Electronic Supplementary Material (ESI) for Chemical Communications.

SUPPORTING INFORMATION

Online desalting and sequential formation of analyte ions for mass spectrometry characterization of untreated biological samples

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1. Experimental section

1.1 Mass spectrometer and ion source

The PR-R-nESI MS is implemented on a LTQ linear ion trap mass spectrometer (Thermo Scientific, U.S.) equipped with a homemade pulled glass capillary for all experiments. In a typical experiment, the negative ion detection mode is at first applied for about 20-30 seconds. The maximum voltage magnitude in negative ion mode is -8.0 kV. After that, the positive ion mode is applied at +1.7 kV. Note that the reproducibility and signal intensity is improved when the polarity reversing sequence (-8 kV step followed by +1.7 kV) is applied sequentially two times. The experimental parameters for LTQ-XL mass spectrometer are as follows: capillary temperature, 180 °C; nitrogen drying gas flow rate, 10 L/min; multipole RF amplitude (V_{p-p}), 800 V. The normal and high scan modes of detection are used. The ion trap mass range is set from 50-2000 and 100 to 4000 Th in both positive and negative ion detection mode. The maximum time of ion injection into the linear ion trap of 0.7-0.001 ms is found to provide the highest ion signal intensity.[1,2] Hence, in all the experiments the ion injection time of 0.7-0.001 ms is used. The capillary temperature is maintained at 180 °C. The detailed description of ion source can be found in our recent paper.[3] The schematic diagram and photograph of PR-R-nESI ion source are shown in Fig. 1 and Fig. S1. The experimental parameters for conventional ESI are as follows: flow rate, 5 μL/min; the distance from the emitter to the ion inlet, 5 mm; the tip diameter, 70 μm; high voltage 3.5-4.5 kV.

1.2 Sample preparation

Analytical-grade bovine heart cytochrome c, insulin, 1,2-Dipalmitoyl-sn-glycerophosphocholine, triflouro-acetic acid, formic acid, methanol, NaCl are purchased from Sigma Aldrich (St. Louis, MO, USA). Cytochrome c is prepared in pure water then diluted with 5% methanol. 1,2-Dipalmitoyl-sn-glycerophosphocholine is prepared in methanol then diluted in pure water. Insulin is prepared in 0.1% triflouro-acetic acid aqueous solution then diluted in pure water. Insulin is less soluble in water

compared to 0.1% triflouro-acetic acid aqueous solution. All chemicals are used without further purification. The deionized water is prepared in our laboratory. All biological samples including strawberry, potato, naval orange and yogurt are purchased from a local market of Nanchang, China. The silver-coated Pt wire with o.d. ~50 μm is purchased from Ida Tianjin Co. (Tianjin, China). Commercial gel loading tip (*epT.I.P.S*, Eppendorf, Germany) with inner diameter of 120 μm is used for loading the sample into pulled glass capillary throughout this study. Note that the back side of the pulled glass capillary was cut short in order to enable the insertion of gel loading tip closer to the glass capillary tip. This minimized bubble formation at the tip during sample loading. The pulled glass capillary is made using Sutter Instrument P-1000 (USA). The inner diameter of pulled glass capillary is 1 μm for all experiments.

2. Results and discussion

Naval orange as a fruit sample has been selected and analyzed without sample preparation by PR-R-nESI. In conventional nESI metal ions always interfere the detection of protonated molecular ions. In PR-R-nESI, metal ions move toward the nanotip due to applying negative high voltage (-8 kV). Thus PR-R-nESI allows separation from untreated plant materials at molecular level in aqueous solutions. Fig.S9b (ESI†) shows mainly the protonated peaks (*m/z* 115, 144, 343, 373, 403, 433) from naval orange within 0-0.4 minutes, followed by mostly metal ion peaks (*m/z* 218, 381, 441, 722, 752, 782, 812, 842, 902) after 0.4 minutes. The maximal signal intensity of protonated peaks is higher than that of metal (sodiated and potasiated) ion peaks. These results indicate that naval orange displays sequential ionization profile by PR-R-nESI.

Viscous samples are particularly difficult for chemical analysis by conventional nESI or ESI due to capillary clogging. Here, yogurt, a viscous sample, is tested using PR-R-nESI applying maximum negative voltage (20-30 s). The solid probe was used to pick up a tinny amount of yogurt sample by dipping and then inserted into solution contained nanocapillary. The separation time for

protonated peak is increased when separation voltage is applied to the maximal value (-8.0 kV) allowed for LTQ-XL mass spectrometer. The observed signals for yogurt sample are left without chemical identification due to the lack of reference data by other methods. Fig.S10b (ESI†) indicate that almost all peaks correspond to protonated species until 1.5 min, followed by the metal ion adducts ($[M+Na]^+$, $[M+K]^+$, $[M+Ca]^+$). The spatial separation between metal ion (sodiated, potasiated or calcium adduct ion) and protonated ion for complex sample is very useful for MS/MS analysis.

