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Application News

MALDI-TOF Mass Spectrometry Analysis MALDI-8030

Quality Control of Synthetic Peptides using the MALDI-8030 Dual Polarity Benchtop MALDI-TOF Mass Spectrometer

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User Benefits

- Easy, simple, negative ion quality control of peptides on affordable benchtop MALDI-TOF
- Useful for peptides with labile functional groups, thus facilitating the detection of the intact species
- Mass spectra are cleaner and easier to interpret since no detection of salt adducts

■ Introduction

Synthetic peptides are nowadays increasingly used in the fields of biochemistry, immunology and medicine. They fulfil a number of purposes, such as cancer diagnosis and treatment, drug and delivery systems development, epitope mapping, production of antibodies, and vaccine design.

The synthesis of peptides is a stepwise process which involves a reaction between the activated carboxylic group of one amino acid and the amino group of another, thus creating the so-called peptide bond. The crude peptide product obtained typically contains impurities (e.g., side-products formed during synthesis), hence requiring a purification step.

Within the manufacturing process of synthetic peptides, quality control (QC) plays a critical role in providing high purity products. MALDI-TOF mass spectrometry is widely used to confirm the molecular identity of the final peptide as well as its purity.

Here, we present the dual polarity MALDI-8030 benchtop linear mass spectrometer for the QC analysis of synthetic peptides in positive and negative ion modes (Fig 1). The benefits of the negative ionisation mode are demonstrated in terms of: i) preserving the integrity of species carrying labile functional groups; ii) simplifying the interpretation of the mass spectra through elimination of salt adduct interferences.

■ Measurement Conditions and Samples

Samples of synthetic peptides were kindly provided by Bachem (United Kingdom). Two different scenarios are herein presented: 1) Peptide D, which contains two labile sulfo groups bound to Tyrosine amino acids; 2) Peptide A, provided in the semi-pure and pure forms. Samples were prepared in 1:1 acetonitrile/water, with/without acid (0.1% trifluoroacetic acid (TFA)) depending on the instrument polarity.

For the MALDI analysis, all samples were spotted with alpha-cyano-4-hydroxycinnamic acid (CHCA), 5 mg/mL in 1:1 acetonitrile/water, with/without acid (0.1% TFA) depending on the instrument polarity.

■ Results – Peptide D

Fig 2 shows positive and negative mode MALDI spectra obtained for Peptide D. Analysis in positive ionisation mode (Fig 2a) results in the loss of both labile sulfo groups, producing a positively-charged species. As a result, only the species corresponding to the loss of both sulfo groups (m/z 2375.068, calculated) is detectable.

In the negative mode spectrum (Fig 2b), the two labile sulfo groups are retained, making the detection of the intact peptide successful (m/z 2532.966, calculated), along with the species corresponding to the loss of one and both sulfo groups (m/z 2453.010 and 2373.052, calculated). All species detected were isotopically resolved (resolution of the monoisotopic peak shown in { }) along with good mass accuracy.

