Analysis of trimethoprim, lincomycin and sulfadoxin using Laser Diode Thermal Desorption Atmospheric Pressure Chemical Ionization Tandem Mass Spectrometry in manure

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Overview

Purpose

- Develop an ulta-fast analysis method for the determination of antibiotics in manure.
- Develop a simple analysis method
- Avoid time consuming steps like derivatization, liquid chromatography and solid phase extraction prior to analysis.

Method

- Use of Laser Diode Thermal Desorption Athmospheric Pressure Chemical Ionization as ionization source
- LDTD parameters have to be optimize : Laser power, laser pattern, gas flow, sample volume in each well…
- MS parameters

Results

- Good recovery values for solid-liquid extraction between 77-86%
- Intra and inter day precision were evaluated with RSDs < 18%
- Calibration curves show good linearity with an R^2 between 0.985 and 0.961
- Limits of detection ranged from 8.3 to 15.1 µg/L

Introduction

Context

- In the last several decades, antibiotics have been widely used in swine, cattle and poultry breeding. They have been administered routinely in therapeutic doses to prevent disease, improve feed efficiency and accelerate growth.
- A significant percentage of these administered antibiotics are not absorbed by animals and are excreted into the environment and found in agricultural soils. This can contribute to bacterial resistance in the environment.
- Trimethoprim, lincomycin and sulfadoxin are three families of antibiotics that are used in animal feed and that can limit bacterial growth. A novel ultra-fast method has been developed to analyze antibiotics using a novel sample introduction method, the laser diode thermal desorption-atmospheric pressure chemical ionization (LDTD-APCI), coupled to tandem mass spectrometry (MS/MS).

Laser Diode Thermal Desorption

- LDTD is an MS interface, which is combined with atmospheric pressure chemical ionization (APCI), could reduce analysis time to seconds by removing the chromatographic step and introducing charged analytes directly into the mass spectrometer.

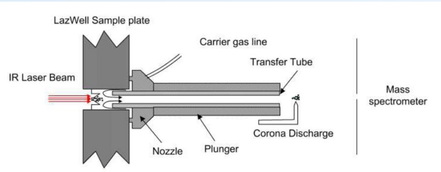


Figure 1 : Schematic of the LDTD source

- **LDTD source :**
  - Ultra-fast analysis time (15s per sample)
  - Indirect thermal desorption by laser diode (980 nm)
  - Minimal sample preparation

Method

Instrumentation

- LDTD model T-960 controlled by LazSoft 4.0 software, Phytronix Technologies.
- TSQ Quantum Ultra AM controlled by Xcalibur 2.0 software, Thermo Fisher Scientific.

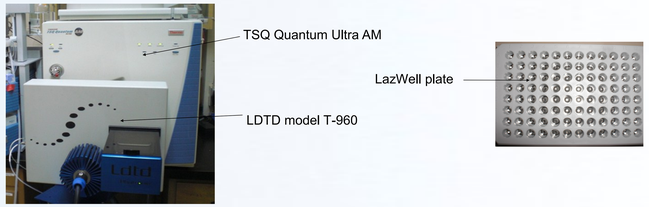
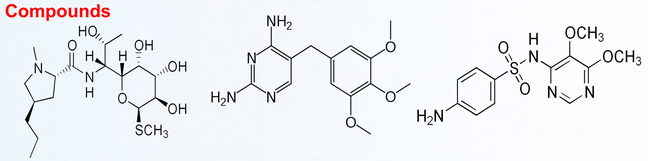


Figure 2 : LDTD-APCI-MS/MS setup and LazWell plate

Compounds



Lincomycin M.W. 406.54 g.mol^{-1} | Trimethoprim M.W. 290.32 g.mol^{-1} | Sulfadoxin M.W. 310.33 g.mol^{-1}

Figure 3 : Chemical structures of Trimethoprim, Lincomycin and Sulfadoxin with molecular weight

- Trimethoprim-13C_3 as internal standard

MS Paremeters

- APCI (+)
- Scan time : 0.005 s
- Q1 width : 0.70 amu
- Q3 width : 0.70 amu
- Corona discharge : 4 µA
- Corona voltage : 5000 V

Table 1 : SRM parameters of the LDTD-APCI-MS/MS analysis

	Precursor ion (m/z)	Product ion (m/z)	Collision Energy (V)	Tube Lens (V)
Lincomycin	407.2	126.1*	25	86
	407.2	359.1	18	86
Sulfadoxin	311.3	156.1*	17	90
	311.3	92.1	28	90
Trimethoprim	291.1	230.0*	23	77
	291.1	123.1	25	77