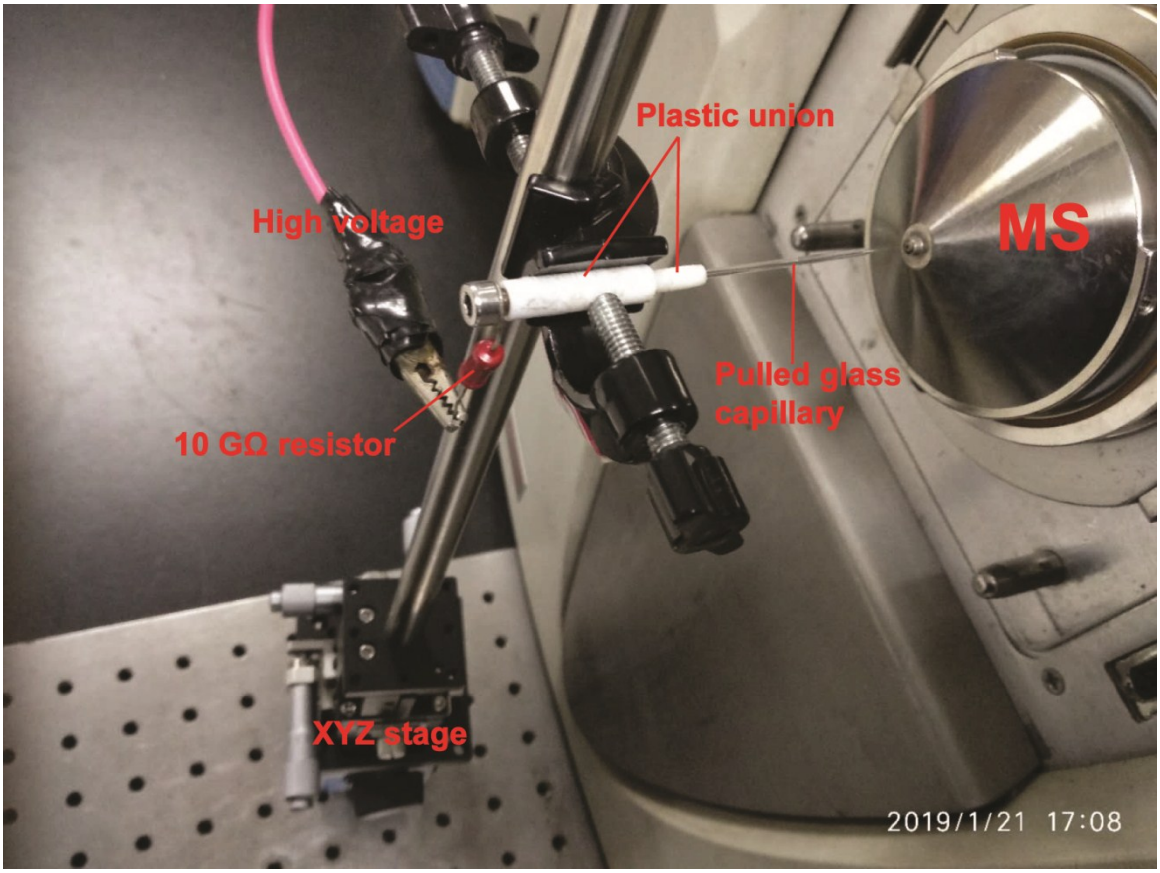

Fig. S1 The photograph of PR-R-nESI ion source installed on ion trap mass spectrometer

