	133.1/74.2*	4.55	380	53/73	+	
Cyproheptadine	288.2	215.1/96.2*	13.34	380	65/25	+	
Carbofuran	222.1	165.1*/123.0	13.62	380	13/25	+	Carbofuran- <i>d</i> <sub>5</sub>
Chlordimeform	197.1	125.0/117.1*	6.55	380	33/29	+	
Fipronil	434.9	278.0/250.0*	16.16	380	37/33	-	Fipronil- <sup>13</sup> C <sub>4</sub> <sup>15</sup> N <sub>2</sub>
IS							
Cimbuterol- <i>d</i> <sub>9</sub>	243.2	160.9	4.35	380	17	+	
Zilpaterol- <i>d</i> <sub>7</sub>	269.0	202.9	3.62	380	21	+	
2-QCA- <i>d</i> <sub>4</sub>	179.0	132.9	5.22	380	21	+	
Metronidazole-hydroxy- <i>d</i> <sub>2</sub>	190.1	127.9	3.76	380	17	+	
Pefloxacin- <i>d</i> <sub>5</sub>	339.2	321.1	5.47	380	25	+	
Dapsone- <i>d</i> <sub>8</sub>	257.4	159.9	7.76	380	17	+	
Carbofuran- <i>d</i> <sub>5</sub>	225.1	164.8	13.62	380	13	+	
Fipronil- <sup>13</sup> C <sub>4</sub> <sup>15</sup> N <sub>2</sub>	441.0	251.8	16.16	380	29	-	
Leucomalachite green- <i>d</i> <sub>6</sub>	337.3	240.1	17.57	380	37	+	
Malachite green- <i>d</i> <sub>5</sub>	334.2	318.2	14.66	380	41	+	

混合中间标准溶液 用 15% 乙腈溶液稀释上述标准储备溶液, 制备成混合中间标准溶液, 其中  $\beta$ -受体激动剂、氯霉素、镇静剂和其他类别的浓度为  $10 \text{ ng} \cdot \text{mL}^{-1}$ ; 氟喹诺酮类浓度为  $20 \text{ ng} \cdot \text{mL}^{-1}$ ; 硝基咪唑类浓度为  $200 \text{ ng} \cdot \text{mL}^{-1}$ ; 喹噁啉类浓度为  $2\ 500 \text{ ng} \cdot \text{mL}^{-1}$ ; 巴比妥类浓度为  $5\ 000 \text{ ng} \cdot \text{mL}^{-1}$ 。用 15% 乙腈溶液稀释内标标准储备溶液, 制备了混合中间内标溶液, 其中氨苯砜-*d*<sub>8</sub>、齐帕特罗-*d*<sub>7</sub>、西布特罗-*d*<sub>9</sub>、克百威-*d*<sub>3</sub>、氟虫腈-<sup>13</sup>C<sub>4</sub><sup>15</sup>N<sub>2</sub>、孔雀石绿-*d*<sub>5</sub>和隐色孔雀石绿-*d*<sub>6</sub>的浓度为  $10 \text{ ng} \cdot \text{mL}^{-1}$ ; 培氟沙星-*d*<sub>5</sub>的浓度为  $20 \text{ ng} \cdot \text{mL}^{-1}$ ; 羟基甲硝唑-*d*<sub>2</sub>的浓度为  $200 \text{ ng} \cdot \text{mL}^{-1}$ ; 2-QCA-*d*<sub>4</sub>的浓度为  $2\ 500 \text{ ng} \cdot \text{mL}^{-1}$ 。

标准曲线工作溶液 取空白山羊角样品粉末 (未检出 53 种禁用兽药) 0.5 g, 精密称定, 置于 50 mL 聚苯乙烯具塞离心管中, 同供试品溶液制备方法制成空白基质溶液。精密吸取 2、4、10、15 和 20  $\mu\text{L}$  混合中间标准溶液和 10  $\mu\text{L}$  混合中间内标溶液, 用空白基质溶液定容至 200  $\mu\text{L}$ 。

供试品溶液制备方法 取山羊角样品适量, 研细, 准确称取 0.5 g, 置于 50 mL 聚苯乙烯具塞离心管中, 加入 50  $\mu\text{L}$  混合中间内标溶液, 加水 10 mL, 涡旋使充分浸润, 加入 1% 甲酸乙腈溶液 15 mL, 涡旋使其混匀, 置振荡器上剧烈振荡 (每分钟 500 次) 10 min。将 NaCl: Na<sub>2</sub>SO<sub>4</sub> (1:4) 混合粉末 5 g 加入离心管中, 再置振荡器

