

## OVERVIEW

### Purpose

- Fast Cocaine and Benzoylcegonine (BE) analysis in Hair sample using LDTD-MSMS

### Method

- Extraction of Cocaine and BE from Hair using Extraction buffer
- Liquid-Liquid extraction (Cocaine) and SPE in tip (BE)
- Deposit of a small volume of the organic phase in LazWell plate
- Fast Analysis using LDTD-MS/MS system

### Results

- Excellent linearity over the calibration range ( $R^2 > 0.99$ )
- Accuracy ranging from 93.8 and 109.7%
- Precision ranging from 0.7 and 10.4 %
- Good sample stability (Wet and Dry in LazWell)
- All these samples are analyzed with a run time of 9 sec using LDTD-MS/MS system**

## INTRODUCTION

Since hair growth is fed by the bloodstream, the use of illicit drugs can be revealed by analyzing a small sample of hair. To increase the throughput analysis of hair samples, the Laser Diode Thermal Desorption (LDTD) combined with a Mass Spectrometry system were evaluated for the analysis of cocaine and benzoylcegonine (BE).

Detection and quantification of drugs in hair samples is traditionally performed by LC/MS/MS or GC/MS analysis. The Laser Diode Thermal Desorption (LDTD) system combined with Mass Spectrometry (MS/MS) allows analysis with runtimes of 10 seconds sample-to-sample. In this study, our goal was to determine the limit of detection (LOD) for cocaine and BE in hair samples using LDTD coupled with MS/MS.

### LDTD™ Ionization Source:

The LDTD uses a Laser Diode to produce and control heat on the sample support (Figure 1) which is a 96 well plate. The energy is then transferred through the sample holder. The sample gets dried and vaporized prior being carried by a gas in a corona discharge region. This type of ionization is characterized by a strong resistance to ionic suppression because of the absence of solvent. LDTD ionization reduces sample-to-sample analysis time to 9 seconds and allows high throughput capabilities without carry over.