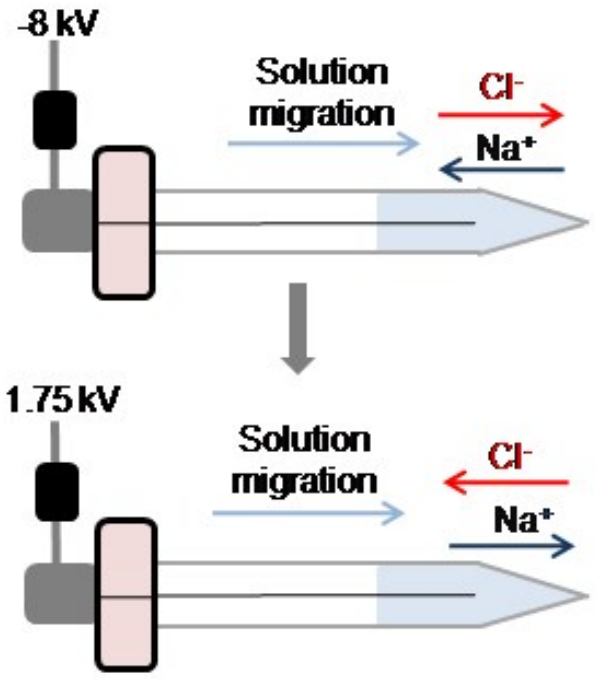

Fig. S2 The electrophoretic migration of solute ions for NaCl in water in PR-R-nESI.

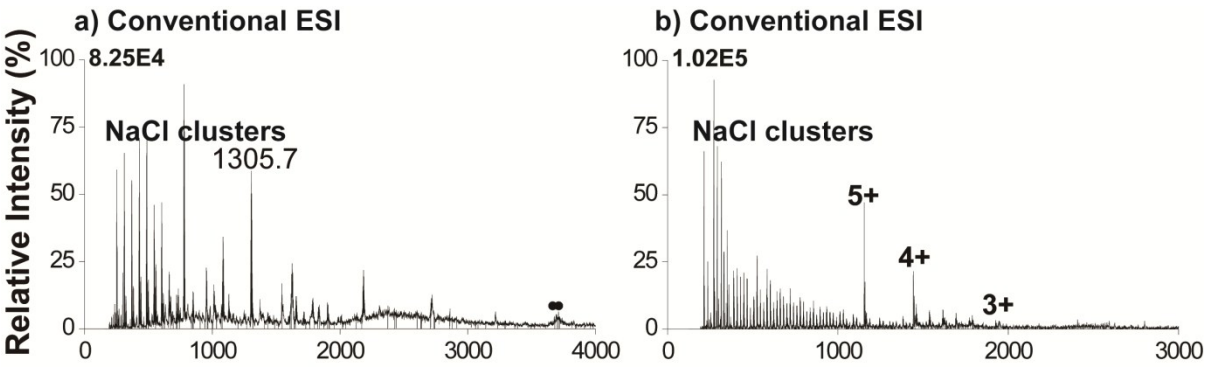

Fig. S3 Conventional ESI-MS analysis of a) cytochrome c (120 µg/mL) in 10 mM aqueous NaCl and b) insulin (58 µg/mL) in 10 mM aqueous NaCl. Two black circles indicate the dimer of cytochrome c.