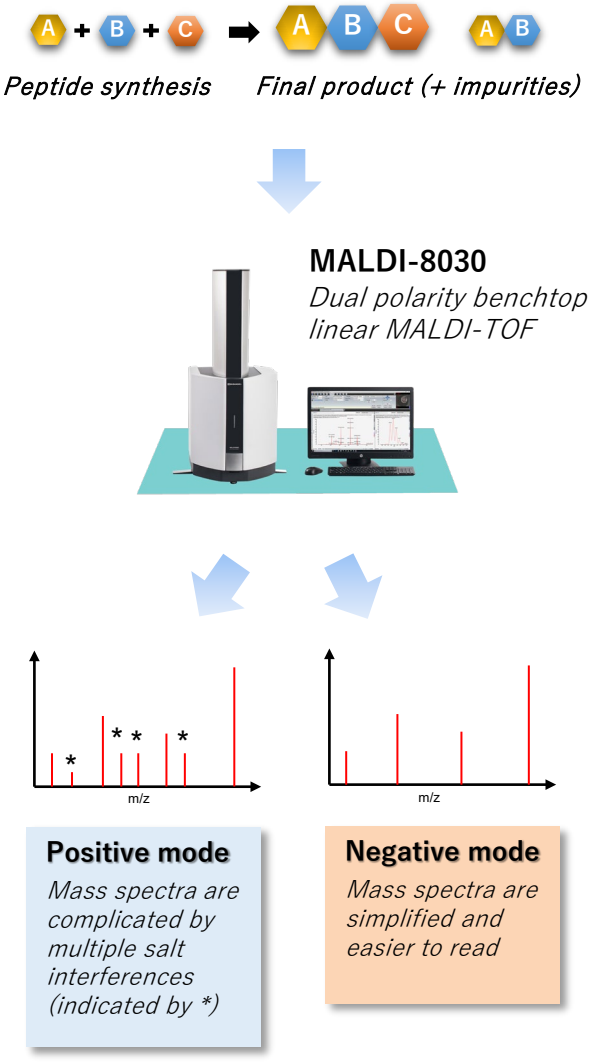

Fig. 1 Dual polarity MALDI-8030 for the QC analysis of synthetic peptides

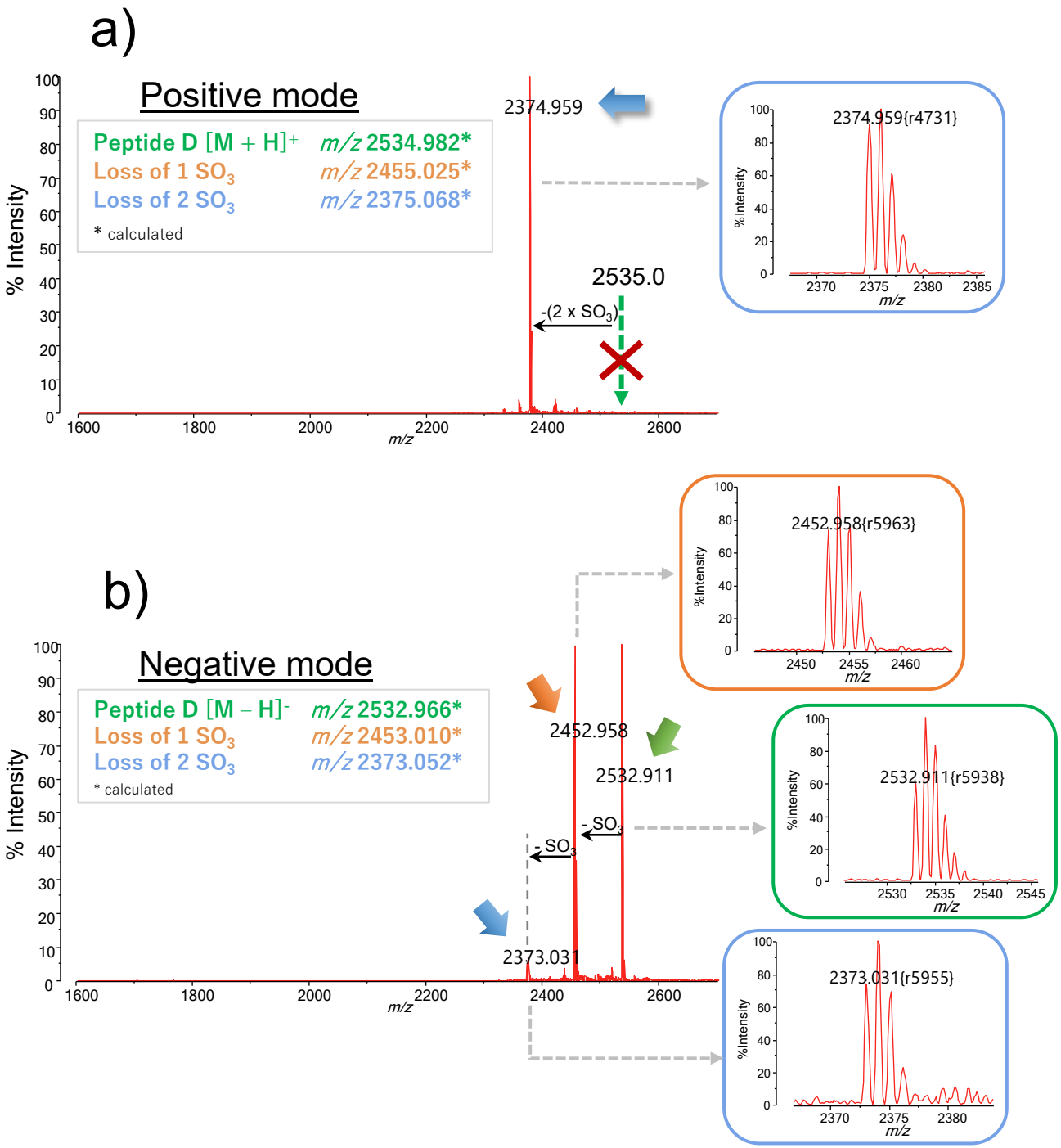

Fig. 2 a) Positive mode MALDI spectrum of Peptide D: only the species corresponding to the loss both sulfo groups was detected. b) Negative mode MALDI spectrum of Peptide D: the intact species is successfully detected, along with the species corresponding to the loss of one and both sulfo groups. Exact calculated monoisotopic masses are provided in the left hand-side inset. Resolution of the detected monoisotopic peaks are shown in { }.