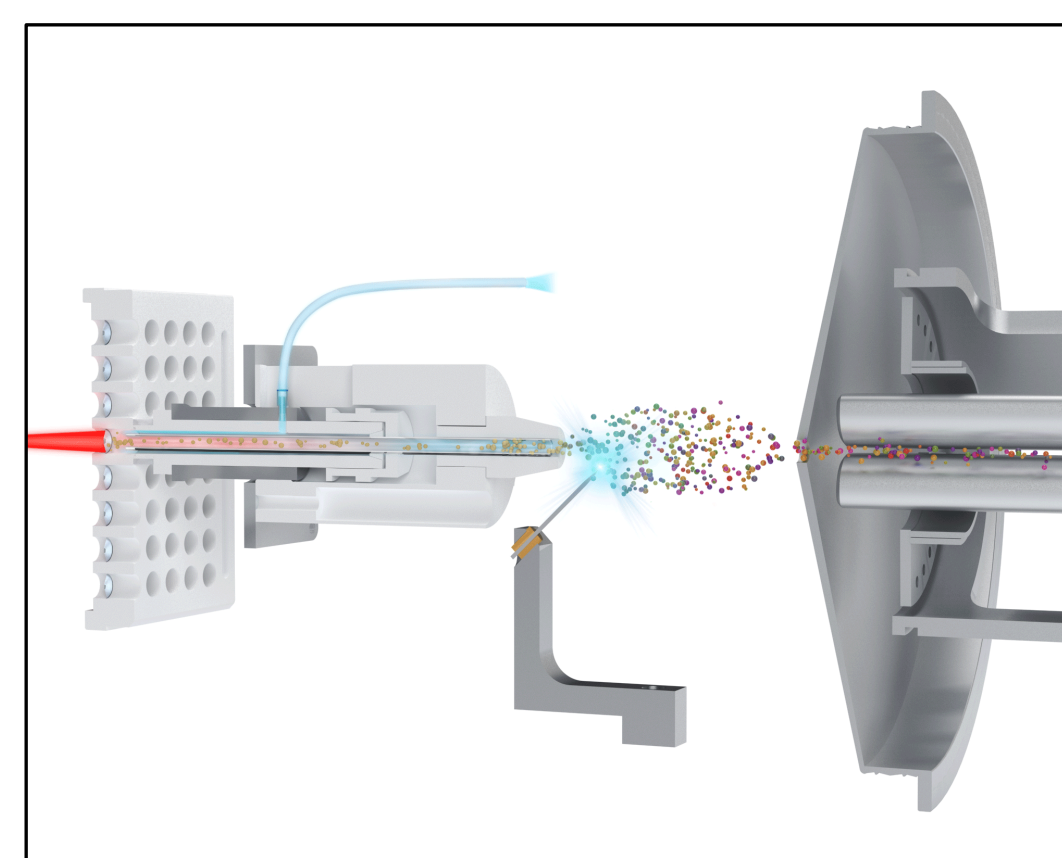


Figure 1 Schematic of the LDTD ionization source.

## METHOD

### Hair Extraction procedure

- 20 mg Hair sample (powder form)
- 1000 µL Hair Extraction Buffer
  - Vortex 30 seconds
- Incubate at 70°C for 1 hour.
  - Centrifuge 6000 rpm / 2 minutes

### Cocaine Extraction procedure

- 100 µL Hair extract sample
- 10 µL Internal Standard (Cocaine-d3, 20 ng/ml in MeOH)
- 100 µL EDTA Buffer (0.5M, pH8)
  - Vortex 30 seconds
- 600 µL MTBE
  - Vortex 30 seconds
- Centrifuge 6000 rpm / 2 minutes
- Spot 3 µL of upper phase in LazWell plate.
- Let evaporate at room temperature

### Instrumentation

- Phytronix Technologies LDTD ion source (model T-960);
- Thermo Vantage Mass Spectrometer

### LDTD Parameters

#### Cocaine

- Laser power pattern :
  - Increase laser power to 45 % in 3.0 s
  - Hold for 2 seconds
  - Decrease laser power to 0 %
- Carrier gas flow: 3 L/min (Air)

#### Benzoylcegonine

- Laser power pattern :
  - Increase laser power to 25 % in 3.0 s
  - Hold for 2 seconds
  - Decrease laser power to 0 %
- Carrier gas flow: 3 L/min (Air)



Figure 2 LDTD system on Thermo Vantage Mass Spectrometer

### BE Extraction procedure

Cartridge: DPX Polar (1 mL / 20 mg)  
 Activation

: 0.4mL MeOH :Water (30 :70)

Load: 100 µL sample

100 µL IS (Benzoylcegonine-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 400 µL EDTA Buffer (200 µg/ml in MeOH/Water/NH4OH (75/20/5))  
 Spot: 3 µL in LazWell plate

### MS/MS Parameters

	Transition	CE	S-Lens
Cocaine	304→182	20	80
Cocaine-d3	307→185	20	80
Benzoylcegonine	290→168	20	80
Benzoylcegonine-d8	298→171	20	80
Mode:	Positive		

## Precision and Accuracy

As shown in following Table 3, 4, 5 and 6 the intra-run and inter-run precision/accuracy for Cocaine and BE

	LLOQ	QC-Low	QC-Med	QC-High	ULOQ
Conc. (pg/mg Hair)	10	50	500	5000	10000
N	6	6	6	6	6
Mean (pg/mg Hair)	11.0	46.3	484.2	5058.2	10127.9
%RSD	5.8	6.1	4.4	2.0	0.7
%Nom	110.2	92.7	96.8	101.2	101.3

Table 3 Intra-run precision and accuracy for Cocaine

	LLOQ	QC-Low	QC-Med	QC-High	ULOQ
Conc. (pg/mg Hair)	10	50	500	5000	10000
N	4	4	4	4	4
Mean (pg/mg Hair)	10.1	54.4	497.8	5028.6	11051.9
%RSD	13.2	6.8	4.5	5.9	9.5
%Nom	100.7	108.8	99.6	100.6	110.5

