

OVERVIEW

Purpose

- High Throughput determination of Melamine with accuracy, sensitivity and repeatability
- Laser Diode Thermal Desorption technology (LDTD)

Method

- Melamine spiked into Infant Milk Powder Formula
- Protein precipitation with ACN/Water (50/50)
- Method 1:
 - Liquid-liquid extraction with Ethyl acetate in basic pH condition
 - Nominal calibration range : 0.25 to 100 µg/g
- Method 2:
 - Solid phase extraction (SPE-PCX)
 - Nominal calibration range : 0.25 to 100 µg/g
- LDTD-APCI-MS/MS analysis : Laser Diode Thermal Desorption coupled with triple quadrupole mass spectrometer

Results

- Excellent linearity over the calibration range ($R^2 > 0.99$)
- Excellent accuracy ranging from 90 and 108% using area value
- Excellent precision ranging from 2.7 and 14.5 % using area value
- No carryover and good recovery (52% and 100% for method 1 and 2, respectively)
- **Ultra-fast sample-to-sample analysis : 8 seconds per sample (4 seconds for sample desorption).**

INTRODUCTION

High-throughput determination of Melamine has become an important part for the food industry. It is also an economical issue for many company. LDTD-MS/MS is used for high-throughput analysis of Melamine to increase the analytical run-time of a factor of 100 (10 seconds versus 15 minutes). The characteristics of the LDTD system are :

LDTD (Figure 1)

- Plug-and-play ionization source interface to most popular mass spectrometer
- Thermal desorption induced by a laser diode
- The sample is carried by a carrier gas to a corona discharge region for APCI
- Loader up to 10 LazWell™ plates

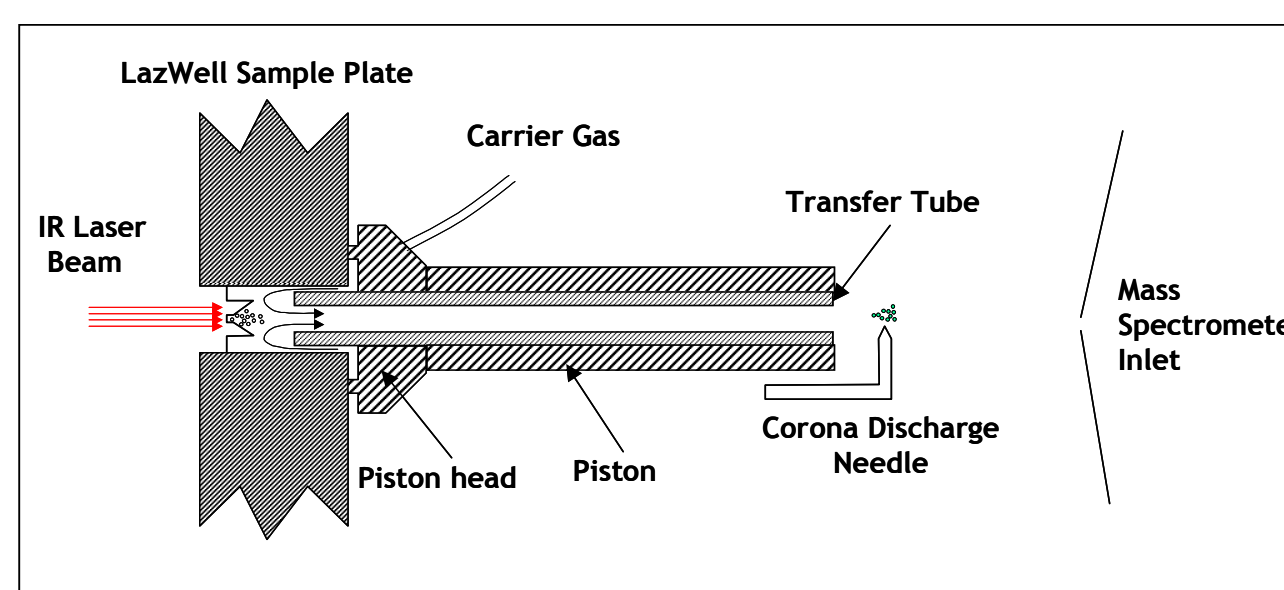


Figure 1 Schematic of the LDTD ionization source.

