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Introduction

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) is a powerful tool to analyze large biomolecules and synthetic polymers. However, qualitative and quantitative MALDI-MS analysis of small analytes ($m/z < 500$ Da) remains a challenge. Our strategy to analyze small targeted molecules was to use a simple and efficient coupling reaction to derivatize analytes with a large charged tag. A pair of isotopically coded light and heavy tag reagents of tris(2,4,6-trimethoxyphenyl)phosphonium acetic acid *N*-hydroxysuccinimide ester (TMPP-Ac-OSu) were developed, adding 573 and 600 Da respectively to each analyte with a fixed positive charge. A variety of primary and secondary amines, amino acids, peptides, drugs, antibiotics, and stimulants were derivatized and analyzed by MALDI-MS in the low femtomole range successfully. Quantitative analysis of mixture of antibiotics such as norfloxacin, ciprofloxacin, lomefloxacin by MALDI using isotopically coded light and heavy tag labeling method was demonstrated. Linear calibration curves of antibiotics from 0.3 to 30 pmol/ μ L with *r*-squared values greater than 0.9995 were achieved. In this presentation, a systematic evaluation of analyzing small molecules by MALDI-MS through charge derivatization is described.

Experimental

Isotopically light and heavy tag reagents: TMPP-Ac-OSu reagents were prepared in-house using the similar method reported by the research group of Watson¹. The reagents were characterized by ¹H, ¹³C, ³¹P NMR, ESI-MS, and MALDI-MS.

TMPP-Ac-OSu stock solution (10 nmol/ μ L): Dissolved in anhydrous CH₃CN and stored in desiccator for months without decomposition.

Buffers:

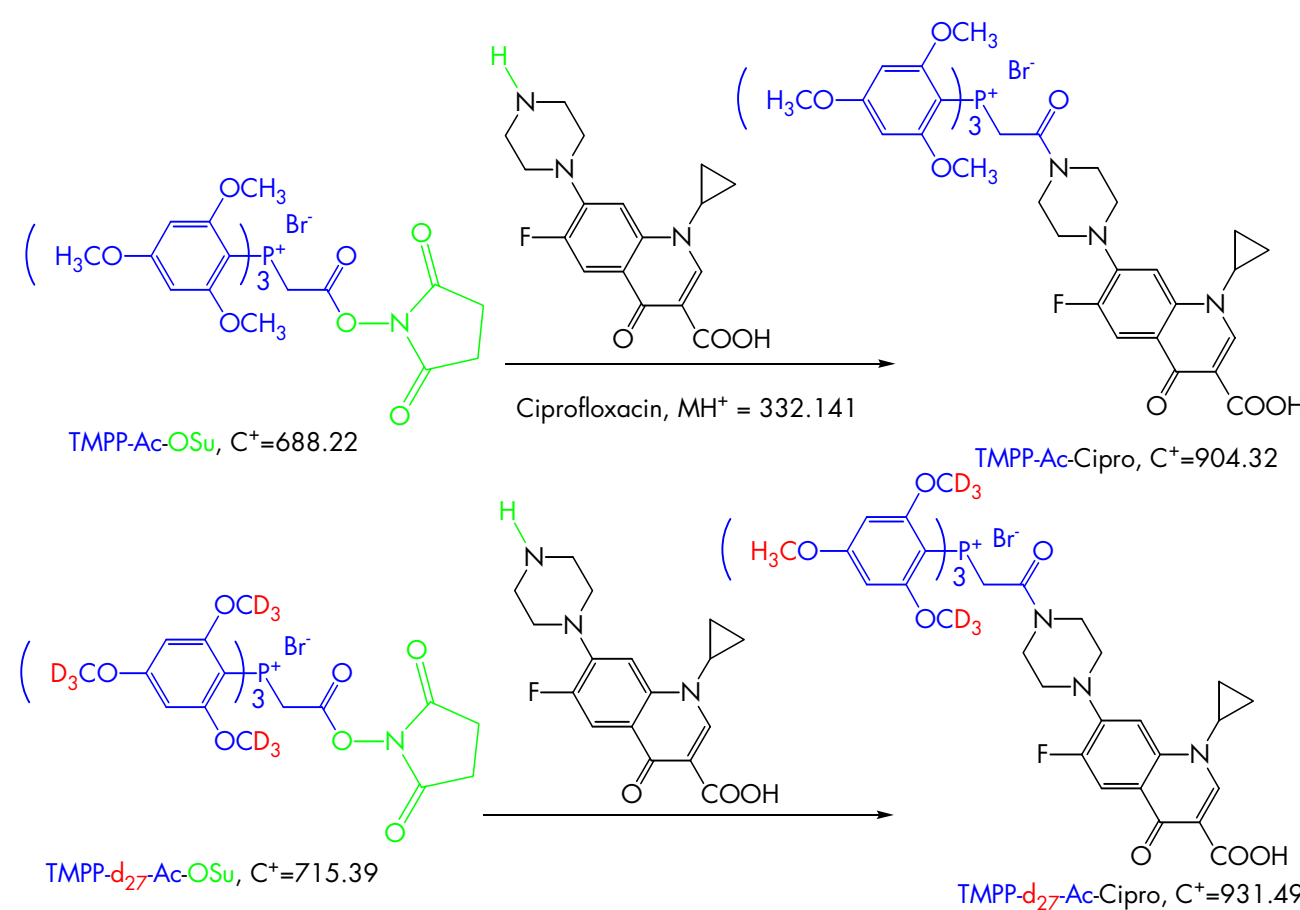
pH 11.7: 20 mM triethylamine aqueous with 20 % CH₃CN.
pH 9.9: 20 mM triethanolamine aqueous with 20 % CH₃CN.
pH 9.2: 20 mM triethylaminonium bicarbonate with 20% CH₃CN.