Table 5 Intra-run precision and accuracy for BE

	QC-Low	QC-Med	QC-High
Conc. (pg/mg Hair)	50	500	5000
N	12	12	12
Mean (pg/mg Hair)	48.1	502.8	5115.3
%RSD	11.7	5.6	3.3
%Nom	96.3	100.6	102.3

Table 4 Inter-run precision and accuracy for Cocaine

	QC-Low	QC-Med	QC-High
Conc. (pg/mg Hair)	50	500	5000
N	10	10	10
Mean (pg/mg Hair)	50.3	503.7	4869.7
%RSD	9.1	5.1	7.4
%Nom	100.7	100.7	97.4

Table 6 Inter-run precision and accuracy for BE

### Wet stability

Following the extraction process, all samples were stored at 4°C to evaluate the wet stability of the drugs. After a given time, all samples were re-spotted and analyzed. Linearity, precision and accuracy are verified for the stability run. Table 7 shows that a wet stability of drug is obtained with good precision and accuracy at the LOQ level for both drug.

Wet stability		
Drug	Cocaine	BE
Time (h)	120	120
Temp. (°C)	4°C	4°C
Conc. (pg/mg hair)	10	10
N	4	4
Mean (pg/mg Hair)	9.8	8.7
%RSD	8.3	1.0
%Nom	98.3	87.3

Table 7 Wet stability results

### Dry sample in LazWell plate Stability

The stability of dry samples in LazWell plate was also verified. All standards and QCs are spotted, dried and kept in specific stability conditions. After the stability time, standards and QCs were re-analyzed and the linearity, precision and accuracy are verified. Table 8 shows the dry stability given the storage conditions of the LazWell plate. Good precision and accuracy at LOQ levels were obtained for both drugs.

Dry stability		
Drug	Cocaine	BE
Time (h)	48	48
Temp. (°C)	RT	RT
Conc. (pg/mg Hair)	10	10
N	4	4
Mean (pg/mg Hair)	10.0	10.4
%RSD	10.1	15.0
%Nom	99.9	103.8

Table 8 Dry stability results

## RESULTS:

### Linearity

As shown in Figure 3 and 4, excellent linearity ( $r^2 > 0.99$ ) with no signs of carryover effect is achieved within the quantification range (10 to 10000 pg/mg Hair).

	$r^2$	Slope (ratio area / concentration)	y-Intercept
Run 1	0.9905	0.0344	-0.0365
Run 2	0.9949	0.0573	0.1041
Run 3	0.9980	0.0066	0.1339