LDTD Paremeters

- Laser power : 40 %
- Carrier gas flow : 3L/min
- 2 µL of sample in each well

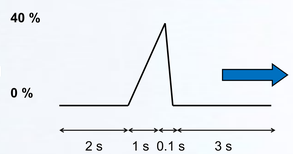


Figure 4 : Laser pattern

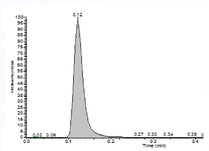


Figure 5 : LDTD peak shape of thermic desorption

Extraction

Figure 6 : Extraction method

Results

Laser power

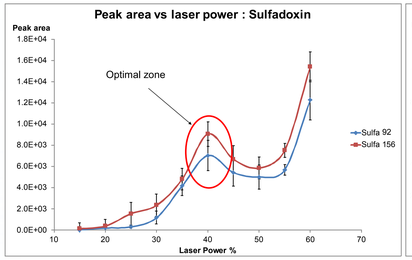


Figure 7 : Average peak area of 250 µg/L of sulfadoxin standard. n = 5 for each laser power (%) used. Vertical error bars represent standard deviations from the mean.

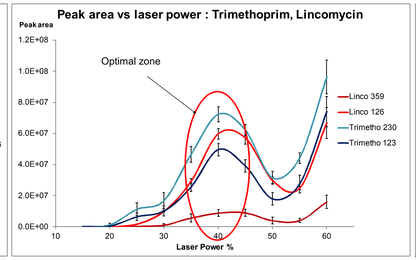


Figure 8 : Average peak area of 250 µg/L of lincomycin and trimethoprim standards. n = 5 for each laser power (%) used. Vertical error bars represent standard deviations from the mean.

Solvent

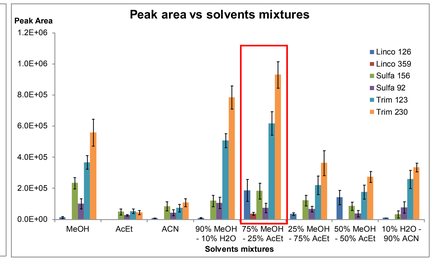


Figure 9 : Comparison of average peak area of 250 µg/L of lincomycin, sulfadoxin and trimethoprim standards. n = 5 for all differents solvents mixture. Vertical error bars represent standard deviations from the mean.

Internal standards

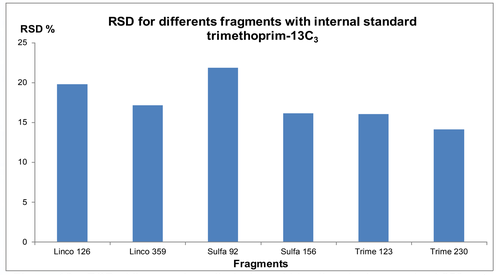


Figure 10 : RSD of lincomycin, sulfadoxin and trimethoprim fragments of 250 µg/L of standards. Internal standard (trimethoprim-13C_3) concentration = 150µg/L. n=5; methanol/ethyl acetate 75/25 v/v with laser power at 40%.

Method validation parameters

Table 2 : Validation parameters including intra-day RSD (%), Inter-day RSD (%), R^2 and LOD (µg/L). Internal Calibration with 150 µg/L Trimethoprim-13C_3 , n=5.

Compounds	Intra-day RSD (%)	Inter-day RSD (%)	R^2	Linearity range (µg/L)	LOD (µg/L)
Trimethoprim	7	9	0.985	5-250	8.3
Lincomycin	8	11	0.969	6-250	10.4
Sulfadoxin	8	18	0.961	10-250	15.1

Calibration curves

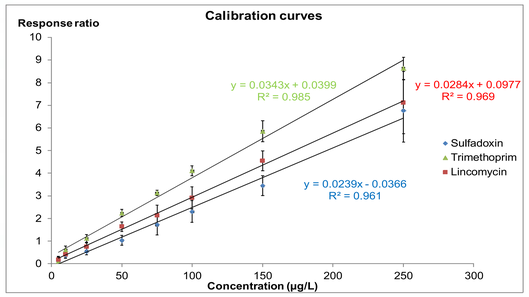


Figure 11 : Calibration curves for lincomycin, sulfadoxin and trimethoprim. Internal standard (trimethoprim-13C_3) concentration =150µg/L . SRM 407.2 → 126.1; 311.3 → 156.1; 291.1 → 230.0; n=5; methanol/ethyl acetate 75/25 v/v with laser power at 40%. Vertical error bars represent standard deviations from the mean.

Conclusion

- LDTD-APCI source coupled to MS/MS is a high-throughput method for the analysis of antibiotics in a complex matrix.
- Simple, less expensive, faster method (15s per sample).
- Solid-liquid exctraction step show good recovery values between 77% and 86% for all three antibiotics in manure
- The intra-day and inter-day precision with RSDs < 18%
- Calibration curves in spiked manure showed linearity in a complex matrix with an R^2 between 0.985 and 0.961 with a dynamic range between 3.0 and 250 µg/L.
- The limits of detection (LOD) ranged from 8.3 to 15.1 µg/L.

Future research direction :

- Test the method in a non-spiked sample
- Test the method with others veterinary antibiotics.

Acknoledgments

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