Matrix: α -cyano-4-hydroxycinnamic acid (10 to 20 mg/mL) in 50/50 of CH₃CN/EtOH.

Derivatization and MALDI sample preparation:

1. Dissolve analytes in a buffer solution containing CH₃CN.
2. Add 10 to 40 times molar excess of TMPP-Ac-OSu solution.
3. Vortex the solution for 10 seconds, then incubate at room temperature for 5 minutes.
4. Add TFA, then mix with matrix solution.
5. Spot 1 μ L on a stainless steel target for MALDI analysis

TMPP-Ac-OSu and Example of Derivatiza-



$\Delta m/z$ of heavy and light tagged analytes = 27

Results

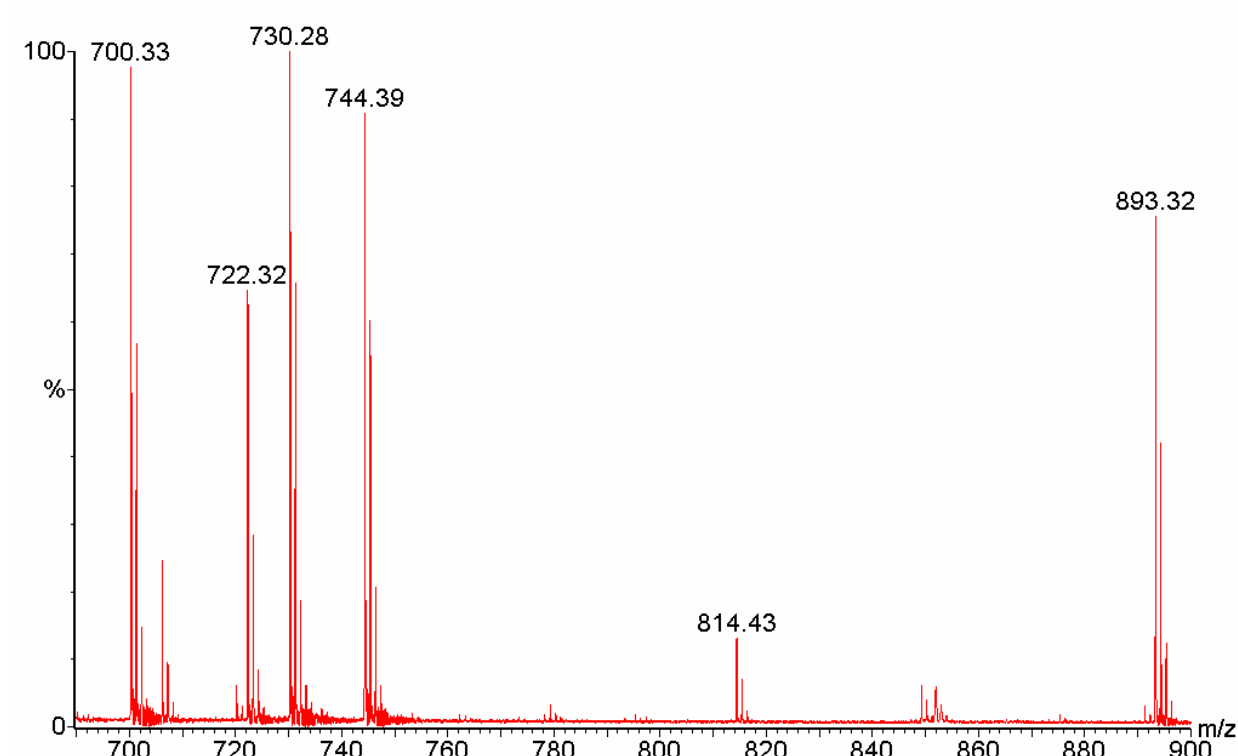
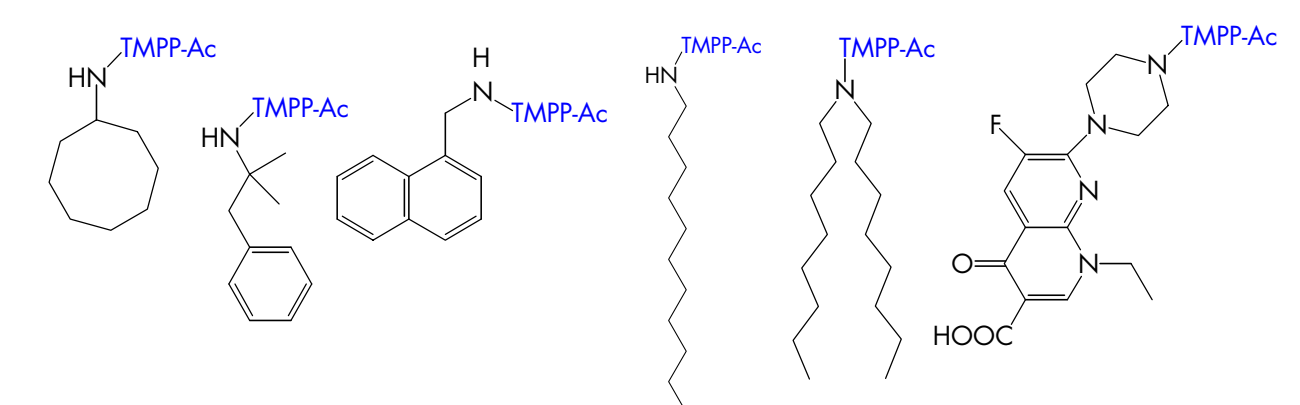


Figure 1. MALDI spectrum of derivatized amines. Mixed 8 μ L of TMPP-Ac-OSu (12 nmole/ μ L) and 400 μ L of pH 11.7 buffer with 4 pmole/ μ L of cyclooctylamine, phentermine, naphthalenemethylamine, undecylamine, dioctylamine and