Table 1 Calibration Curve Parameters for Cocaine

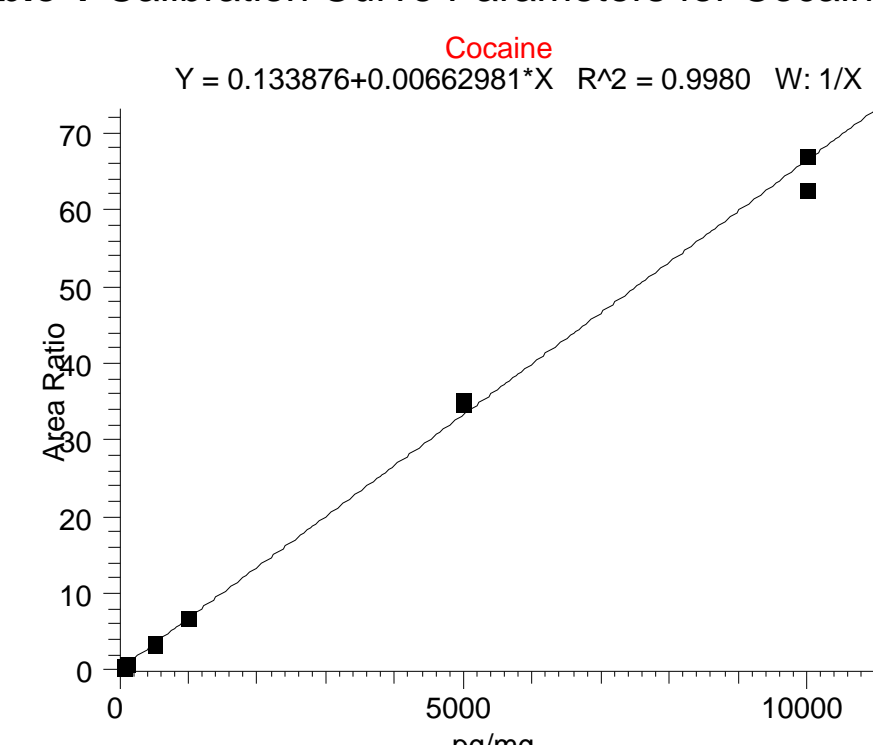


Figure 3 Typical Standard curve of Cocaine

	$r^2$	Slope (ratio area / concentration)	y-Intercept
Run 1	0.9954	0.0048	0.0188
Run 2	0.9940	0.0085	0.0371
Run 3	0.9978	0.0033	0.0115

Table 2 Calibration Curve Parameters for BE

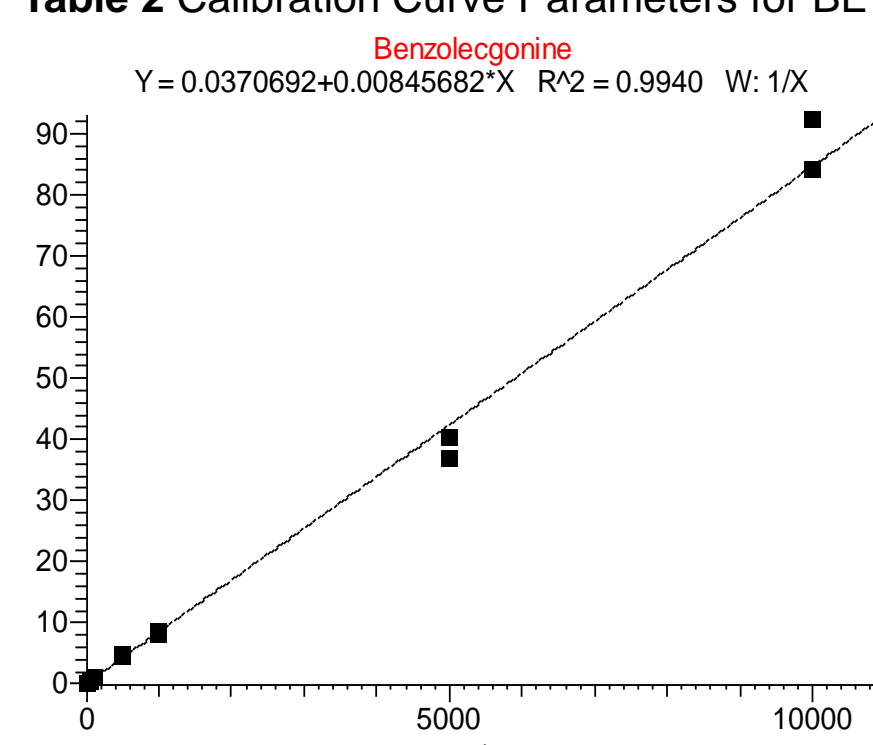


Figure 4 Typical Standard curve of BE

### Real sample comparison

18 real patient hair samples have been tested with this method to correlate with GC-MS results. Figure 5 and 6 shows a correlation >95% between results using both methods.