LazWell™ Plate (Figure 2)

- Standard 96-well plate format
- Low volume delivery (from 1 to 10 µL of sample per well)
- No carryover
- No enhancement matrix needed
- No sample desalting needed
- No liquid mobile phase needed
- Sample dried at room temperature

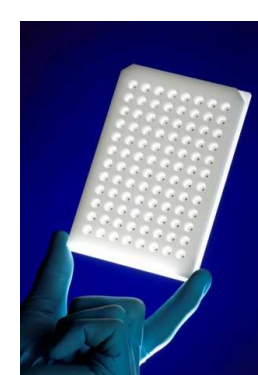


Figure 2 LazWell™ sample plate

METHOD

Instrumentation (Figure 3)

- LDTD model T-960, Phytronix Technologies
- TSQ® Vantage, Thermo Fisher Scientific



Figure 3 LDTD-MS/MS analytical system.

MS Parameters

- APCI (+)
- Scan time : 0.02 s
- Q1 width : 0.70 amu
- Q3 width : 0.70 amu
- SRM: 126.89 - 68.08
- Tube lens : 61 V
- CE : 30 V (Ar)

LDTD Parameters

- Laser power pattern :
 - Increase laser power to 25 % in 2.0 s
 - Hold at 25 % for 2.0 s
 - Decrease laser power to 0 %
- Carrier gas flow : 3 L/min (Air)
- Corona voltage value : 5 kV
- Deposited sample volume: 4 µL

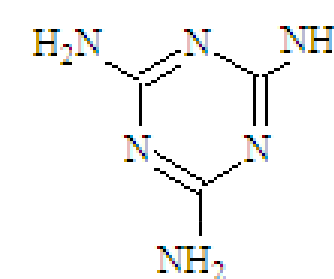


Figure 4 Chemical structure

Sample Preparation (Method 1)

- Protein precipitation
 - 0.125g of Infant Milk Powder Formula in eppendorf tube (1.5ml)
 - 1000 µL of Acetonitrile/Water (1/1)
 - Vortex 1 min.
 - 12.5µL of stock solution (2.5, 5, 10, 25, 50, 100, 500 and 1000 µg/mL)
 - Vortex 1 min. and sonicate 2 min
 - Vortex 1 min and centrifuge 5 min at 14000g

– Liq-Liq extraction

- 200 µL of supernatant of protein precipitation in eppendorf tube (1.5ml)
- 200 µL of Buffer (Borate buffer, 0.1M / pH10 saturated with NaCl)
- Vortex 1 min.
- 1 ml Ethyl acetate
- Vortex 1 min and centrifuge 5 min at 14000g.
- Transfer 4.0 µL of organic phase onto LazWell™
- Evaporate solvent at room temperature
- Perform LDTD-MS/MS analysis

Sample Preparation (Method 2)

- Protein precipitation
 - 0.125g of Infant Milk Powder Formula in eppendorf tube (1.5ml)
 - 1000 µL of Acetonitrile/Water (1/1)
 - Vortex 1 min.
 - 12.5µL of stock solution (1, 5, 10, 25, 50 and 100 µg/mL)
 - Vortex 1 min. and sonicate 2 min.
 - 50 µL of HCl solution (1N)
 - Vortex 1 min and centrifuge 5 min at 14000g.

– Automated Solid phase extraction (Bond Elut Plexa, PCX, 3cc, 30mg)

- Activation: 1 mL Methanol and 1 ml HCl (0.1N) by gravity
- Loading: 400 µL of supernatant of protein precipitation by gravity
- Wash: 1 mL HCl (0.1N) and 1 ml Methanol by gravity
- Elution: 1 ml Methanol/Water/Ammonium hydroxide (75/20/5) by gravity
- Transfer **Manually** 4.0 µL of elution onto LazWell™
- Evaporate solvent at room temperature
- Perform LDTD-MS/MS analysis

Calibration Curves

The calibration curve were evaluated over a nominal range of 0.25 to 100 µg/g for method 1 and 0.25 to 100 µg/g for method 2 (Figure 5). The linearity were excellent for all methods (Table 1).