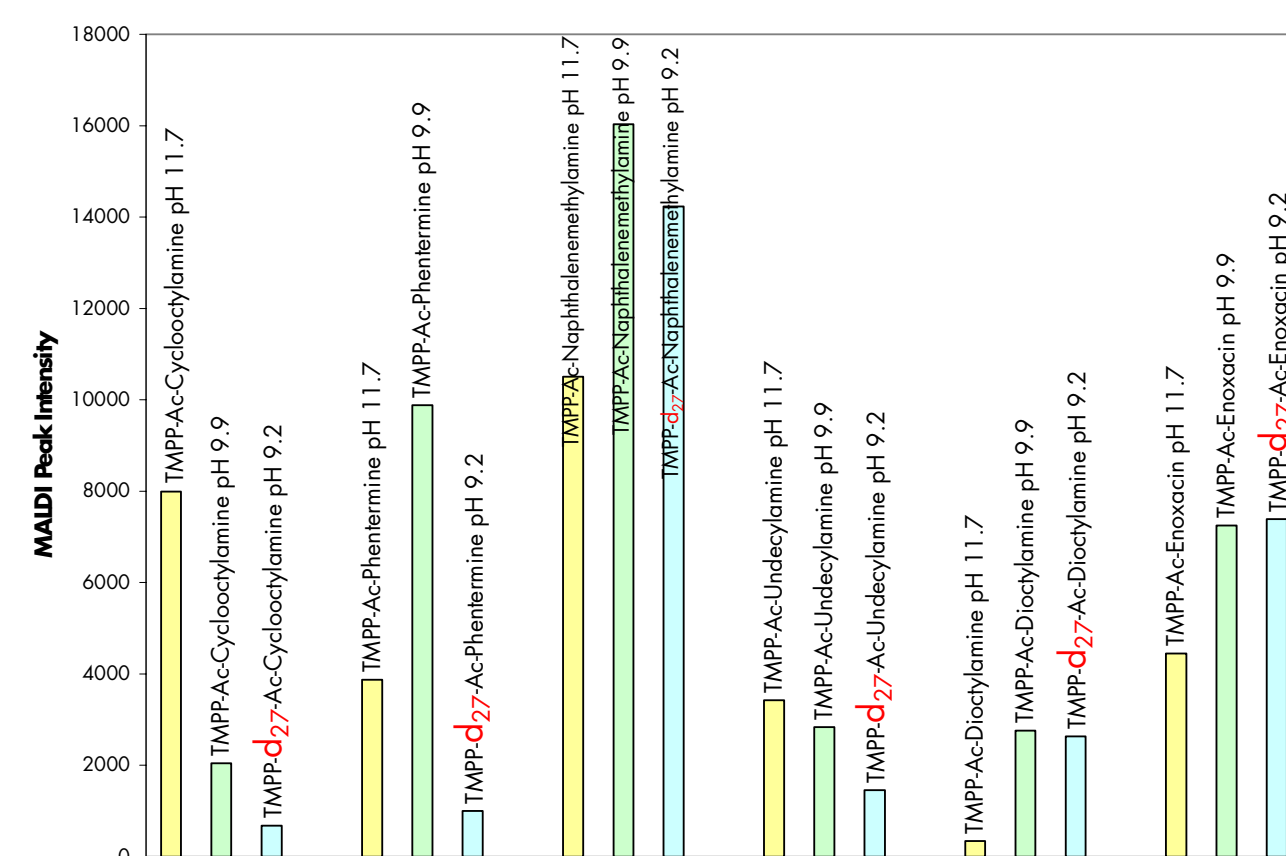


Figure 2. Effect of pH on coupling reactions. A mixture of amines was dissolved in pH 9.2, 9.9 and 11.7 buffers, respectively. The pH 9.2 solution was treated with TMPP-d₂₇-Ac-OSu and solutions of pH 9.9 and 11.7 were treated with TMPP-Ac-OSu. Spotted a mixture of equal volume of pH 11.7 and 9.2 reaction solutions in a well and pH 9.9 and 9.2 solutions in another well for MALDI. Normalized intensity of TMPP-Ac tagged peaks with TMPP-d₂₇-Ac