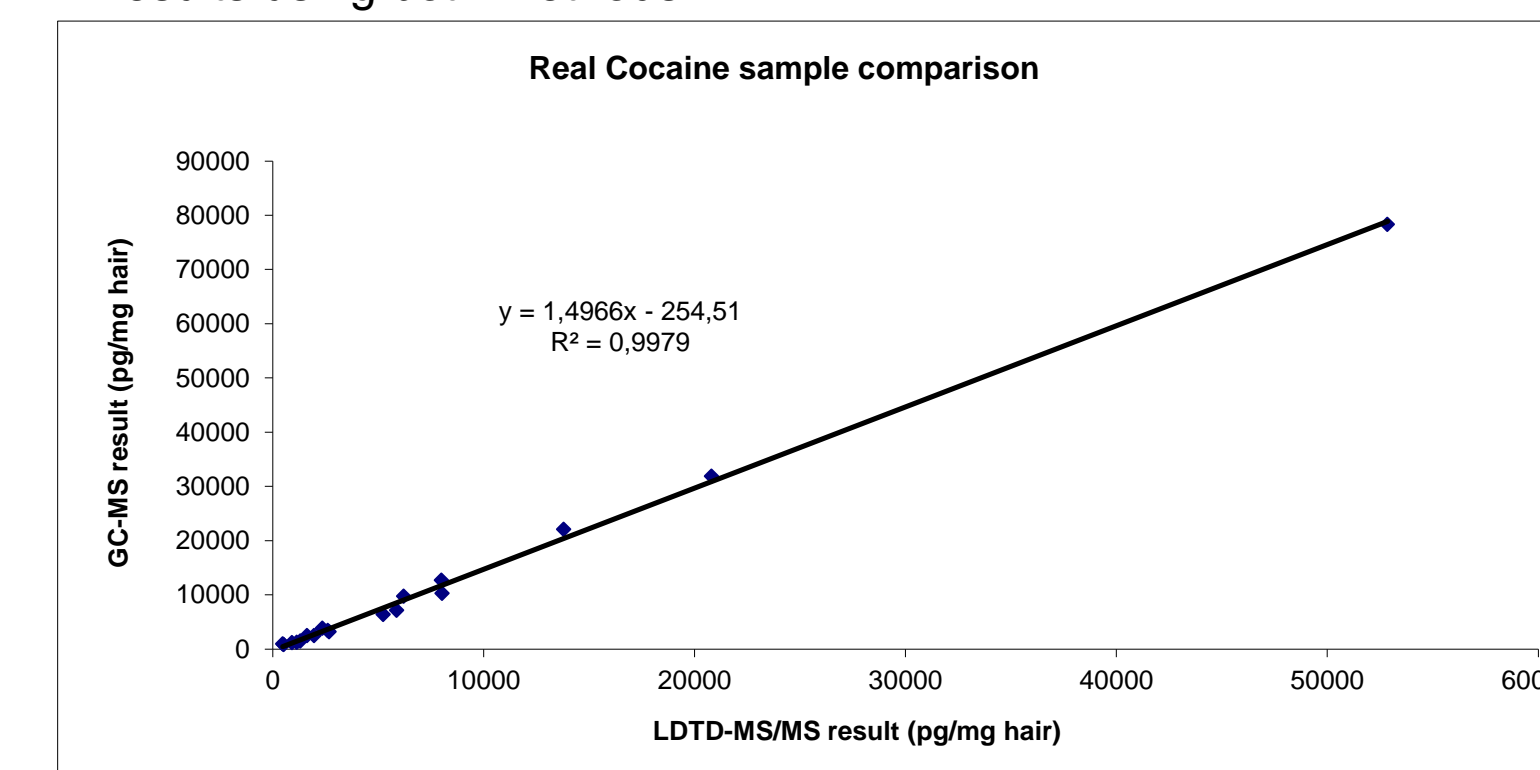


Figure 5 Correlation between Cocaine concentrations in real hair samples obtained with LDTD-MS/MS and GC-MS

