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Simple and Sensitive Analysis of Clenbuterol in Urine Matrices by UHPLC-MS/MS Method with Online-SPE Sample Preparation

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Supplementary Material

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Table S1. Gradient of the mobile phase and positions of the switching valve in the SPE–UHPLC–MS/MS method.

t [min]	Solvent B [%]	Valve Position	Process
0.00	5	1	loading & washing
6.00	5	2	separating & measuring
6.50	5	2	
11.00	95	2	
13.00	95	2	
13.01	5	2	re–equilibrating
17.00	5	1	
20.00	5	1	

Solvent B—methanol

Table S2. Recovery and matrix effect of the developed SPE–UHPLC–MS/MS method from the analysis of QC samples in Surine™.

Nominal [ng/mL]	Found [ng/mL]	Recovery [%], $n = 5$	Matrix effect without IS [%], $n = 5$	Matrix effect with IS [%], $n = 5$
0.5	0.45	101.56	15.84	1.56
2.5	2.35	106.82	15.35	6.82
25	23.64	100.18	-10.53	-0.18

IS—internal standard

Table S3. Recovery and matrix effect of the developed SPE–UHPLC–MS/MS method from the analysis of QC samples in pooled urine.

Nominal [ng/mL]	Found [ng/mL]	Recovery [%], $n = 5$	Matrix effect without IS [%], $n = 5$	Matrix effect with IS [%], $n = 5$
0.5	0.47	106.08	23.04	6.08
2.5	2.47	112.28	20.67	12.28
25	23.77	100.73	-0.44	0.73

IS—internal standard

Table S4. Stability of clenbuterol from the analysis of QC samples in pooled urine.

Conditions	Nominal [ng/mL]	Found [ng/mL]	Recovery [%], $n = 5$
Autosampler stability in urine at 8°C after 24 h	0.5	0.47	98.09
	2.5	2.41	98.05
	25	25.66	97.67
Freeze–thaw stability in urine (-80°C, after the third cycle)	0.5	0.47	98.68
	2.5	2.36	95.94
	25	25.86	98.40
Benchtop stability in urine after 24 h	0.5	0.47	97.35
	2.5	2.46	100.24
	25	26.44	100.63

Table S5. Comparison of retention time of two MRM transitions for blinded samples in Surine™.

Blinded sample	Retention time IS [min] $n = 3$		Retention time analyte [min] $n = 3$		Difference [%] Max. tolerated: 0.5%
	Sample	Reference	Sample	Reference	
1	9.516	9.526	9.537	9.545	0.09
2	9.480	9.526	9.499	9.545	0.49
3	9.486	9.526	9.506	9.545	0.44
4	9.513	9.526	9.534	9.545	0.13
5	9.488	9.526	9.508	9.545	0.39
6	9.485	9.526	9.496	9.545	0.40
7	9.482	9.526	9.502	9.545	0.45
8	9.518	9.526	9.539	9.545	0.07
9	9.487	9.526	9.497	9.545	0.45
10	9.483	9.526	9.503	9.545	0.44

IS—internal standard

Table S6. Relative abundances of two MRM transitions for blinded samples in Surine™.

Blinded sample	Transition [m/z] = 277	Relative abundance [%] $n = 3$		Difference [%]	Max. tolerated: Window ±5 (reference)
		Sample	Reference		
1	→ 203	100	100	0.96	>0–9.63%
	→ 132.1	5.59	4.63		
2	→ 203	100	100	0.65	>0–9.63%
	→ 132.1	5.28	4.63		
3	→ 203	100	100	0.32	>0–9.63%
	→ 132.1	4.95	4.63		
4	→ 203	100	100	0.98	>0–9.63%
	→ 132.1	5.61	4.63		
5	→ 203	100	100	0.18	>0–9.63%
	→ 132.1	4.81	4.63		
6	→ 203	100	100	0.54	>0–9.63%
	→ 132.1	5.17	4.63		
7	→ 203	100	100	0.67	>0–9.63%
	→ 132.1	5.3	4.63		
8	→ 203	100	100	1.35	>0–9.63%
	→ 132.1	5.98	4.63		
9	→ 203	100	100	0.68	>0–9.63%
	→ 132.1	5.31	4.63		
10	→ 203	100	100	0.92	>0–9.63%
	→ 132.1	5.55	4..63		

Table S7. Comparison of retention time of two MRM transitions for blinded samples in urine.