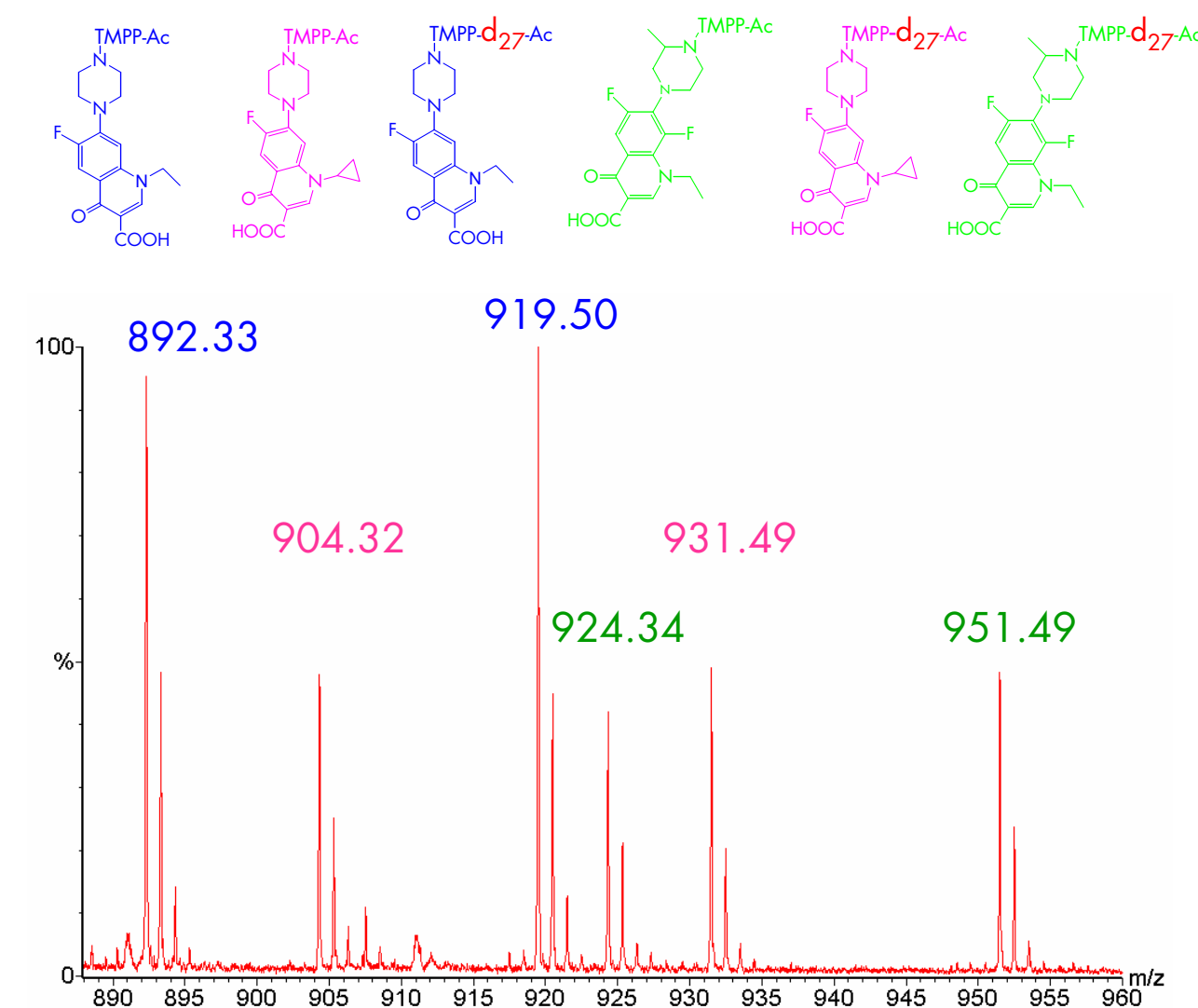


Figure 3. Quantitative MALDI analysis of antibiotics. Solutions of 3 pmol/ μ L of norfloxacin, ciprofloxacin and lomefloxacin in pH 9.2 buffer were treated with TMPP-Ac-OSu and TMPP-d₂₇-Ac-OSu, respectively. Mixed equal volume of solution from both reactions and combined with equal volume of matrix for