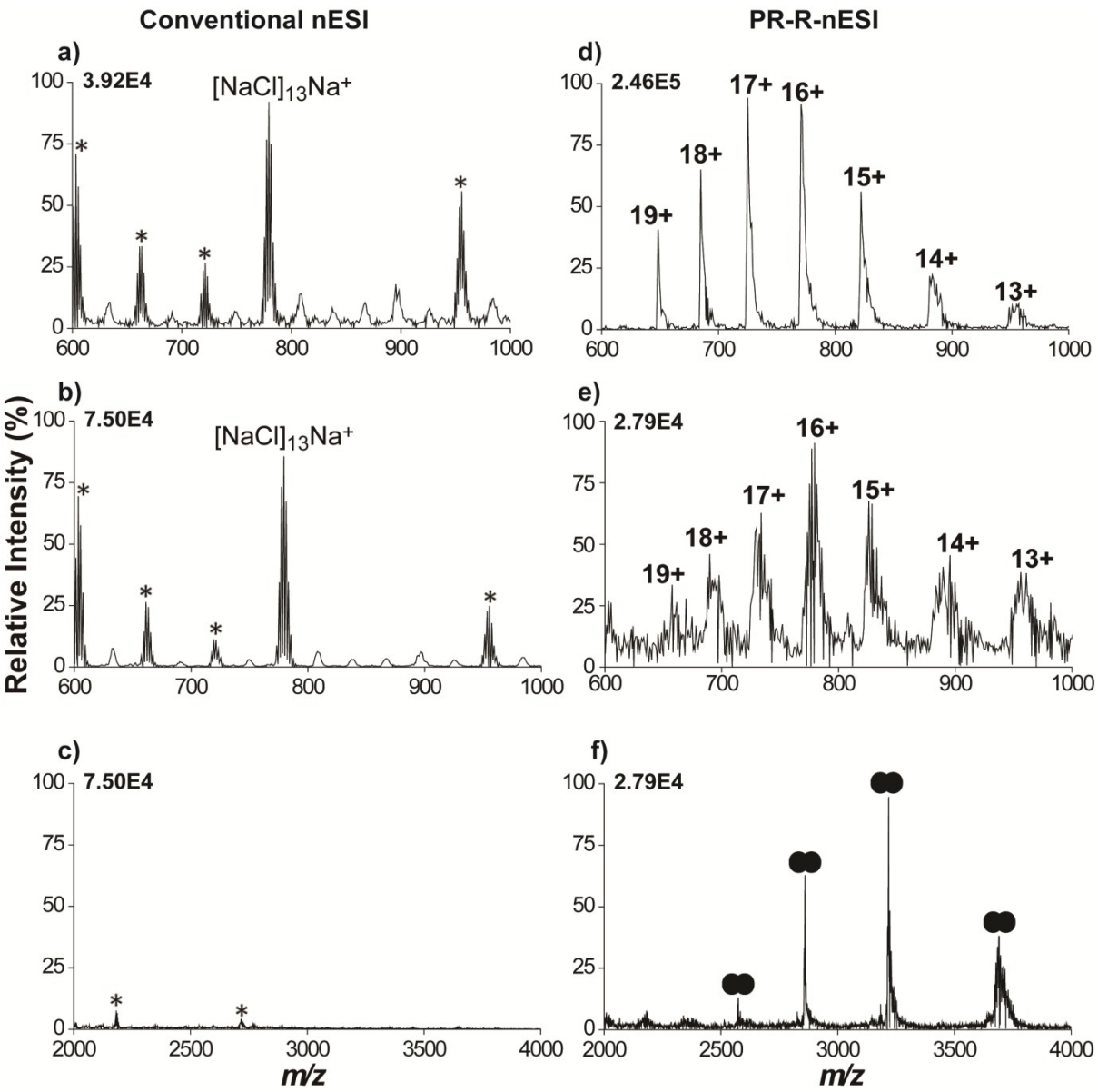

Fig. S4 The comparison of spectra for cytochrome c (120 µg/mL) in aqueous NaCl using PR-R-nESI-MS analysis (a-c) vs. conventional nESI-MS (d-f). The panels (a&d) correspond to 10 mM concentration of NaCl. The panels (b, e, c& f) correspond to 100 mM NaCl. The panels c and f correspond to the high mass range. The asterisks and double black circles correspond to sodium cluster ions and dimers of cytochrome c, respectively. The spectral shapes of cytochrome c in PR-R-nESI are broader at 100 mM NaCl than at 10 mM NaCl due to the higher degree of protein-metal adduct formation.

