Analysis of peptides by laser diode thermal desorption – triple quadrupole mass spectrometry (LDTD-QqQMS)

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Introduction

- Analysis of peptides using high-throughput techniques such as LDTD-QqQMS is of interest for quality control of peptide therapeutics and biological drugs in the pharmaceutical industry, monitoring of food allergens in the food industry, and detection of viruses, including SARS-CoV-2.
- **Objectives**: Identify the main ions generated by rapid thermal desorption and atmospheric pressure chemical ionization of target peptides in LDTD and determine if those ions can be used for quantitative analysis.

Method

- **Target peptides**: endomorphin-2 (YPFF-NH_2), leu-enkephalin (YGGFL), bradykinin (RPPGFSPFR) and representative peptides of the SARS-CoV-2 virus spike protein (GVYYPDK, IADYNY, QIAPGQTGK).
- **Sample preparation**: peptides were dissolved in water and mixed in 25% water and 75% methanol with 15 mM of dibasic phosphate buffer.
- **LDTD-QqQMS parameters**:
  - Ion source (Figure 1): LDTD model W-960 (Phytronix)
  - Laser pattern: 100% in 6 s then held for 4 s
  - Gas flow: 3 L/min
  - Corona needle current: 3µA
  - QqQMS: Xevo TSQ-micro triple quadrupole (Waters)
- **Quantification**: internal calibration using deuterated isotopologues of the target peptides

Results

Detection of peptides

- $[M+H]^+$ and $[M-H_2O+H]^+$ ions were observed for endomorphin-2 (Figure 2) and leu-enkephalin.
- a_n, b_n, c_n and y_n-ions are the most observed precursor and product ions.

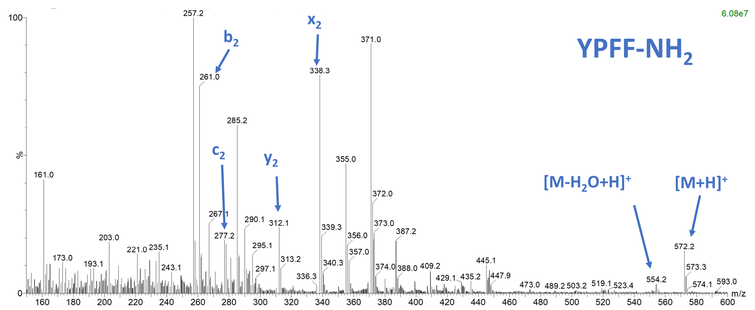

Figure 2. Full scan of endomorphin-2 generated by LDTD in the positive mode.

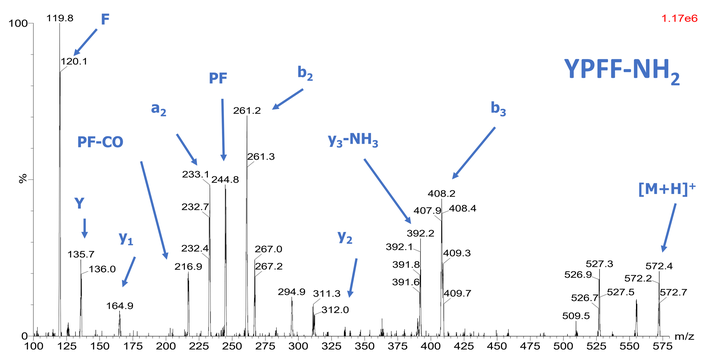

Figure 3. Product ion scan of *m/z* 572 the $[M+H^+]$ ion of endomorphin-2 generated by LDTD in the positive mode.

Peptides can be **detected** and **quantified** in **a few seconds** using LDTD-QqQMS

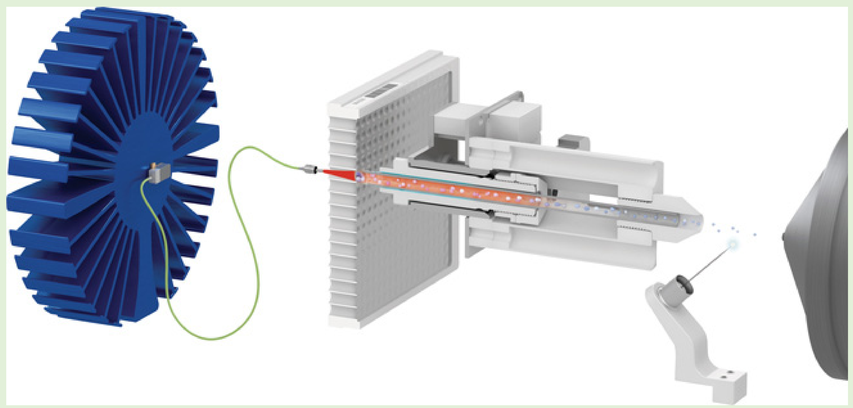

Figure 1. Diagram of the LDTD source.