■ Results – Peptide A

Peptide A was provided in the semi-pure and pure forms following sequential stages of purification of the crude product. The semi-pure is an intermediate form which still contains a significant amount of impurities. Fig 3a shows the positive mode MALDI spectrum of Peptide A (semi-pure). As it can be observed, the spectrum interpretation is complicated by the presence of salt (sodium and potassium) adducts of both impurities and final product.

In contrast, the negative mode MALDI spectrum looks cleaner and easier to interpret due to the absence of salt adducts (Fig 3b). The final peptide was detected successfully in both modes with good mass accuracy.

Similar considerations are applied to the pure form of Peptide A (Fig 4). Again, a significant reduction in the salt adducts of the target peptide product in the negative mode spectrum result in a cleaner spectrum.

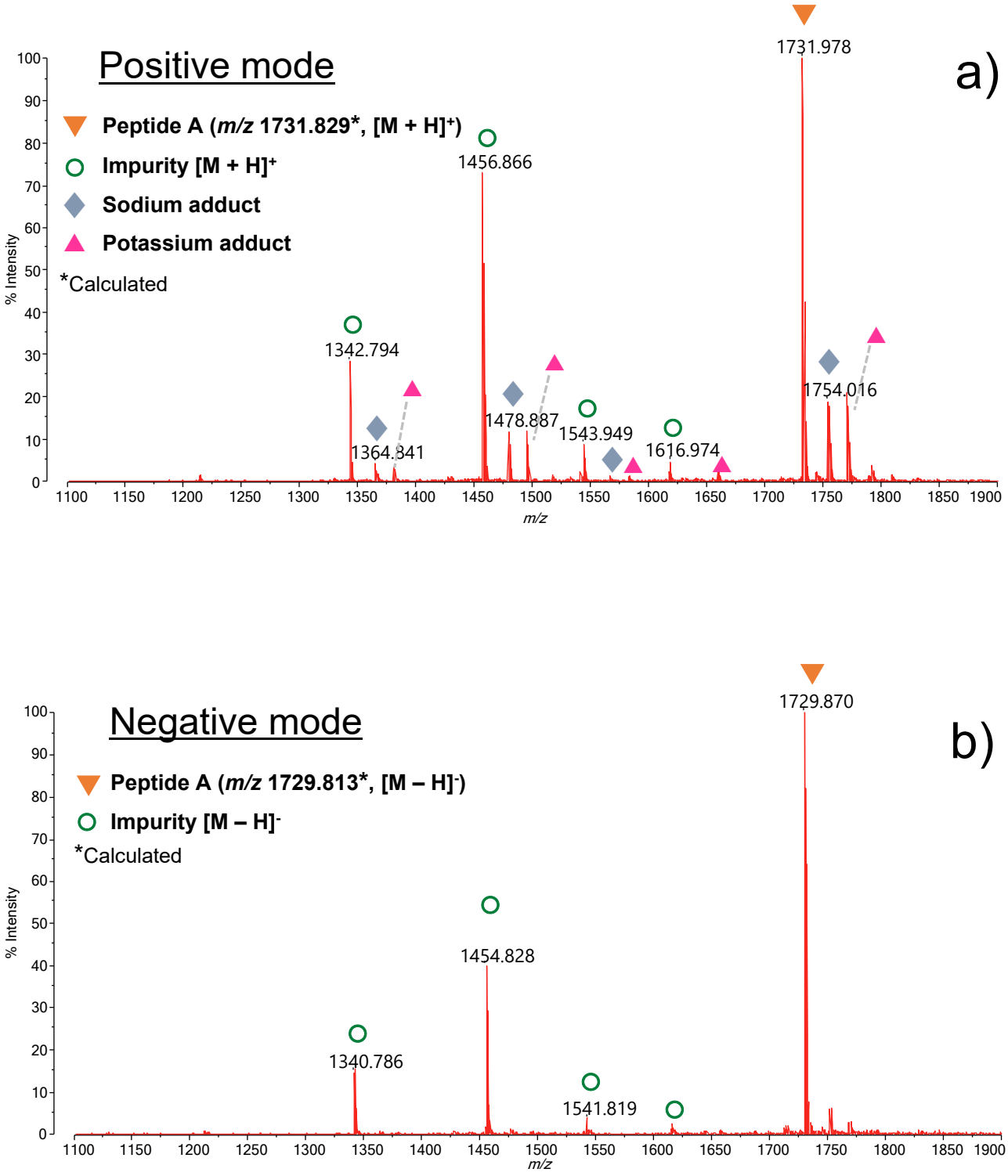

Fig. 3 a) Positive mode MALDI spectrum of Peptide A (semi-pure): the spectrum interpretation is complicated by the salt adduct signals of both impurities and final peptide. b) Negative mode MALDI spectrum of Peptide A (semi-pure): the spectrum interpretation is easier thanks to the absence of salt adduct signals.

