

Dispersive Pipette Extraction (DPX) of Benzoylecgonine in Urine with analysis by LDTD-MS/MS

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Introduction

Analysis of certain drugs of abuse in urine can require a sample clean-up step to reduce the interference effect from the matrix. To obtain an optimal sample clean-up, Dispersive Pipette eXtraction (DPX) technology is used. This technology uses a pipette tip to provide fast and efficient sample clean-up with extractions in less than 1 minute. The extraction is simply aspirate and dispense using a pipette tip containing loose sorbent. The tips can be used with automated liquid handling systems or with a semi-automated extractor. The DPX Polar tips 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.

Dispersive Pipette Extraction (DPX)

The DPX Polar tip is used for the sample extraction procedure.



Figure 1: DPX WAX tips using Tecan freedom EVO™



Figure 2: DPX semi-automated extractor

LDTD-MS/MS System



Figure 2: LDTD system on Thermo Vantage Mass Spectrometer

Sample Method

Extraction procedure

- DPX Tip:** DPX Polar (1 mL / 20 mg)
- Activation:** 0.4mL MeOH :Water (30 :70)
- Load:** 100 µL sample
100 µL IS (Benzoylecgonine-d8 at 25 ng/mL in MeOH: Na Acetate (100 mM, pH 3), (30:70))
- Wash 1:** 0.4 mL Water
- Elution:** 0.4 mL MeOH
After elution, add 200 µL Water. Mix*
Spot: 2 µL in LazWell plate

*Organic phase can be evaporated and reconstituted to further concentrate the sample

LDTD-MS/MS Parameters

LDTD

Gas Flow:	3 L/min	
Laser pattern:	Time (s)	Power (%)
	0	0
	2	0
	5	25
	7	25
	7.1	0
	8	0

MS/MS Method

	Transition	CE	S-Lens
Benzoylecgonine	290->168	20	80
Benzoylecgonine-d8	298->171	20	80
Mode:	Positive		

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 (0.5 to 50 ng/ml).

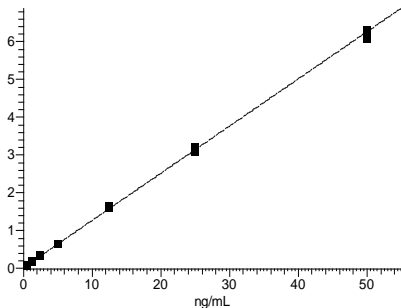


Figure 3: Benzoyllecgonine standard curve

	r^2	Slope (ratio area / concentration)	y- Intercept
Run 1	0.9992	0.1249	0.0201
Run 2	0.9991	0.1270	0.0190
Run 3	0.9986	0.1281	0.0207

Table 1: Calibration Curve Parameters

Accuracy and Precision

As shown on **Table 2 and 3**, the inter-run and intra-run accuracy and precision are between 97.3 to 101.2% and 1.4 to 13.8% respectively.

	QC-Low	QC-Med	QC-High
Conc. (ng/mL)	1.25	5	25
N	18	18	18
Mean (ng/mL)	1.25	5.01	24.96
%RSD	9.3	2.4	1.7
%Nom	100.3	100.3	99.8

Table 2: Inter-run precision and accuracy for Benzoyllecgonine

	LLOQ	QC-Low	QC-Med	QC-High	ULOQ
Conc. (ng/mL)	0.5	1.25	5	25	50
N	6	6	6	6	6
Mean (ng/mL)	0.49	1.22	5.06	24.97	49.45
%RSD	13.8	10.4	1.8	1.6	1.4
%Nom	97.7	97.3	101.3	99.9	98.9

Table 3: Intra-run precision and accuracy for Benzoyllecgonine

Stability Verification

Following the SPE extraction process, all samples were stored at 4°C to evaluate the wet stability of the drugs. After 21h, all samples were re-spotted and analyzed.

Linearity, precision and accuracy were evaluated to determine the stability. **Table 4** shows that a wet stability of 21h 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 21h. Then, standards and QCs were analyzed and the linearity, precision and accuracy are verified. **Table 4** shows the dry stability results and the storage conditions of the LazWell.

	Wet Stability	Dry in LazWell (RT)
Time (h)	21	21
Temp. (°C)	4°C	RT
Conc. (ng/mL)	0.5	0.5
N	3	3
Mean (ng/mL)	0.44	0.47
%RSD	12.5	10.7
%Nom	87.7	94.8

Table 4: Stability Results for Benzoyllecgonine

Conclusions

A fast analysis can be reached using LDTD-MS/MS system. This system allows a total sample-to-sample analysis time of **8 seconds**. The combination of DPX technology and LDTD provides raw sample to final result of 96 samples in **less than 1 hour**.

The very fast extraction using **DPX Polar** tips ensures accurate and precise results with a linear standard curve ($r^2 > 0.99$).