

OVERVIEW

Purpose:

- The development of an accurate LDTD-MS/MS method for a light sensitive pharmaceutical ligand is challenging
- LDTD represents a technological revolution that omits the chromatographic step and significantly increases the analytical throughput for the quantitation of chlortetracycline (CTC)

Method:

- Chlortetracycline spiked in wastewater (WW) and wastewater sludge (WWS) samples
- Solid phase extraction (SPE) of wastewater samples using C₁₈ cartridges
- Calibration range: 5 – 200 µg/L
- LDTD-APCI-MS/MS analysis

INTRODUCTION

Chlortetracycline antibiotic is a potential WW contaminant. It is readily soluble in water. Its characteristic complexation property with metals makes it difficult to retain it on cartridge during clean up process and also desorption of CTC from LazWell™ plate (96-well plate). To overcome this problem, Na₂H₂EDTA was added to WW sample to make CTC free from metal complex and to improve chlortetracycline desorption from plate.

A MS interface LDTD combined with atmospheric pressure chemical ionization (APCI), has shown great potential to reduce analysis time from minutes to seconds by removing the chromatographic step and introducing charged analytes directly into the mass spectrometer.

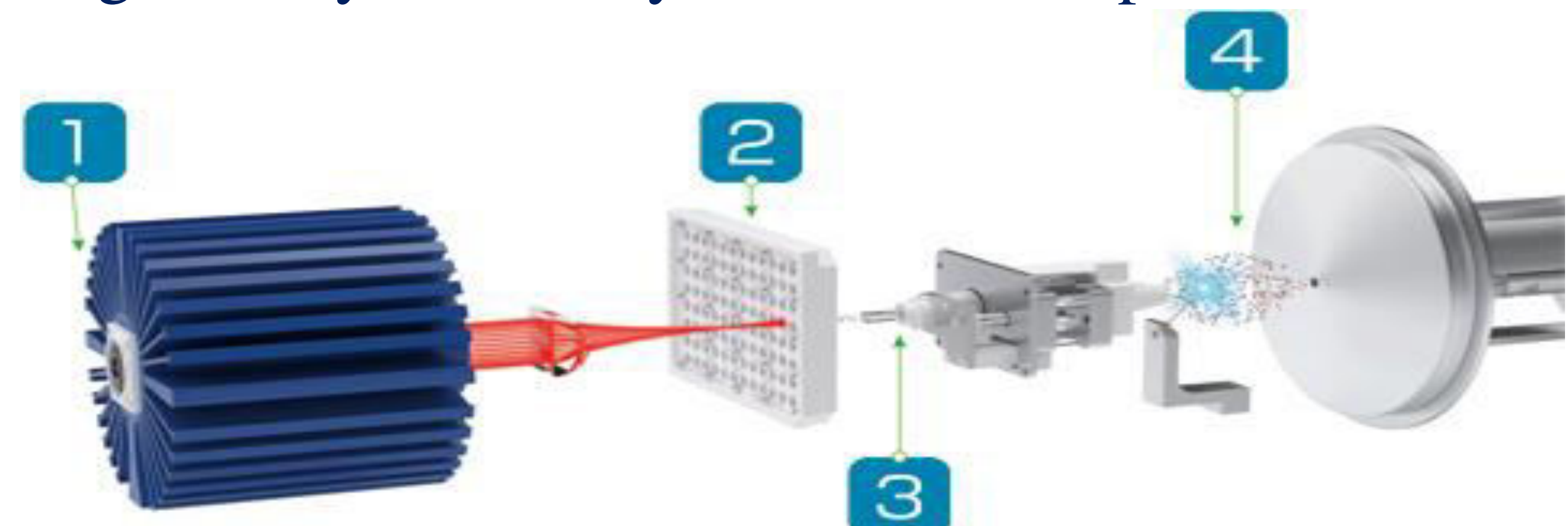


Figure 1: Schematics of Path of ionization of sample in LDTD

- Laser continuously illuminated on the back of the bottom surface of the well plate (LazWell).
- LazWell plate is designed to hold only a sample of 1-10µL and for maximum heat transfer for quick sample evaporation.
- Transfer tube to deliver vaporized samples without loss.
- Sample ionization by corona discharge at the transfer tube exit.