上剧烈振荡 (每分钟 500 次) 3 min, 离心 5 min, 使溶液分层。吸取上清液 3 mL, 在 40 °C 水浴下浓缩至干, 精密加 0.2 mL 15% 乙腈溶液, 涡旋 1 min, 混匀, 转速离心 ( $15\ 000 \text{ r} \cdot \text{min}^{-1}$ ) 2 min, 取上清液, 即得。

系统适用性试验 取最低浓度水平的标准曲线工作溶液, 进样测定, 按上述条件测定的 MRM 色谱峰的信噪比均应大于 10:1。

#### 方法学验证

线性关系 取标准曲线工作溶液进样测定, 齐帕特罗-*d*<sub>7</sub>作为沙丁胺醇和齐帕特罗的内标; 西布特罗-*d*<sub>9</sub>作为西马特罗、特布他林、克仑特罗、西布特罗、溴布特罗和福莫特罗的内标; 培氟沙星-*d*<sub>5</sub>作为氟喹诺酮类的内标; 羟基甲硝唑-*d*<sub>2</sub>作为二甲硝咪唑、2-羟基二甲硝咪唑、羟基甲硝唑、甲硝唑、罗硝唑和异丙硝唑的内标; 2-QCA-*d*<sub>4</sub>作为喹噁啉类的内标; 氨苯砜-*d*<sub>8</sub>、克百威-*d*<sub>3</sub>、氟虫腈-<sup>13</sup>C<sub>4</sub><sup>15</sup>N<sub>2</sub>、孔雀石绿-*d*<sub>5</sub>和隐色孔雀石绿-*d*<sub>6</sub>分别作为氨苯砜、克百威、氟虫腈、孔雀石绿和隐色孔雀石绿的内标; 其余化合物以外标法定量, 绘制标准曲线。

专属性 通过检测不含 53 种兽药的空白样品来评估该方法的选择性。

基质效应 样品的基质成分可能会干扰目标化合物的电离效率。为了评价基质效应 (ME), 制备了空白基质匹配标准曲线和溶剂标准曲线, 以  $\text{ME} = (\text{空白基质匹配标准曲线斜率} / \text{溶剂标准曲线斜率}) \times 100\%$  的

值来表示基质效应的大小。

**检测限** 定量限 (LOQ) 是准确度和精确度均可接受的最低加样水平。检出限 (LOD) 是能从背景噪声中检测到信号的最低加样水平 ( $S/N = 3$ , 且相对离子丰度比符合要求)。

**准确度** 取样品, 分别添加低 (定量限)、中 (2倍定量限)、高 (10倍定量限) 3个水平的对照品溶液, 每个水平做6个平行样, 共18份, 按上述供试品溶液制备方法和色谱质谱条件进行分析, 计算回收率。

**重复性** 取同一批供试品溶液, 按准确度加样平行制备6份供试品溶液, 按色谱和质谱条件进行测定, 计算重复性 RSD。

**稳定性** 取同一水平加样溶液, 在室温下于 24 h 内每间隔 4 h 进样测定, 共进样 6 次, 计算稳定性 RSD。

## 结果

### 1 方法学验证

**1.1 线性关系** 空白基质匹配标准曲线在各自的浓度范围内表现出良好的线性,  $r$  值均  $\geq 0.99$ , 线性范围及相关系数见表 3。

**1.2 专属性** 空白基质色谱图在目标化合物的保留时间处, 均未检测到干扰峰。

**1.3 基质效应** 53 种化合物的 ME 值为 36.07%~268.90%。其中 30 种化合物的 ME 值为 70%~130%, 基质效应较小; 17 种化合物呈现出基质抑制作用; 6 种化合物呈现出基质增强作用, 结果见表 3。

**1.4 检测限** 53 种化合物的检出限 (LOD) 为 0.1~50  $\mu\text{g}\cdot\text{kg}^{-1}$ , 定量限为 (LOQ) 为 0.2~100  $\mu\text{g}\cdot\text{kg}^{-1}$ , 结果见表 3。