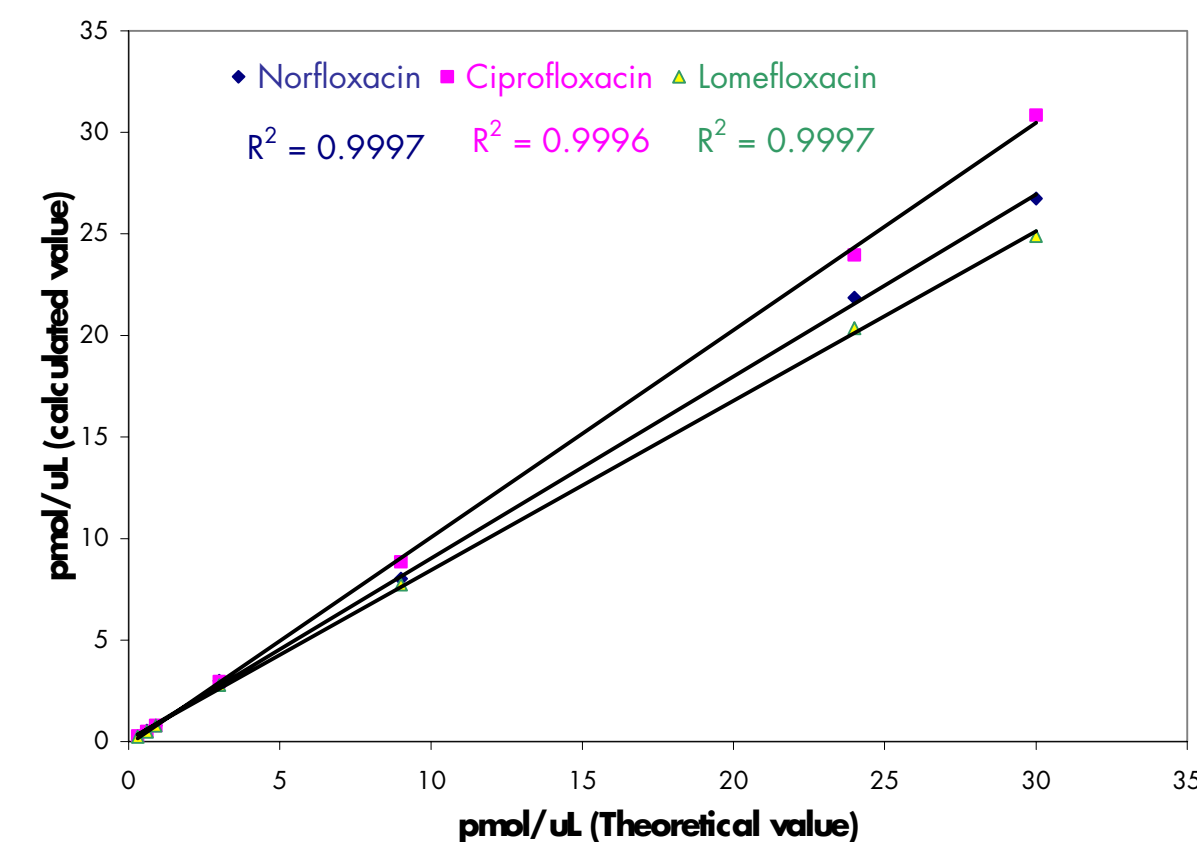


Figure 4. MALDI calibration curves of antibiotics. Test solutions with a mixture of antibiotics concentrations ranging from 0.3 to 30 pmol/ μ L were treated with TMPP-Ac-OSu. A 3 pmol/ μ L antibiotics standard solution was treated with TMPP-d₂₇-Ac-OSu. Equal volumes of standard and test solutions were mixed for MALDI analysis. Antibiotics in test solutions were calculated as intensity of TMPP-Ac tagged peak/TMPP-d₂₇-Ac tagged peak X 3 pmol/ μ L.