METHOD

Sample Preparation

- Centrifugation of sludge sample
- Lyophilization of solid residue
- Microwave extraction of lyophilized solid

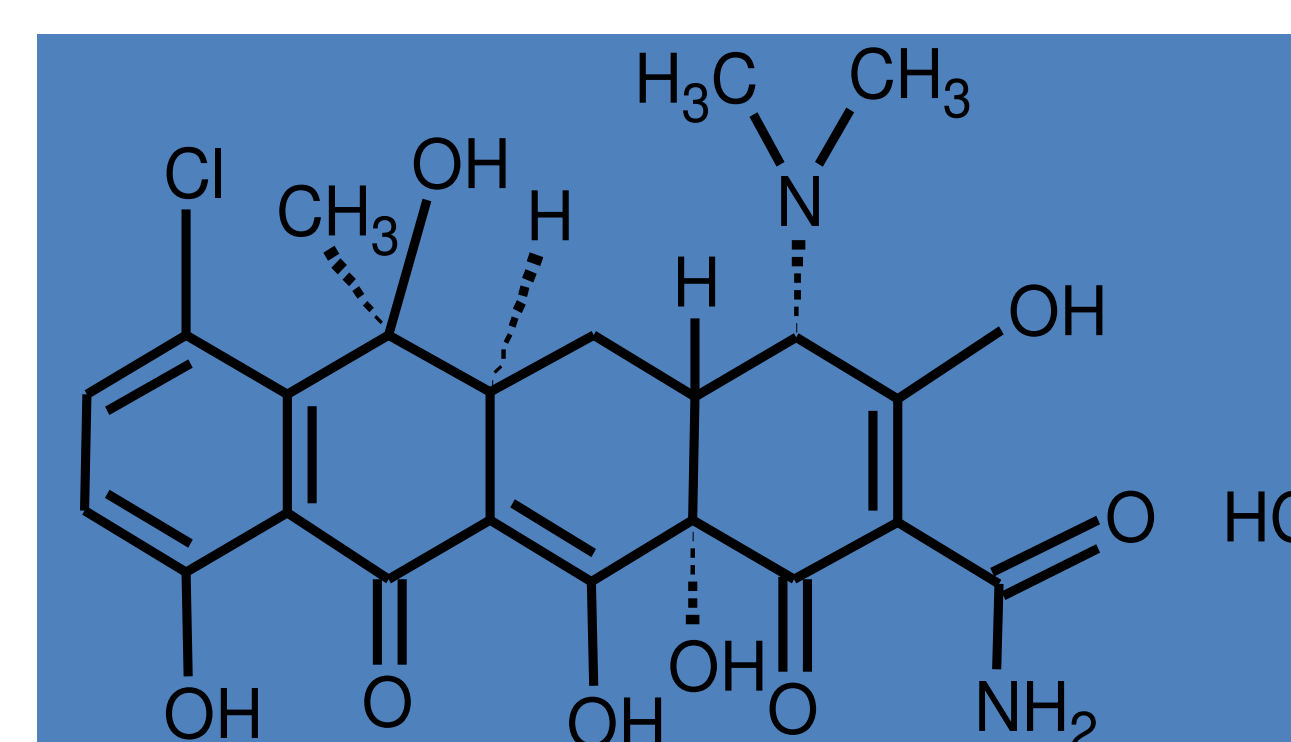


Figure 2: CTC chemical structure

Clean up

- Adjust the pH of sample to 3.5 – 4.0
- Add Na₂H₂EDTA to the sample to make CTC free from metal complex
- SPE of sample using C₁₈ cartridges and methanol as eluent
- Dry the extract under a gentle stream of N₂
- Dried residue was reconstituted with 1 mL of methanol/water mixture (75:25, v/v) before analysis

Instrumentation:

- LDTD S-960, Phytronix Technologies
- API 5500 QTrap, AB SCIEX

LDTD Parameters

- Laser power pattern :
The laser power is ramped from 0 to 65% in 6.0 s and hold at 65% for 2.0 s
- Carrier gas (Air) temperature -- 30 °C and flow rate -- 3 L/min
- The optimum sample volume deposited into a well is 4 µL

Mass Spectrometry parameters

- Ion source --- APCI (+)
- Mode --- MRM
- Corona discharge current --- 3 µA

Table 1: Precursor and product ions and collision energy (CE) for the pharmaceutical compound CTC

| Compound | Precursor ion Mass, [M + H] ⁺ , (m/z) | Product ion Mass | Collision energy (volts) |
|------------|--|------------------|--------------------------|
| CTC | 479 | 211 | 45.00 |
| Clomiphene | 406 | 100 | 35.00 |