**1.5 准确度** 根据 CAC/GL 71-2009《与食用动物的兽药使用有关的国家食品安全保障监管方案的设计与执行指南》<sup>[32]</sup> 要求, 当兽药残留水平小于 1  $\mu\text{g}\cdot\text{kg}^{-1}$  时, 可接受的回收率为 50%~120%, 兽药残留水平 1~10  $\mu\text{g}\cdot\text{kg}^{-1}$  的回收率应为 60%~120%, 10~1 000  $\mu\text{g}\cdot\text{kg}^{-1}$  的回收率应为 70%~120%。结果表明, 在三个加样水平下各化合物的回收率范围为 70.4%~118.7%, 回收率均良好 (表 4)。

**1.6 重复性** 在三个加样水平下, 一式六份重复性 RSD 为 0.69%~12.07%, 重复性良好 (表 4)。

**1.7 稳定性** 53 种化合物的稳定性 RSD 为 2.16%~13.22%, 结果表明 24 h 内稳定性良好。

### 2 样品测定

采用所建立的方法对 27 批山羊角样品进行了测定, 其中产地为内蒙古的 2 批样品分别检出艾司唑仑 0.92  $\mu\text{g}\cdot\text{kg}^{-1}$  和氧氟沙星 10.78  $\mu\text{g}\cdot\text{kg}^{-1}$ , 产地为浙江的

2 批样品分别检出氧氟沙星 7.04、4.73  $\mu\text{g}\cdot\text{kg}^{-1}$ 。禁用兽药检出率为 14.8%。

在我国, 艾司唑仑被禁止用于饲料和动物饮用水中; 氧氟沙星已停止用于食用动物, 其最大残留限量在 GB31650.1-2022 中规定为 2  $\mu\text{g}\cdot\text{kg}^{-1}$ 。但在药用动物中是否禁用尚无明确规定。

## 讨论

山羊角主要由蛋白质类、多肽类、核苷类和无机元素类组成, 几乎不含脂肪。文献<sup>[33]</sup>报道, 纯乙腈作提取溶剂时添加一定的水相, 可以提高萃取效率, 并更有效地去除蛋白质或其他基质组分, 本方法采用乙腈水溶液作为提取溶剂。经实验比较, 于乙腈水溶液中加入无机盐可使两相分离, 去除水溶性基质成分干扰, 因此采用盐析辅助液液萃取作为提取方法。

研究中考察了 3 种提取溶剂 [乙酸乙酯、乙腈、乙腈-乙酸乙酯 (1:1)], 结果表明乙酸乙酯对于氟喹诺酮类提取效率较差; 比较了提取溶剂的 pH 对提取效率的影响, 结果表明在 1% 甲酸乙腈溶液条件下提取效率最好。在盐析步骤中, 考察了  $\text{NaCl-Na}_2\text{SO}_4$  (1:4)、 $\text{NaCl-MgSO}_4$  (1:4)、 $\text{MgSO}_4\text{-NaOAc}$  (4:1)、 $\text{NaCl-MgSO}_4$ -柠檬酸三钠-柠檬酸氢二钠 (1:4:1:0.5) 四种盐包对回收率的影响, 结果表明  $\text{NaCl-Na}_2\text{SO}_4$  的回收率最好, 而加入  $\text{MgSO}_4$  后释放大量的热导致部分化合物损失。

在评估净化方法时, 比较了 5 种方法: MCX、MAX、d-SPE、HLB 和不净化。结果表明, MCX 和 MAX 专属性较强, 不适用于多残留同时筛查; d-SPE 和 HLB 会造成部分化合物的损失; 而提取后不净化的回收率普遍较好。

沙丁胺醇和莱克多巴胺等  $\beta_2$ -受体激动剂类易在动物体内代谢发生醛酸化和硫酸化, 以游离态和结合态两种形式存在<sup>[34]</sup>。文献多采用酶解方式处理, 也有采用酸性条件下直接提取的报道<sup>[35]</sup>。本研究基于禁用兽药筛查的测定目的, 兼顾多数兽药的前处理, 采用酸性提取的方式主要针对游离态进行测定。如筛查为阳性结果, 可另行建立酶解方法准确测定。