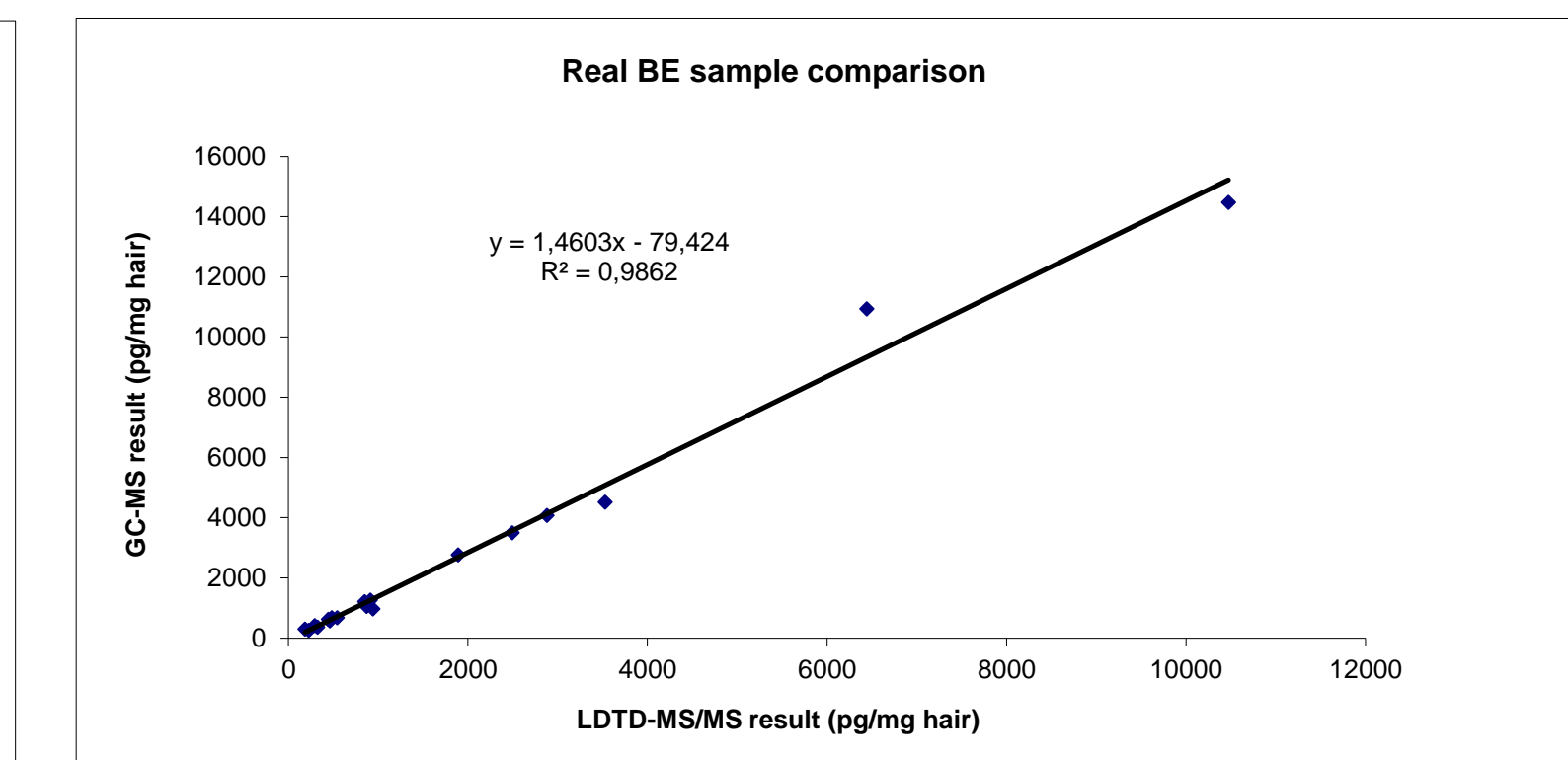


Figure 6 Correlation between BE concentrations in real hair samples obtained with LDTD-MS/MS and GC-MS

## CONCLUSIONS

- Fast extraction of Cocaine and Benzoylcegonine from hair sample
- High Selectivity, Sensitivity and Specificity using Tandem Mass Spectrometry
- Versatility of LDTD Technology proven with real hair samples
- LDTD provides the High-Throughput analysis of sample extract in **9 seconds sample-to-sample**.