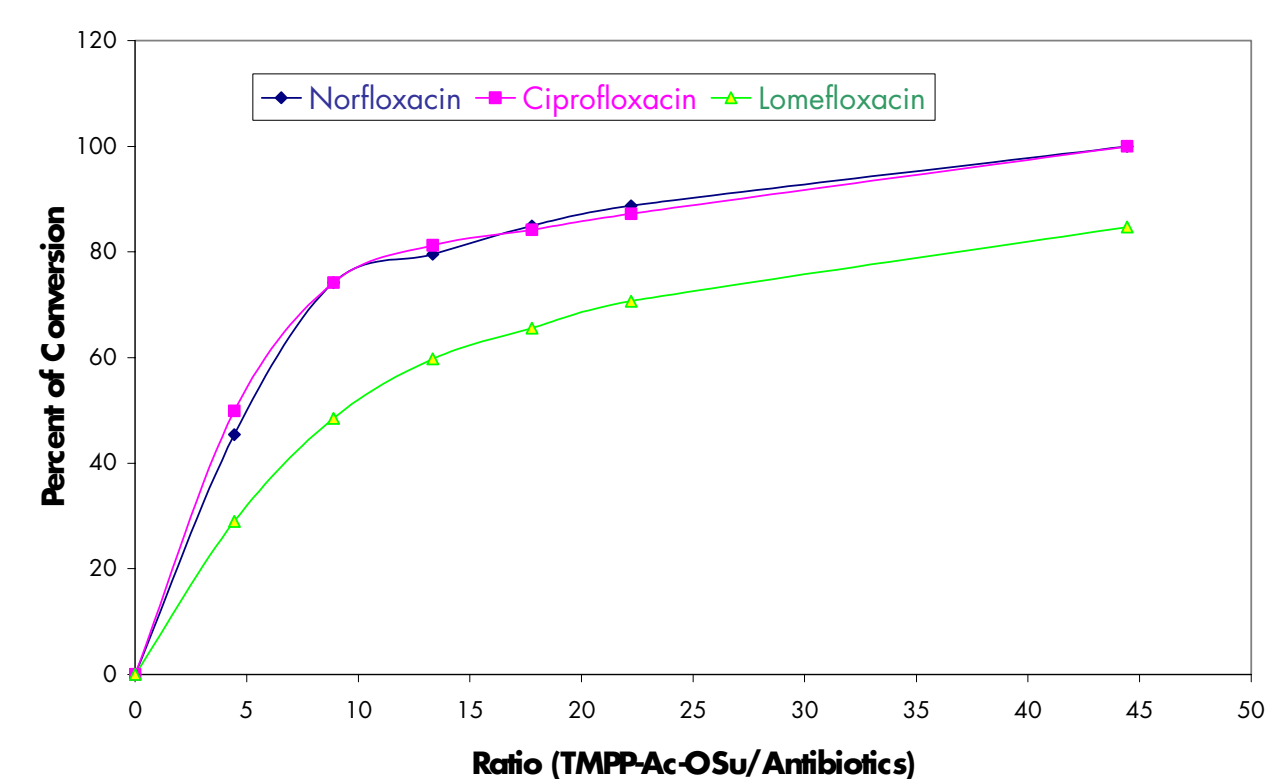


Figure 5. Examining tag reagent needed for derivatization. Antibiotic solution was first treated with 4 to 40 times excess TMPP-Ac-OSu for 5 minutes, then treated with excess ratios of TMPP-d₂₇-Ac-OSu again. Percent conversion was calculated as intensity of TMPP-Ac tagged peak/(intensity of TMPP-Ac + TMPP-