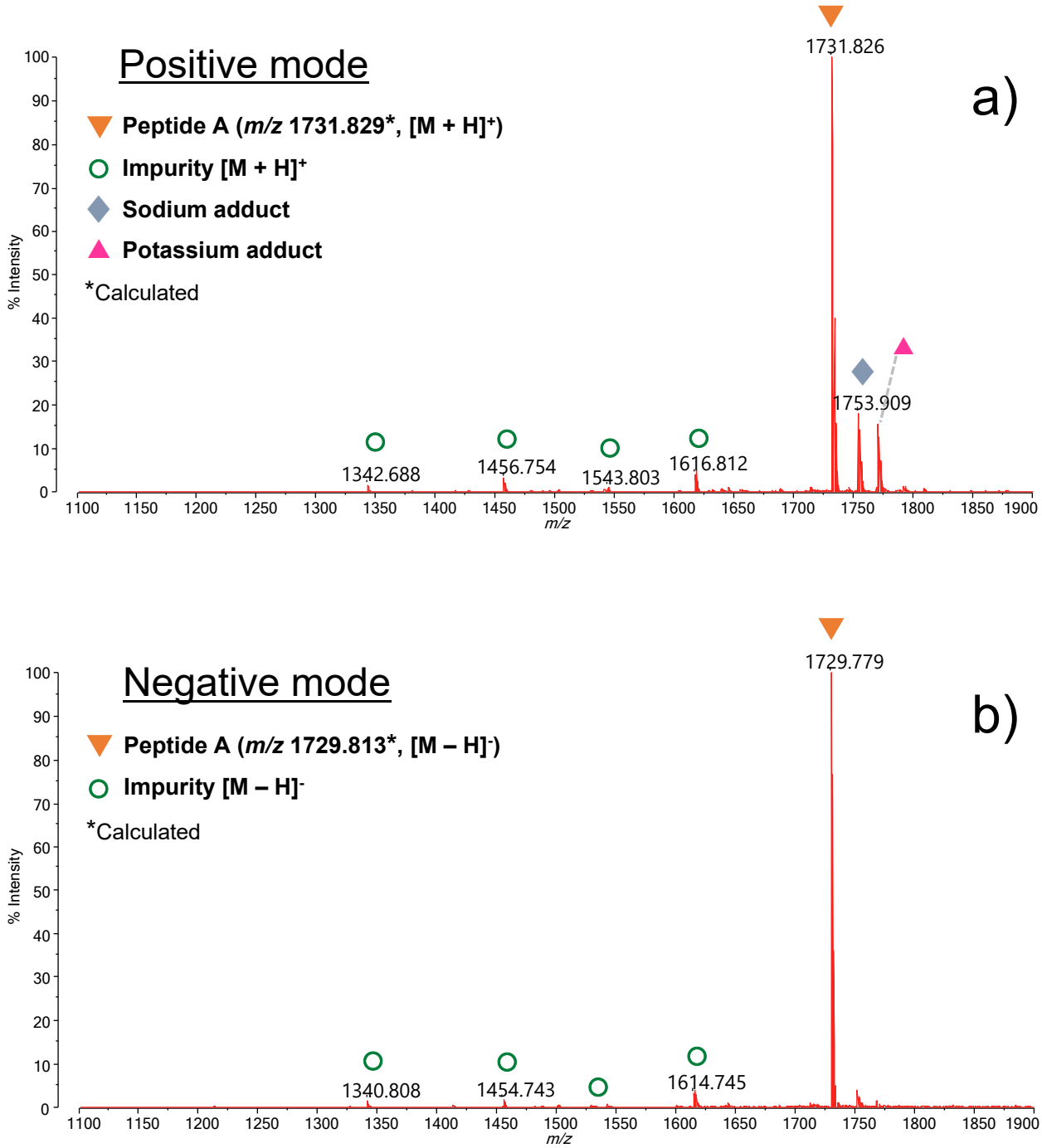

Fig. 4 a) Positive mode MALDI spectrum of Peptide A (pure): the target peptide is detected along with salt adducts. b) Negative mode MALDI spectrum of Peptide A (pure): there is a significant reduction in salt adducts detected for the target peptide species, resulting in a cleaner spectrum.

■ Conclusion

This application demonstrates the benefits of negative ion mode for the QC analysis of synthetic peptides.

Despite positive ion mode detection being more popular for the analysis of peptides, for the examples shown, the negative ion mode proved to be more beneficial for two reasons: 1) it can help prevent the loss of labile functional groups, thus facilitating the detection of the intact species; 2) due to the significantly reduced formation of salt adducts in negative ion mode, the obtained mass spectra are cleaner and easier to interpret.

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