Solid-Phase Extraction of Clenbuterol in Plasma and analysis by LDTD–MS/MS

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Introduction

Analysis of performance-enhancing drug in plasma can require a sample clean-up step to reduce the interference effect from the matrix. To obtain an optimal sample clean-up, the Silia*Prep*™ CleanDrug SPE cartridges are used in the extraction procedure prior to the Laser Diode Thermal Desorption (LDTD) analysis.

The LDTD ion source uses an infrared laser diode to desorb samples that have been previously dried onto a 96-well LazWell™ plate after sample preparation extraction. The rapid desorption produces neutral species which are carried into a corona discharge region to undergo an efficient protonation and are subsequently transferred directly into the mass spectrometer for detection.

Solid Phase Cartridge

The Silia*Prep* CleanDrug cartridge is used for the sample extraction procedure.

Figure 1: Silia*Prep* CleanDrug SPE cartridge

Silia*Prep* CleanDRUG Formats		
Formats	Qty / Box	Product Number
Silia*Prep* SPE Cartridges		
1 mL / 50 mg	100	SPEC-R651230B-01B
1 mL / 100 mg	100	SPEC-R651230B-01C
3 mL / 200 mg	50	SPEC-R651230B-03G
3 mL / 500 mg	50	SPEC-R651230B-03P
6 mL / 500 mg	50	SPEC-R651230B-06P
6 mL / 1g	50	SPEC-R651230B-06S
Silia*Prep* 96-Well Plates		
2 mL / 50 mg	1	96W-R651230B-B
2 mL / 100 mg	1	96W-R651230B-C

Table 1: Silia*Prep* CleanDRUG Product Number

LDTD-MS/MS System

Figure 2: LDTD system on Thermo Vantage Mass Spectrometer.

Sample Method

Extraction procedure

Cartridge:	Silia*Prep* CleanDrug (1 mL / 100 mg)
Pre-Wash:	2 x 1 mL EtOAc/IPA/NH_4OH (78/20/2)
Activation:	1 mL MeOH
	1 mL Water
	1 mL Na Acetate (100 mM, pH 6) in Water
Load:	500 µL sample
	50 µL IS (Clenbuterol-d9 at 20 ng/mL in MeOH)
	500 µL Na Acetate (100 mM, pH 6) in Water
Wash 1:	1 mL Water
Wash 2:	1 mL Acetic Acid (1N in Water)
Wash 3:	2 x 1 mL MeOH
Elution:	1 mL EtOAc/IPA/NH_4OH (78/20/2)
	Evaporate to dryness
	Reconstitute with 100 µL of MeOH/Water (75/25)
	Spot: 4 µL in LazWell plate

LDTD-MS/MS Parameters

LDTD

Gas Flow:	3 L/min	
Laser pattern:	Time (s)	Power (%)
	0	0
	2	0
	5	45
	5.1	0
	6.6	0

MS/MS Method

	Transition	CE	S-Lens
Clenbuterol	277->132	25	85
Clenbuterol-d9	286->133	25	85
Mode:	Positive		

For Research Use Only. Not for use in diagnostic procedures

Results and Discussion

Linearity Results

As shown in Figure 3, excellent linearity ($r^2 > 0.99$) with no signs of carryover effect is achieved within the quantification range (50 to 10,000 pg/mL).

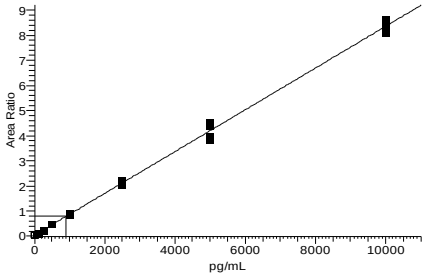

Figure 3: Clenbuterol Standard Curve

	r^2	Slope (ratio area / concentration)	y-Intercept
Run 1	0.9947	0.0008	0.0369
Run 2	0.9972	0.0008	0.0393
Run 3	0.9975	0.0009	0.0283

Table 2: Calibration Curve Parameters

Accuracy and Precision

As shown on Table 3 and 4, the inter-run and intra-run accuracy and the precision are between 93.6 to 109.2% and 2.7 to 8.3% respectively.

	QC-Low	QC-Med	QC-High
Conc. (pg/mL)	100	500	2500
N	18	18	18
Mean (pg/mL)	94.10	545.78	2532.09
%RSD	5.2	4.8	5.5
%Nom	94.1	109.2	101.3

Table 3: Inter-run Precision and Accuracy for Clenbuterol

	LLOQ	QC-Low	QC-Med	QC-High	ULOQ
Conc. (pg/mL)	50	100	500	2500	10000
N	6	6	6	6	6
Mean (pg/mL)	48.49	93.64	545.33	2549.59	1002.86
%RSD	8.3	2.8	2.7	7.8	7.0
%Nom	103.3	93.6	109.1	102.0	99.7

Table 4: Intra-run Precision and Accuracy for Clenbuterol

Matrix effect

Matrix effect was evaluated at 100 and 1000 pg/mL in 3 different plasmas, as shown in Table 5. No matrix effect was detected, and the accuracy and precision are between 84.9 to 99.5% and 3.87 to 13.31% respectively.

	Plasma 1		Plasma 2		Plasma 3	
Conc. (pg/mL)	100	1000	100	1000	100	1000
N	9	9	9	9	9	9
Mean (pg/mL)	89.7	849.3	85.4	959.7	88.3	995.3
%RSD	11.13	4.66	4.66	3.87	13.31	4.26
%Nom	89.7	84.9	85.4	96.0	88.3	99.5

Table 5: Calibration Curve Parameters

Stability Verification

Following the SPE extraction process, all samples were stored at 4°C to evaluate the wet stability of the drugs. After 72h, all samples were re-spotted and analyzed. Linearity, precision and accuracy were evaluated to determine the stability. Table 6 shows that a wet stability of 72h is obtained with good precision and accuracy of LOQ standard.

The stability of dry samples in LazWell plate was also determined. All standards and QCs are spotted, dried and kept at room temperature for 48h. Then, standards and QCs were analyzed and the linearity, precision and accuracy are verified. Table 5 shows the dry stability results and the

	Wet Stability	Dry in LazWell (RT)
Time (h)	72h	48h
Temp. (°C)	4°C	RT
Conc. (pg/mL)	50	50
N	6	6
Mean (pg/mL)	48.25	49.91
%RSD	11.67	8.80
%Nom	96.5	99.8

Table 6: Stability Results for Clenbuterol

Conclusions

The sample solid extraction using Silia*Prep* CleanDrug SPE cartridges ensures accurate and precise results with a linear standard curve ($r^2 > 0.99$).

A fast analysis can be reach using LDTD-MS/MS system. This system allows a total sample-to-sample analysis time of 8 seconds.

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