Table 1 Calibration curves parameters.

| | Method 1 | Method 2 |
|------------------------------|----------|----------|
| R^2 | 0.9940 | 0.9900 |
| Slope (area / concentration) | 3212.2 | 9353.8 |
| y-Abciss | 1177.14 | 1676.32 |

Weighting factor 1/x

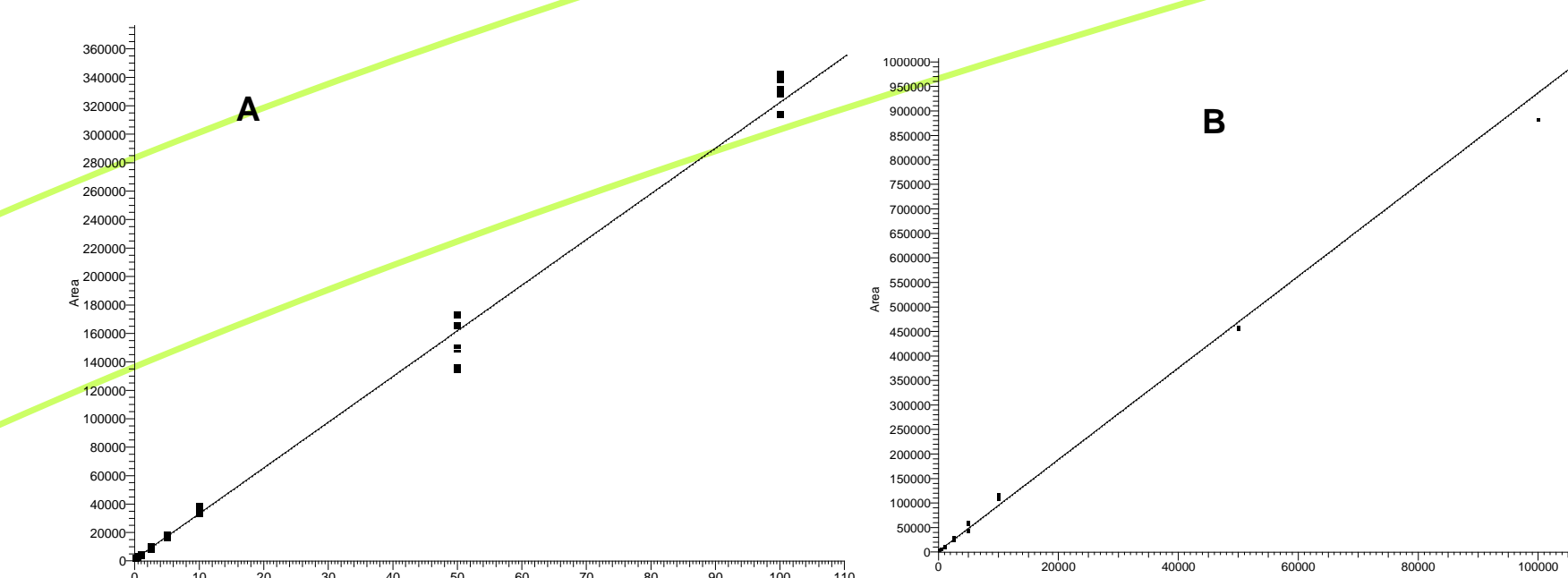


Figure 5 Calibration curves for A) Extraction method 1, and B) Extraction method 2

Within-run Accuracy and Precision

The within-run accuracy and precision were evaluated with quality control at 3 different levels. The LDTD shows an excellent accuracy of 89.5 % to 92.0 % for Method 1 and from 92.0 % and 108.0 % for Method 2. The precision was excellent with values from 11.0 to 14.5% (RSD) for Method 1 and from 2.7 to 3.4% for Method 2 knowing that no internal standard was used.