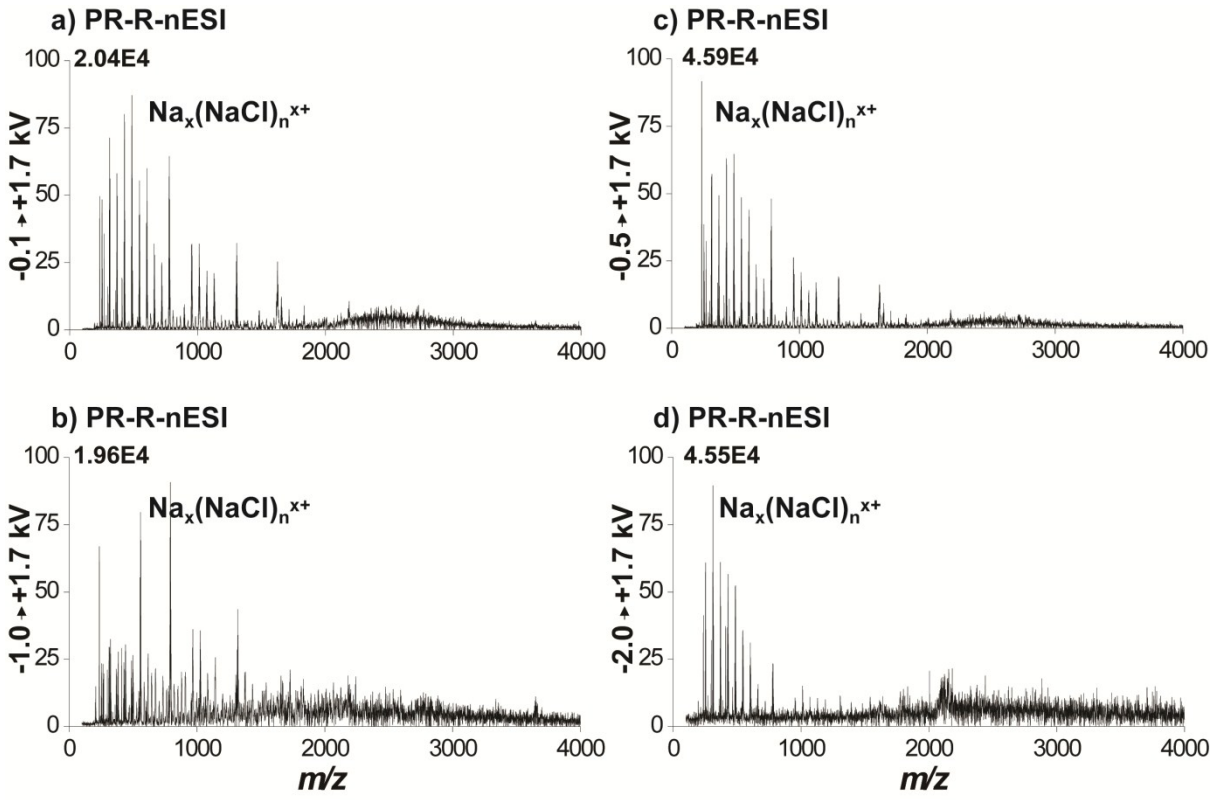

Fig. S5 PR-R-nESI-MS for the analysis of cytochrome c (120 µg/mL) with 100 mM NaCl. Mass spectra of cytochrome c with 100 mM NaCl obtained by PR-R-nESI at different voltages a) -0.1 to 1.75 kV b) -0.5 to 1.75 kV c) -1.0 to 1.75 kV d) -2.0 to 1.75 kV respectively.