Blinded sample	Retention time IS [min] $n = 3$		Retention time analyte [min] $n = 3$		Difference Max. tolerated: 0.5%
	Sample	Reference	Sample	Reference	
1	9.509	9.526	9.527	9.545	0.18
2	9.549	9.526	9.525	9.545	0.23
3	9.542	9.526	9.568	9.545	0.21
4	9.516	9.526	9.536	9.545	0.10
5	9.571	9.526	9.539	9.545	0.26
6	9.545	9.526	9.565	9.545	0.21
7	9.534	9.526	9.554	9.545	0.09
8	9.581	9.526	9.512	9.545	0.46
9	9.519	9.526	9.541	9.545	0.06
10	9.524	9.526	9.527	9.545	0.11

IS—internal standard

Table S8. Relative abundances of two MRM transitions for blinded samples in urine.

Blinded sample	Transition [m/z] = 277	Relative abundance [%] $n = 3$		Difference [%]	Max. tolerated: Window ±5 (reference)
		Sample	Reference		
1	→ 203	100	100	0.36	>0–9.63%
	→ 132.1	4.96	4.63		
2	→ 203	100	100	1.06	>0–9.63%
	→ 132.1	5.69	4.63		
3	→ 203	100	100	0.6	>0–9.63%
	→ 132.1	4.03	4.63		
4	→ 203	100	100	1.24	>0–9.63%
	→ 132.1	5.87	4.63		
5	→ 203	100	100	0.24	>0–9.63%
	→ 132.1	4.87	4.63		
6	→ 203	100	100	0.28	>0–9.63%
	→ 132.1	4.91	4.63		
7	→ 203	100	100	0.75	>0–9.63%
	→ 132.1	5.38	4.63		
8	→ 203	100	100	0.39	>0–9.63%
	→ 132.1	5.02	4.63		
9	→ 203	100	100	0.1	>0–9.63%
	→ 132.1	4.73	4.63		
10	→ 203	100	100	2.16	>0–9.63%
	→ 132.1	6.89	4.63		

Table S9. Precision (%RSD) and accuracy (%RE) of the developed SPE–UHPLC–MS/MS method from analysis of blinded samples in Surine™.

Blinded Sample	Nominal [ng/mL]	Found [ng/mL]	RSD [%] *n* = 3	RE [%] *n* = 3
1	2	1.99	0.56	0
2	40	38.75	1.87	-3
3	1	0.99	3.10	-1
4	2.5	2.71	0.64	-8
5	0.5	0.57	0.36	-12
6	5	5.60	0.92	-11
7	0.2	0.22	1.21	-9
8	1.25	1.21	1.77	-3
9	50	51.64	0.85	-3
10	12.5	13.34	0.46	-6

RSD—relative standard deviation; RE—relative error

Table S10. Precision (%RSD) and accuracy (%RE) of the developed SPE–UHPLC–MS/MS method from analysis of blinded samples in urine.

Blinded Sample	Nominal [ng/mL]	Found [ng/mL]	RSD [%] *n* = 3	RE [%] *n* = 3
1	10	10.02	1.34	0
2	2	2.05	0.67	-2
3	0.5	0.48	0.97	-4
4	12.5	12.63	1.33	-1
5	1.25	1.18	0.69	-6
6	1	1.07	5.40	-7
7	50	48.95	0.31	-2
8	0.2	0.21	2.75	-5
9	2.5	2.48	0.17	-1
10	0.4	0.45	1.29	-11

RSD—relative standard deviation; RE—relative error