RESULTS

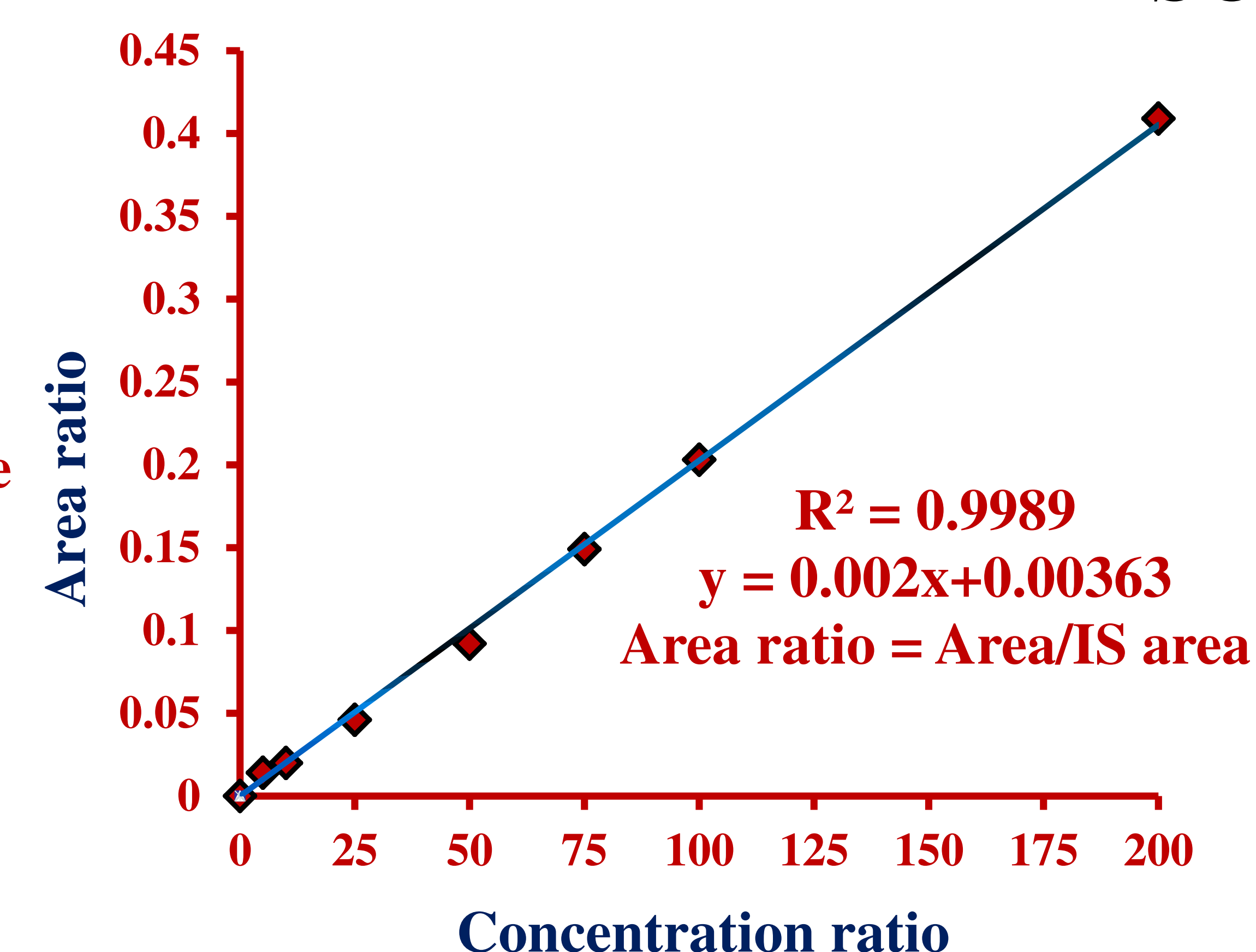


Figure 3: Calibration curve of CTC

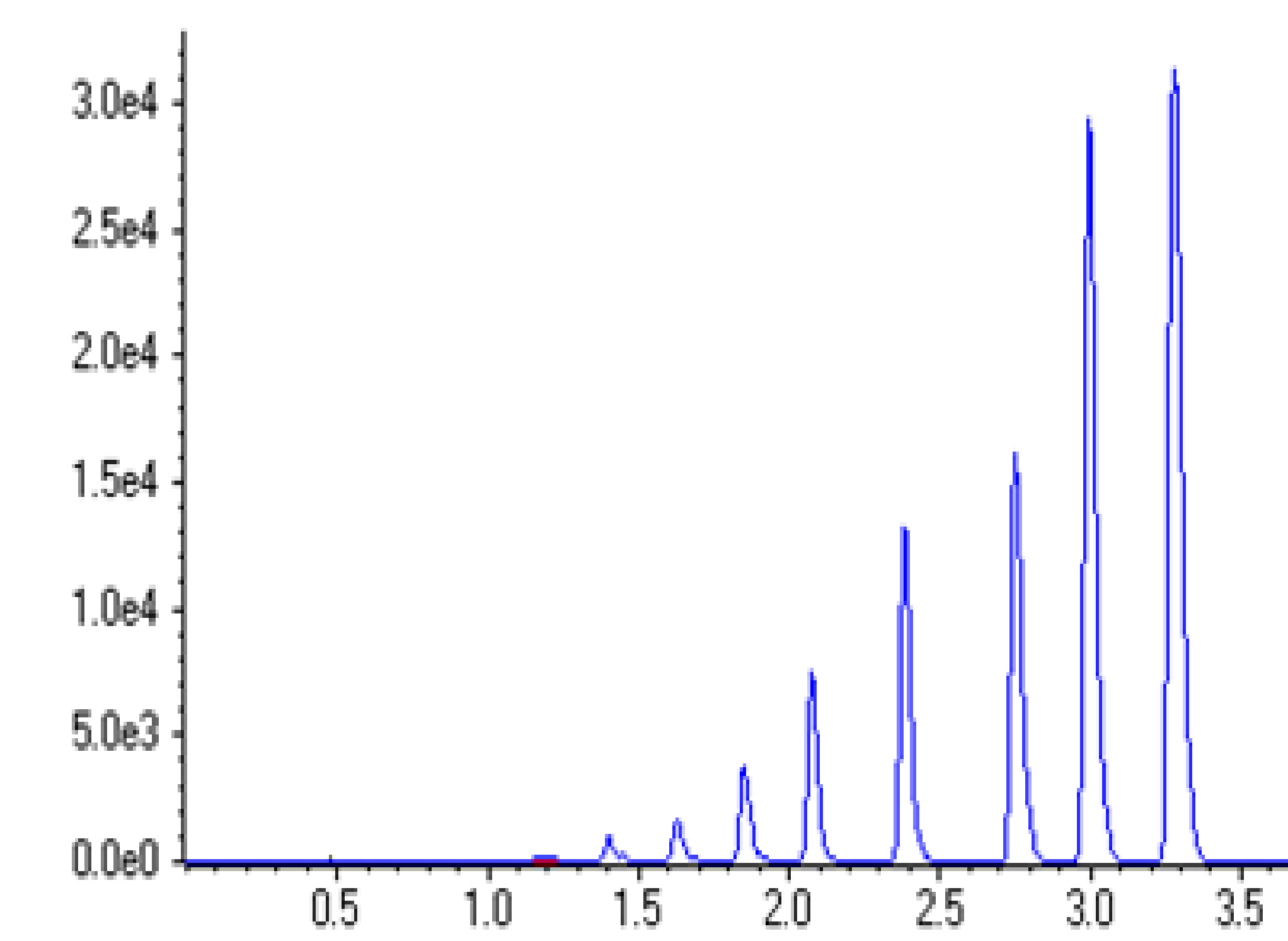


Figure 4: LDTD intensity peaks for CTC calibration curve (multiple sample desorption in one file)

Table 2: Chlortetracycline method validation parameters

| Sample ID | R ² | Intra-day precision (%RSD) | Inter-day precision (%RSD) | IDL (µg/L) | MDL (µg/L) | |
|-------------------|----------------|----------------------------|----------------------------|------------|------------|-------|
| | | | | | LOD | LOQ |
| Wastewater | 0.998 | 4.22 | 7.53 | 1.67 | 5.13 | 17.54 |
| Wastewater sludge | 0.998 | 9.18 | 12.39 | 2.49 | 7.49 | 25.92 |

Table 3: Concentrations of chlortetracycline in wastewater samples collected from Quebec Urban Community (QUC) WWTP (n = 3)

| Sample ID | Concentration ±%RSD (n=3) (µg/L) | Spike Recovery | Sample ID | Concentration ±%RSD (n=3) (µg/g) | Spike Recovery |
|-----------------|----------------------------------|----------------|------------------|----------------------------------|----------------|
| Influent | 61.46±3.84 | 92.46 | Primary sludge | 0.804±4.9 | 85.32 |
| Screened Sample | 57.63±2.91 | 97.56 | Secondary sludge | 0.638±6.7 | 90.42 |
| Effluent | 7.28±1.71 | 95.81 | Mixed sludge | 0.469±5.4 | 87.32 |

DISCUSSION AND CONCLUSIONS

A simple, rapid and sensitive method was developed for the quantification of CTC in wastewater samples. The applicability of this method in environmental samples was confirmed by analyzing CTC in WW and WWS and its recovery values. Since there is no chromatographic step involved in the analysis process, the analysis time was reduced from minutes to seconds compared to traditional LC-MS/MS system.

Instrumental parameters were optimized to give the maximal signal for CTC. Laser power and sample volume spotted were optimized and combined with appropriate MRM conditions for accurate quantification. The ultra-fast analysis time of 10 seconds/sample, combined with the high specificity power of mass spectra instrument makes this method suitable alternative for rapid environmental screening of emerging contaminants.



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