由于外标法在定量某些化合物时准确性较差, 因此有必要采用内标法定量。内标的理化性质应与被定量化合物的性质相似, 因此使用稳定的同位素标记物作为内标是最佳选择。由于结构相似的化合物通常表现出相近的理化性质, 某一化合物的同位素内标可作为具有相似结构化合物的共用内标, 从而有助于降低检测成本。例如,  $\beta$ -受体激动剂的基本结构是  $\beta$ -苯乙醇胺, 西布特罗的  $[\text{M}+\text{H}]^+$  母离子容易失去一个  $\text{H}_2\text{O}$  和一个  $\text{C}_4\text{H}_8$  分子, 形成  $m/z$  160 的碎片离子。而大多数  $\beta$ -

**Table 3** The LODs, LOQs, linear equations, ranges, and MEs for 53 analytes

Compound	LOD/ $\mu\text{g}\cdot\text{kg}^{-1}$	LOQ/ $\mu\text{g}\cdot\text{kg}^{-1}$	Linear equation	Linear range/ $\mu\text{g}\cdot\text{L}^{-1}$	<i>r</i>	ME/%
<i><math>\beta</math></i> -Agonist						
Clorprenaline	0.1	0.2	$y = 5\,841\,216.26x + 87\,425.55$	0.1-1	0.995	95.64
Tulobuterol	0.1	0.2	$y = 6\,027\,646.87x + 18\,522.54$	0.1-1	1.000	86.96
Ractopamine	0.1	0.2	$y = 2\,747\,991.54x + 29\,099.74$	0.1-1	1.000	66.30
Bambuterol	0.1	0.2	$y = 5\,194\,463.70x + 44\,581.13$	0.1-1	0.998	89.90
Phenylethanolamine A	0.1	0.2	$y = 4\,057\,591.55x + 99\,910.70$	0.1-1	0.998	128.25
Mabuterol	0.1	0.2	$y = 5\,193\,449.89x + 48\,044.08$	0.1-1	0.999	69.61
Salbutamol	0.1	0.2	$y = 13.89x - 0.53$	0.1-1	0.993	73.17
Zilpaterol	0.1	0.2	$y = 3.15x - 0.27$	0.1-1	0.991	65.09
Cimaterol	0.1	0.2	$y = 0.41x - 0.01$	0.1-1	0.995	49.36
Terbutaline	0.1	0.2	$y = 1.19x - 0.01$	0.1-1	0.997	55.38
Clenbuterol	0.1	0.2	$y = 0.88x + 0.02$	0.1-1	0.996	78.58
Cimbuterol	0.1	0.2	$y = 1.26x + 0.09$	0.1-1	0.997	73.32
Brombuterol	0.1	0.2	$y = 0.20x + 0.01$	0.1-1	0.997	62.23
Formoterol	0.1	0.2	$y = 1.48x + 0.02$	0.1-1	0.993	101.37
Amphenicol						
Chloramphenicol	0.1	0.2	$y = 95\,516.35x - 14.83$	0.1-1	0.999	69.46
Tranquilizer						
Chlorpromazine	0.1	0.2	$y = 4\,311\,198.01x - 123\,375.27$	0.1-1	0.998	91.62
Methaqualone	0.1	0.2	$y = 5\,181\,626.41x - 67\,016.44$	0.1-1	0.998	76.57
Xylazine	0.1	0.2	$y = 1\,414\,153.91x + 11\,363.70$	0.1-1	1.000	86.53
Estazolam	0.1	0.2	$y = 1\,079\,542.68x + 12\,236.56$	0.1-1	0.998	116.99
Midazolam	0.1	0.2	$y = 1\,694\,281.78x + 2\,928.75$	0.1-1	0.998	58.64
Triazolam	0.1	0.2	$y = 784\,153.47x - 427.84$	0.1-1	0.999	107.84