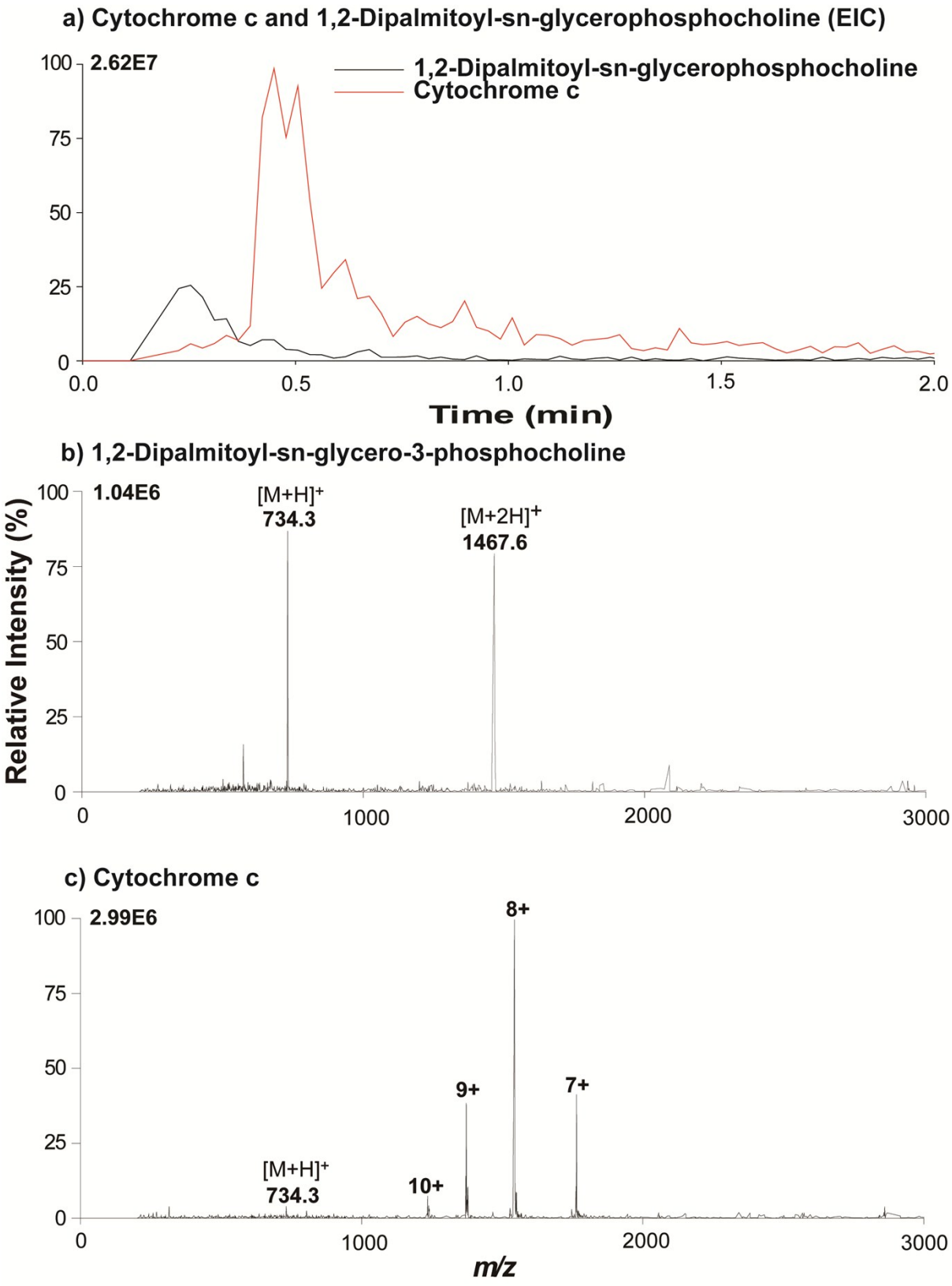

Fig. S6 Sequential PR-R-nESI for the analysis of cytochrome c and 1,2-Dipalmitoyl-sn-glycerophosphocholine (10^{-5} M) prepared in 5% methanol aqueous solution. Other instrumental settings were identical.

