Confirmation of Cotinine in Human Plasma and analysis by LDTD–MS/MS

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Introduction

Cotinine testing in human plasma is a fast and effective way to determine the amount of tobacco exposure, including second-hand smoke. Since Cotinine assays are more reliable than smoking histories, forensic sciences and toxicology can strongly benefit from this cost-effective approach to drug testing. In a clinical environment, blood plasma can be used in patient screening for confirmation of the presence or absence of tobacco exposure, whether by oral, transdermal or inhalation methods.

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.

LDTD-MS/MS System

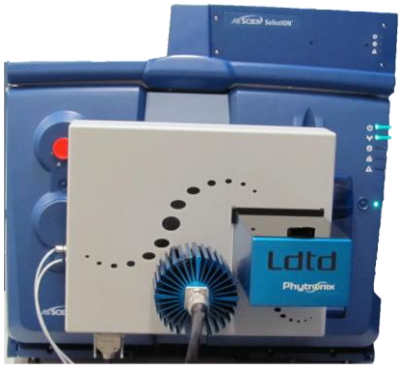

Figure 1: LDTD system on AB SCIEX 5500 Qtrap Mass Spectrometer

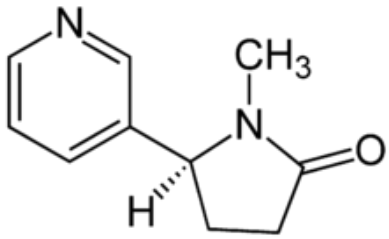

Figure 2: Structure of the Cotinine molecule

Sample Method

Extraction Procedure

100 µL Plasma sample
20 µL IS (100 ng/mL of Cotinine-d3 in MeOH/NaOH(1N) (1/1)
- Mix

400 µL Ethyl Acetate
- Mix and Centrifuge (2 min. / 14000 rpm)

Spot 5 µL of organic phase in LazWell plate
- Evaporate to dryness at room temperature

LDTD-MS/MS Parameters

LDTD

Gas Flow: 3 L/min

Laser pattern:

Time (s)	Power (%)
0	0
2	0
5	45
7	45
7.1	0
8	0

MS/MS Method

	Transition	CE	CXP
Cotinine	177->80	30	11
Cotinine-d3	180->80	30	11

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 in the quantification range (1 to 500 ng/mL).

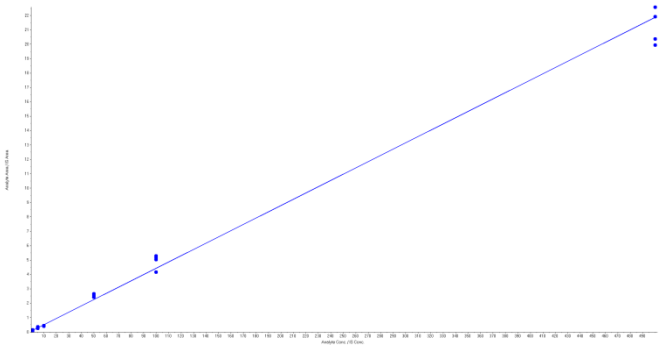

Figure 3: Cotinine standard curve

	r^2	Slope (ratio area / concentration)	y-Intercept
Run 1	0.9956	0.0436	0.0719
Run 2	0.9989	0.0709	0.0251
Run 3	0.9981	0.0464	0.0112

Table 1: Calibration Curve Parameters

Accuracy and Precision

As shown on **Table 2 and 3,** the inter-run and intra-run accuracy and the precision are between 95.8 to 109.1% and 3.5 to 13.5% respectively.

	QC-Low	QC-Med	QC-High
Conc. (ng/mL)	5	50	100
N	12	12	12
Mean (ng/mL)	4.79	51.31	103.13
%RSD	9.2	13.5	8.5
%Nom	95.8	102.6	103.1

Table 2: Inter-run precision and accuracy for Cotinine

	LLOQ	QC-Low	QC-Med	QC-High	ULOQ
Conc. (ng/mL)	1	5	50	100	500
N	4	4	4	4	4
Mean (ng/mL)	1.09	4.64	54.20	106.18	491.36
%RSD	8.3	3.5	4.5	7.13	5.5
%Nom	109.1	92.8	108.4	106.2	98.3

Table 3: Intra-run precision and accuracy for Cotinine

Matrix Effect

No matrix effect was detected within the runs.

Stability Verification

Following the liquid-liquid extraction process, all samples were stored at 4°C to evaluate the wet stability of the drug. After stability time, all samples were re-spotted and analyzed. Linearity, precision and accuracy were evaluated to determine the stability. **Table 5** shows that a wet stability of 43h 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 and 4°C for stability. Then, standards and QCs were analyzed and the linearity, precision and accuracy are verified. **Table 5** shows that the dry stability of 42h is obtained with good precision and accuracy of LOQ standard.

	Wet Stability	Dry in LazWell (RT)
Time (h)	44h45	19h45
Temp. (°C)	4°C	RT
Conc. (ng/mL)	1	1
N	4	4
Mean (ng/mL)	1.19	1.09
%RSD	16.81	14.68
%Nom	119.0	109.0

Table 5: Stability Results for Cotinine

Conclusions

The liquid-liquid extraction procedure ensures accurate and precise results with a linear standard curve ($r^2 > 0.99$).

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