Alprazolam	0.1	0.2	$y = 722\,016.87x - 7\,329.70$	0.1-1	0.999	73.13
Diazepam	0.1	0.2	$y = 563\,673.41x - 10\,515.61$	0.1-1	0.999	81.37
Nitrazepam	0.1	0.2	$y = 75\,022.22x - 1\,339.64$	0.1-1	0.997	57.22
Oxazepam	0.1	0.2	$y = 374\,484.50x + 7\,089.87$	0.1-1	0.999	263.27
Clonazepam	0.1	0.2	$y = 51\,232.53x + 520.24$	0.1-1	0.996	52.90
Lorazepam	0.1	0.2	$y = 261\,090.69x + 2\,415.79$	0.1-1	0.997	234.22
Phenobarbital	50	100	$y = 442.00x + 3\,375.35$	50-500	0.998	91.27
Barbital	50	100	$y = 349.63x - 3\,857.60$	50-500	0.998	63.24
Amobarbital	50	100	$y = 1\,200.29x + 15\,337.73$	50-500	0.997	85.00
Secobarbital	50	100	$y = 554.73x - 4\,923.25$	50-500	0.998	39.30
Fluoroquinolone						
Norfloxacin	0.2	0.4	$y = 0.37x - 0.03$	0.2-2	0.993	268.90
Pefloxacin	0.2	0.4	$y = 0.47x + 0.01$	0.2-2	0.999	177.75
Lomefloxacin	0.2	0.4	$y = 0.13x - 0.00$	0.2-2	0.996	60.97
Ofloxacin	0.2	0.4	$y = 0.42x - 0.01$	0.2-2	0.997	111.81
Nitroimidazole						
Dimetridazole	2	4	$y = 0.17x - 0.04$	1-10	0.999	86.52
Dimetridazole-2-hydroxy	2	4	$y = 0.05x - 0.05$	1-10	0.996	36.07
Metronidazole-hydroxy	2	4	$y = 0.09x + 0.15$	1-10	0.995	57.96
Metronidazole	2	4	$y = 0.52x - 0.08$	1-10	0.996	161.30
Ronidazole	2	4	$y = 0.72x - 0.40$	1-10	0.998	142.78
Ipronidazole	2	4	$y = 0.22x + 0.08$	1-10	0.999	78.37
Tinidazole	2	4	$y = 310\,695.06x + 52\,015.05$	1-10	1.000	91.53
Quinoxaline						
2-QCA	25	50	$y = 0.534x + 0.03$	25-250	0.996	112.92
MQCA	25	50	$y = 5.11x + 0.01$	25-250	0.998	120.07
Carbadox	25	50	$y = 35.29x + 0.40$	25-250	0.999	109.83
Others						
Dapsone	0.1	0.2	$y = 1.46x - 0.01$	0.1-1	0.997	95.36
Leucomalachite green	0.1	0.2	$y = 0.59x - 0.06$	0.1-1	0.995	108.42
Malachite green	0.1	0.2	$y = 1.46x - 0.18$	0.1-1	0.996	60.44
Clonidine	0.1	0.2	$y = 301\,538.77x - 7\,590.75$	0.1-1	0.997	76.02
Cyproheptadine	0.1	0.2	$y = 2\,368\,886.83x - 24\,673.13$	0.1-1	0.998	78.51
Carbofuran	0.1	0.2	$y = 0.82x - 0.00$	0.1-1	0.999	49.77
Chlordimeform	0.1	0.2	$y = 1\,083\,880.45x + 15\,761.62$	0.1-1	0.996	84.15
Fipronil	0.1	0.2	$y = 0.86x - 0.03$	0.1-1	1.000	122.19