Table 2 Within-run accuracy and precision for Method 1

| | QC (Low) | QC (med) | QC (High) |
|----------------|----------|----------|-----------|
| Nominal conc. | 0.5 | 5 | 50 |
| N | 3 | 3 | 3 |
| Mean | 0.448 | 4.602 | 45.334 |
| RSD (%) | 11.2 | 11.0 | 14.5 |
| %Nominal conc. | 89.5 | 92.0 | 90.7 |

Table 3 Within-run accuracy and precision for Method 2

| | QC (Low) | QC (med) | QC (High) |
|----------------|----------|----------|-----------|
| Nominal conc. | 0.5 | 5 | 50 |
| N | 3 | 3 | 3 |
| Mean | 0.526 | 5.398 | 45.999 |
| RSD (%) | 2.66 | 5.15 | 3.44 |
| %Nominal conc. | 105.13 | 107.96 | 92.00 |

RESULTS

Recovery

Recovery of 51.5% was obtain with the Liq-Liq extraction (Method 1) and a recovery of 100% was obtain with the solid phase extraction (Method 2)

Table 4 Recovery with Method 1 and Method 2

| Recovery | |
|----------|----------|
| Method 1 | Method 2 |
| 51.5 | 100.0 |

Blank matrix evaluation

The matrix use have a 56% and 42% interference with the lower standard for Method 1 and Method 2, respectively.

Table 5 Blank matrix area versus lower STD 1

| | Method 1 | | Method 2 | |
|-----------------|----------|-------|----------|-------|
| | Blank | STD 1 | Blank | STD 1 |
| Mean area value | 1128 | 2017 | 1437 | 3459 |

High-Throughput LDTD Analysis

The analysis sequence :

- Carrier gas stabilization (2.0 seconds)
- Sample desorption (4.0 seconds)
- Baseline stabilization (4.0 seconds)

These steps lead to a **sample-to-sample run time of 10 seconds** offering high-troughput Melamine analysis.

Desorption Profiles

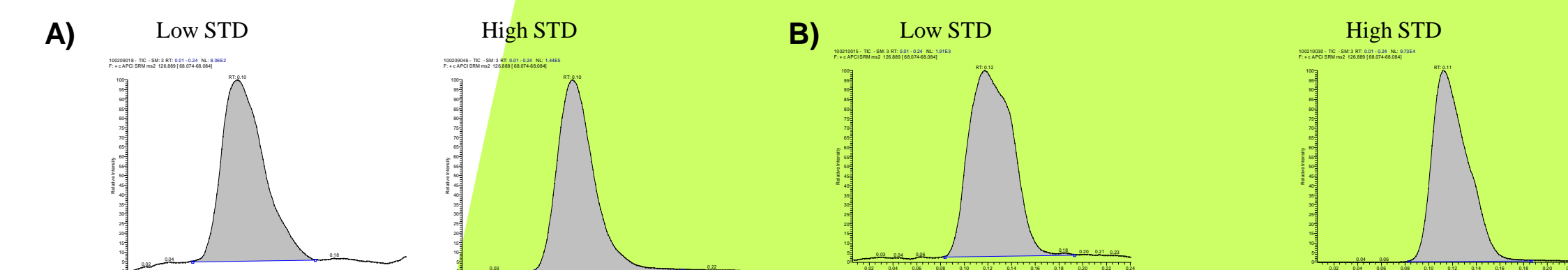


Figure 6 Desorption profile for 2 calibration standards for A) Method 1, and B) Method 2.

CONCLUSIONS

- **Fast extraction with low solvent consumption**
- **Ultra-fast analysis of Melamine using thermal desorption in 4.0 seconds**
- **Excellent signal linearity over 0.25 to 100 µg/g for method 1 and 2**
- **Good Recovery, Accuracy and Precision**
- **A label internal standard (deuterated) are recommended to minimized extraction variation**
- **Suitable methods for Melamine quantification in Infant Milk Powder Formula**

Acknowledgement

- **Varian Inc (Christophe Deckers) for the Bond Elut extraction PCX (3cc,30mg)**