For more detailed information please scan the QR code

Quantification of peptides

- Endomorphin-2, bradykinin, IADYNY and QIAPGQTGK can be quantified in the solvent (15 mM K_2HPO_4 in H_2O-MeOH 1:3) with a $R^2 > 0.99$.
- Endomorphin-2, IADYNY and QIAPGQTGK can be quantified in a 20 mg/L digested BSA matrix with $R^2>0.99$.
- Calculated LOQ were between 0.4 and 2 mg/L depending on the peptide.

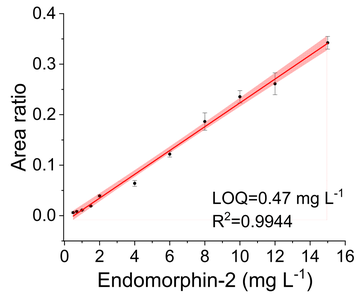

Figure 4. Calibration curve of endomorphin-2 in the solvent (MRM transitions used: *m/z* 554 → *m/z* 362 for endomorphin-2 and *m/z* 577 → *m/z* 408 for endomorphin-2-d_5).

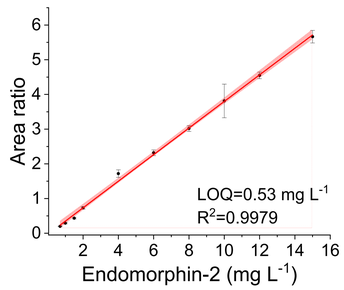

Figure 5. Calibration curve of endomorphin-2 spiked in a 20 mg/L digested BSA (MRM transitions used: *m/z* 572 → *m/z* 245 for endomorphin-2 and *m/z* 577 → *m/z* 233 for endomorphin-2-d_5)

- Generally, coefficients of determination were higher when both peptide MRM transition and deuterated isotopologue MRM transition used isotopologue precursor ions to calculate areas ratios .

Table 1. Coefficients of determination obtained for endomorphin-2 calibration curves in a 20 mg/L digested BSA matrix (in green: R^2 when MRM transitions used isotopologue precursor ions).

		Endomorphin-2-d_5 MRM transitions					
		559→390	**559→362**	**577→120**	**577→261**	**577→233**	**577→408**
Endomorphin-2 MRM transitions	**572→120**	2-15 mg/L R^2 = 0.9615	2-15 mg/L R^2 = 0.9592	0.7-15 mg/L R^2 = 0.9977	0.7-15 mg/L R^2 = 0.9955	0.7-15 mg/L R^2 = 0.9966	0.7-15 mg/L R^2 = 0.9961
	572→408	2-15 mg/L R^2 = 0.9506	2-15 mg/L R^2 = 0.9476	0.7-15 mg/L R^2 = 0.9974	0.7-15 mg/L R^2 = 0.9925	0.7-15 mg/L R^2 = 0.9942	0.7-15 mg/L R^2 = 0.9974
	572→261	2-15 mg/L R^2 = 0.9636	2-15 mg/L R^2 = 0.9610	0.7-15 mg/L R^2 = 0.9976	0.7-15 mg/L R^2 = 0.9950	0.7-15 mg/L R^2 = 0.9969	0.7-15 mg/L R^2 = 0.9954
	572→245	2-15 mg/L R^2 = 0.9644	2-15 mg/L R^2 = 0.9588	0.7-15 mg/L R^2 = 0.9966	0.7-15 mg/L R^2 = 0.9957	0.7-15 mg/L R^2 = 0.9979	0.7-15 mg/L R^2 = 0.9948
	554→390	0.7-15 mg/L R^2 = 0.9951	0.7-15 mg/L R^2 = 0.9933	0.7-15 mg/L R^2 = 0.9827	0.7-15 mg/L R^2 = 0.9896	0.7-15 mg/L R^2 = 0.9910	0.7-15 mg/L R^2 = 0.9791
	554→362	0.7-15 mg/L R^2 = 0.9966	0.7-15 mg/L R^2 = 0.9948	0.7-15 mg/L R^2 = 0.9835	0.7-15 mg/L R^2 = 0.9908	0.7-15 mg/L R^2 = 0.9918	0.7-15 mg/L R^2 = 0.9812
	554→199	2-15 mg/L R^2 = 0.9933	2-15 mg/L R^2 = 0.9925	2-15 mg/L R^2 = 0.9896	2-15 mg/L R^2 = 0.9941	2-15 mg/L R^2 = 0.9963	2-15 mg/L R^2 = 0.9846

Acknowledgments

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