**Table 4** Recoveries and RSDs for 53 compounds at three spiked levels

Compound	1 LOQ		2 LOQ		10 LOQ	
	Recovery/%	RSD/%	Recovery/%	RSD/%	Recovery/%	RSD/%
<i><math>\beta</math></i> -Agonist						
Clorprenaline	81.65	8.99	83.27	5.52	93.53	6.40
Tulobuterol	84.69	7.12	86.37	3.26	90.42	2.34
Ractopamine	74.17	10.29	71.26	8.35	84.82	5.42
Bambuterol	76.66	9.54	87.41	5.04	98.90	1.99
Phenylethanolamine A	70.40	6.93	72.93	5.44	92.14	4.17
Mabuterol	77.92	6.10	87.31	2.51	92.87	2.97
Salbutamol	101.52	9.87	93.61	9.07	94.50	8.53
Zilpaterol	114.34	5.21	99.62	5.73	89.48	10.47
Cimaterol	106.96	7.20	83.02	11.04	92.25	7.08
Terbutaline	94.65	9.77	89.19	9.41	82.22	8.99
Clenbuterol	106.31	5.20	116.42	10.44	117.36	7.95
Cimbuterol	79.27	10.45	86.07	9.81	118.64	7.43
Brombuterol	102.95	9.44	113.33	11.05	117.89	1.95
Formoterol	111.92	4.43	105.16	4.67	118.70	3.44
Amphenicol						
Chloramphenicol	85.65	2.32	88.29	2.71	94.70	3.43
Tranquilizer						
Chlorpromazine	70.92	4.22	81.41	3.42	84.73	9.71
Methaqualone	95.55	8.78	93.92	5.17	96.72	3.60
Xylazine	76.42	5.38	88.01	3.18	97.89	3.97
Estazolam	94.54	6.29	93.84	3.96	102.29	6.10
Midazolam	80.00	7.39	93.12	3.64	102.31	6.14
Triazolam	92.08	7.38	107.75	2.21	116.78	4.37
Alprazolam	110.67	8.49	116.83	2.65	118.03	4.62
Diazepam	97.93	10.26	102.39	6.70	106.23	4.73
Nitrazepam	74.28	7.48	81.54	8.25	86.06	4.63
Oxazepam	78.74	8.78	82.45	8.16	93.86	4.92
Clonazepam	86.37	6.43	81.12	10.44	95.40	8.76
Lorazepam	80.59	8.15	81.34	7.86	91.48	3.71
Phenobarbital	83.52	3.85	96.46	5.93	100.19	1.45
Barbital	93.87	7.54	87.32	5.99	97.23	6.21
Amobarbital	75.73	4.05	87.45	6.94	94.54	3.05
Secobarbital	85.78	4.88	86.79	6.58	86.73	5.35
Fluoroquinolone						
Norfloxacin	100.39	5.02	89.32	1.93	84.17	4.61
Pefloxacin	86.99	2.91	94.55	4.22	95.97	3.97
Lomefloxacin	109.14	4.29	104.26	2.36	104.11	3.60
Ofloxacin	112.11	4.70	101.32	2.14	96.25	7.12
Nitroimidazole						
Dimetridazole	104.62	8.29	89.95	1.55	94.29	9.77
Dimetridazole-2-hydroxy	113.47	8.58	99.60	5.50	95.19	12.07
Metronidazole-hydroxy	97.53	7.23	101.34	4.77	97.18	5.16
Metronidazole	86.93	10.67	90.35	4.39	91.06	7.65
Ronidazole	113.83	7.29	92.73	4.15	94.21	9.84
Ipronidazole	92.84	8.01	81.48	8.47	86.78	10.11
Tinidazole	78.74	8.11	88.82	5.84	101.48	5.16
Quinoxaline						
2-QCA	84.68	10.03	100.67	8.45	98.38	1.21
MQCA	109.14	6.24	100.36	7.68	98.07	0.69
Carbadox	104.18	9.14	101.76	6.11	99.31	6.42
Others						
Dapsone	89.94	4.16	97.97	3.55	96.88	6.76
Leucomalachite green	105.46	10.96	80.71	8.69	94.35	3.47
Malachite green	103.38	8.54	80.43	2.38	71.39	1.33
Clonidine	95.29	9.74	86.11	5.31	87.89	8.25
Cyproheptadine	74.09	3.94	71.91	6.14	83.47	2.67
Carbofuran	88.18	4.05	93.99	5.05	98.36	2.23
Chlordimeform	78.45	5.35	80.89	5.82	101.92	2.03
Fipronil	96.04	4.31	90.39	4.15	91.07	6.02

受体激动剂相似的质谱裂解规律,因此西布特罗- $d_9$ 可用于西马特罗、特布他林、克仑特罗、西布特罗、溴布特罗和福莫特罗的定量。然而,由于共馏出组分引起的基质效应,具有相似保留时间的齐帕特罗和沙丁胺醇选择齐帕特罗- $d_7$ 作为内标。同样,选择培氟沙星- $d_5$ 、羟基甲硝唑- $d_2$ 和2-QCA- $d_4$ 分别作为氟喹诺酮类、硝基咪唑类和喹噁啉类的共用内标。

综上,本研究建立的盐析辅助液液萃取结合UHPLC/MS/MS方法,可用于定性定量检测山羊角中53种禁用兽药残留,方法专属准确,前处理简便,已应用于实际样品的检测,有效弥补了动物源性中药中兽药残留检测的空白,发现了一定的安全风险。

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