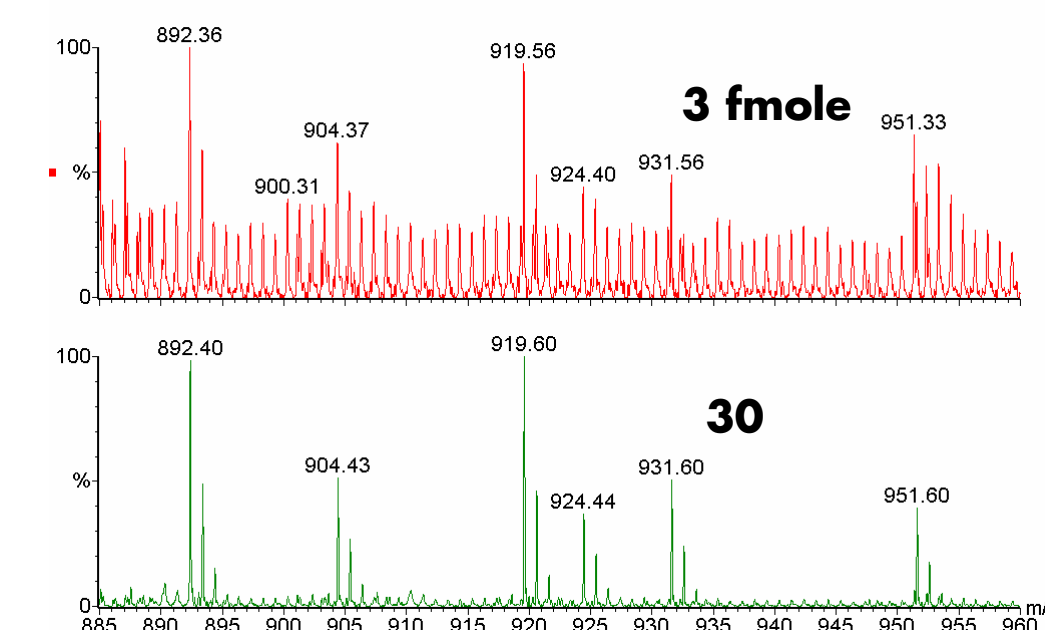


Figure 6. Limit of detection. MALDI analysis of 3 fmole and 30 fmole derivatized norfloxacin, ciprofloxacin and lomefloxacin.

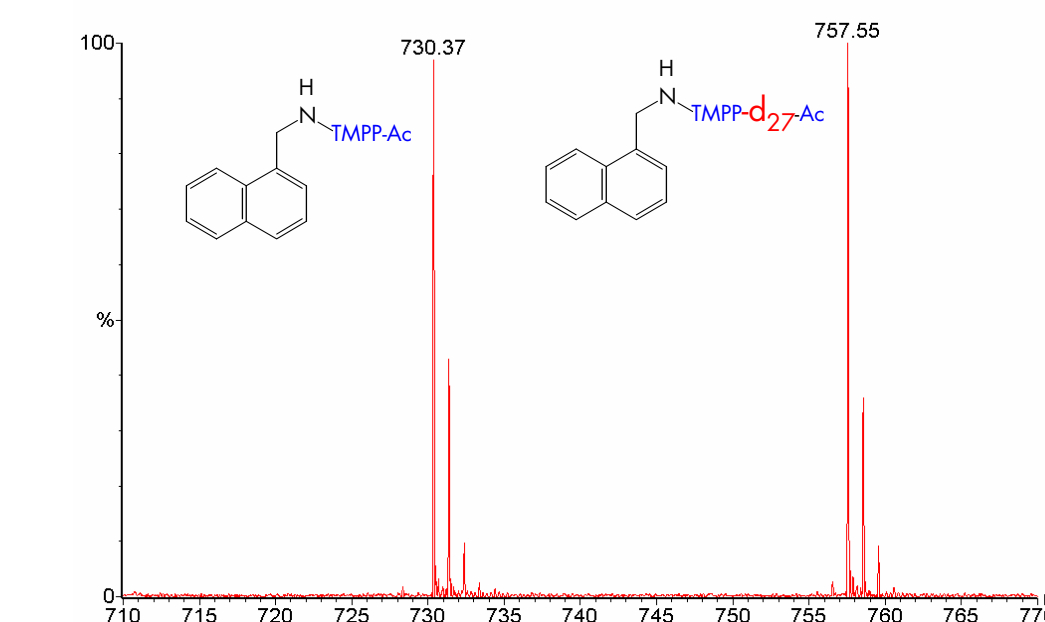


Figure 7. Example of laser desorption/ionization spectrum of derivatized analytes without matrix.

Conclusions

- A simple and efficient charged derivatization was employed.
- Fixed charge TMPP-Ac-derivatization improves efficiency for mass analysis that allows low femtomole detection of small molecules.
- A fast and easy method to evaluate and optimize coupling reaction conditions by MALDI was demonstrated.
- MALDI analysis of target small molecules was achieved.
- Quantitative MALDI analysis of antibiotic mixtures using isotopically light and heavy TMPP-Ac-OSu tags was accomplished.
- MALDI calibration curves of antibiotics from 0.3 to 30 pmol/ μ L with *r*-squared values greater than 0.9995 were achieved.

References

1. Huang Z. *et. al., Analyt.Biochem.* **1999**, *268*, 305-317.