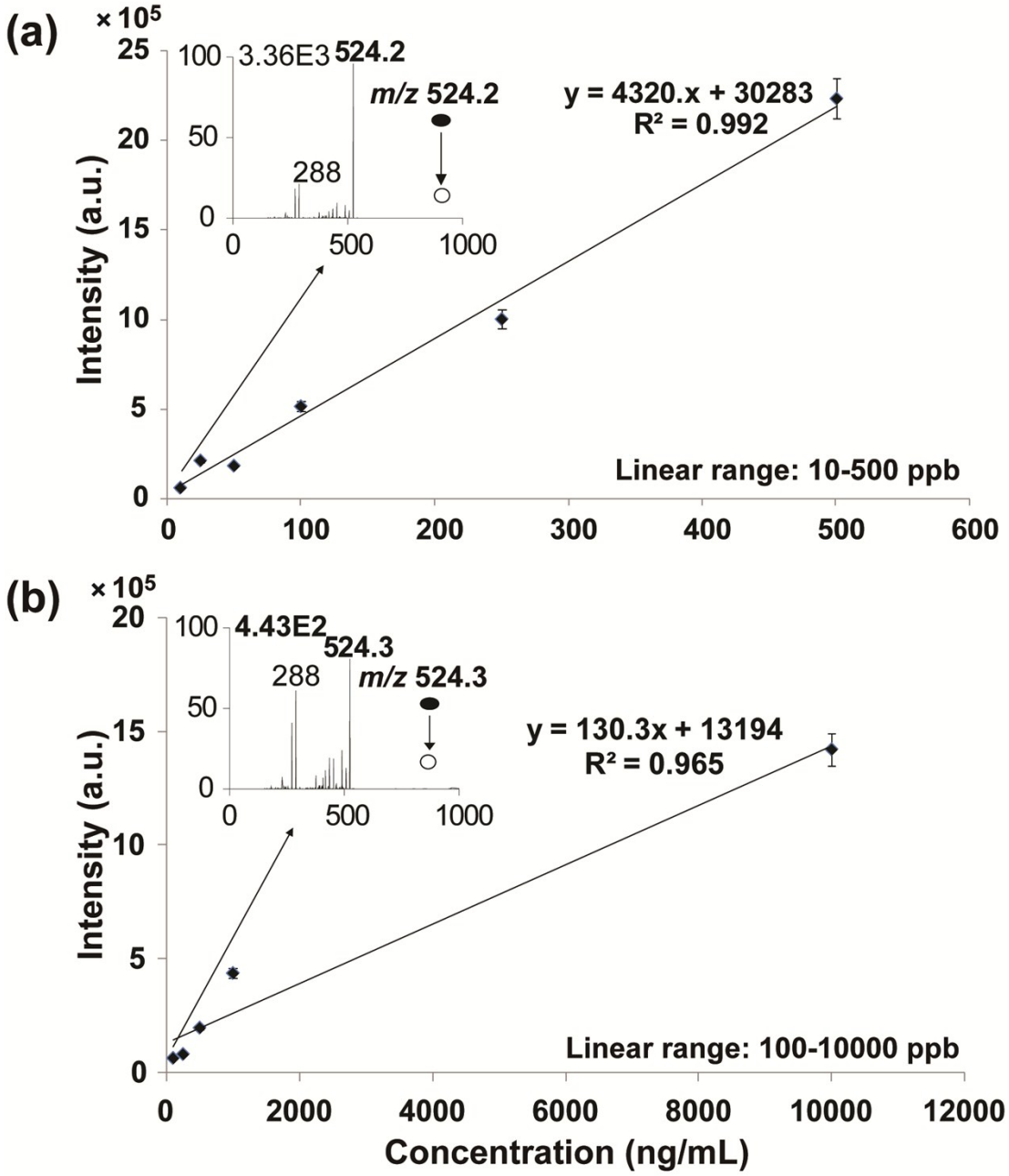

Fig. S7 Quantitative PR-R-nESI analysis of MRFA peptide in a) water/methanol mixture (95:5), b) water/methanol mixture (95:5) with 10 mM NaCl. The linearity range for MRFA in water/methanol mixture was 10–500 ng/mL. The linearity range for MRFA in water/methanol mixture with NaCl was 100–10000 ng/mL. Three independent experiments were performed to estimate the corresponding standard errors. The inset spectrum shows MS/MS pattern of the target signal obtained at analyte concentration 10 ng/mL and 100 ng/mL.

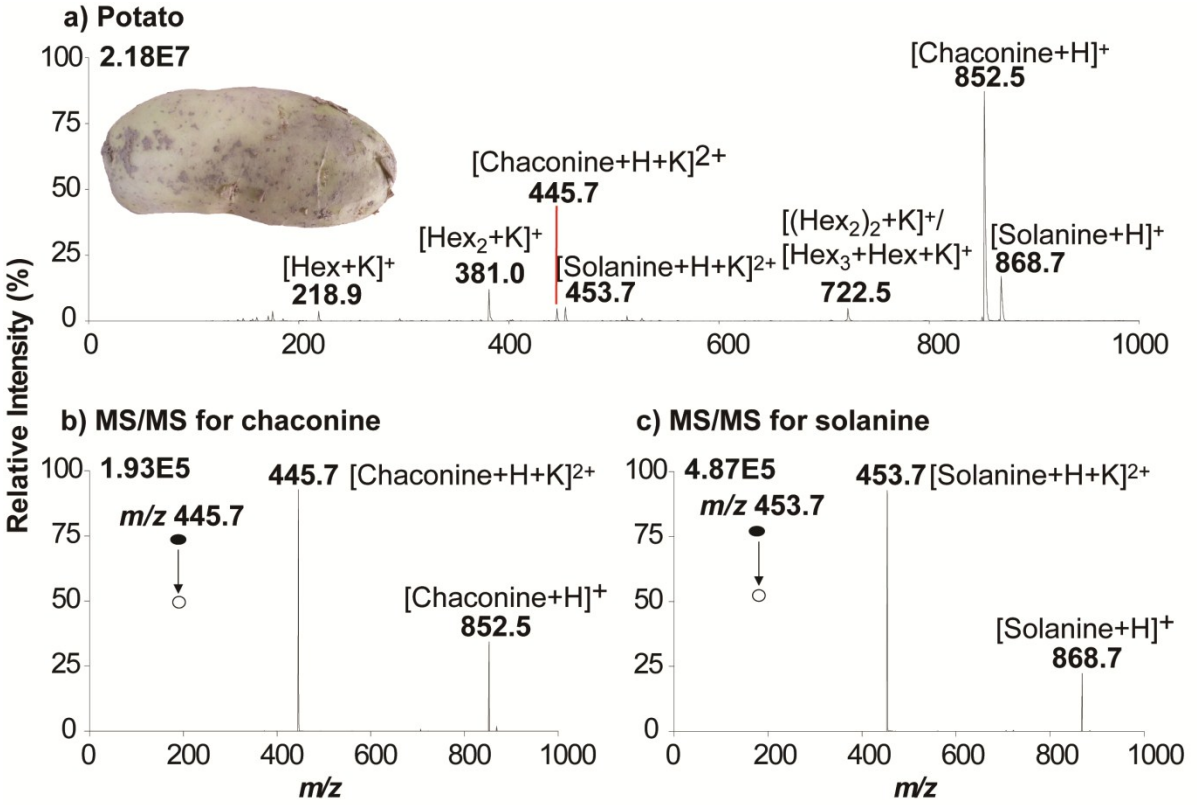

Fig. S8 Full-range mass spectra (a) and tandem mass spectra (b&c) of potato acquired by PR-R-nESI in positive ion mode using 5% methanol aqueous solution. Phytochemicals were identified using tandem operation as solanine and chaconine.

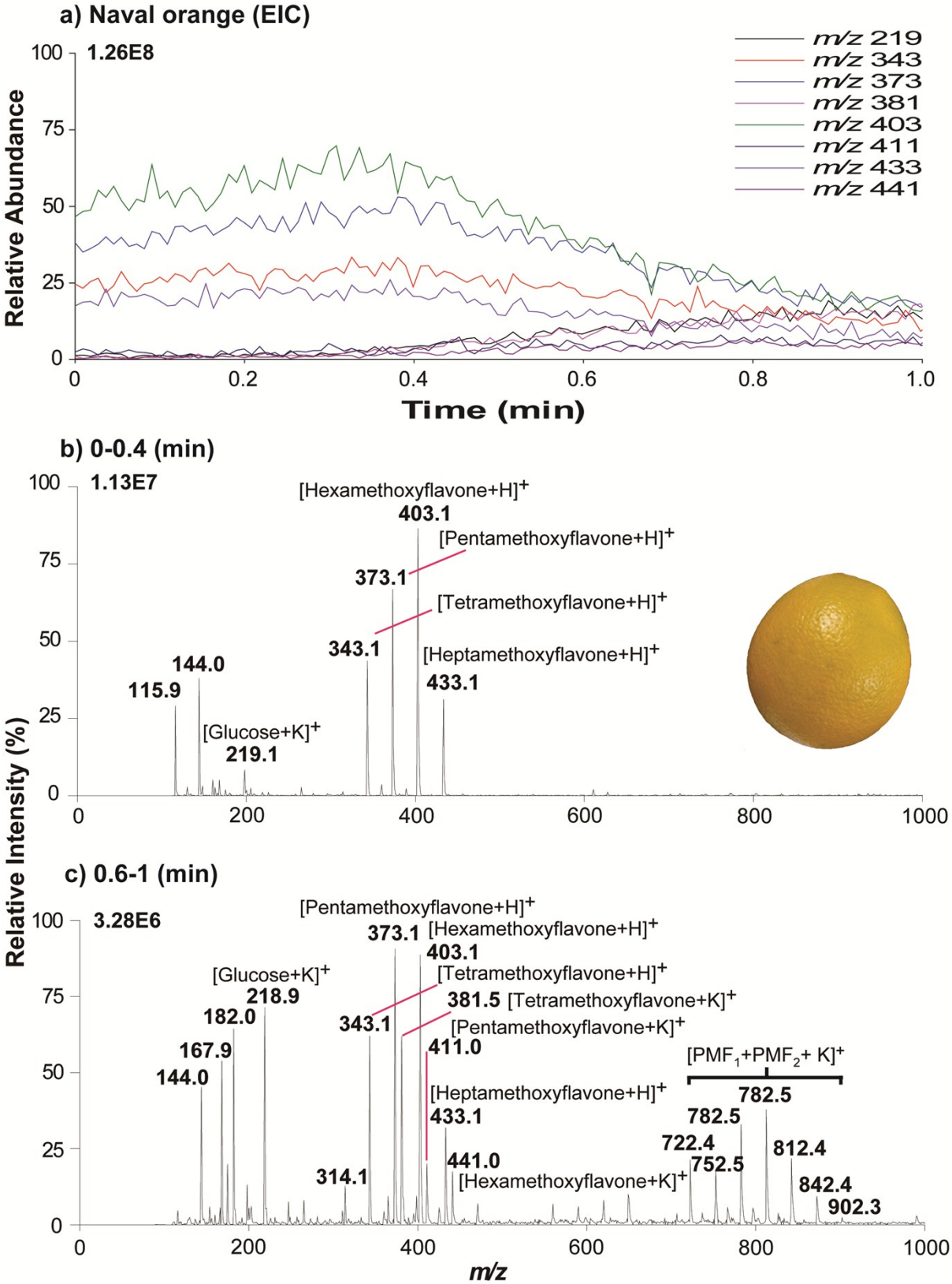

Fig. S9 Direct PR-R-nESI-MS analysis of untreated naval orange: a) EIC b) 0-0.4 min c) 0.6-1 min. $[PMF_1 + PMF_2 + K]^+$ indicate potassium-bound adduct of polymethoxyflavone 1 (PMF_1) and polymethoxyflavone 2 (PMF_2).

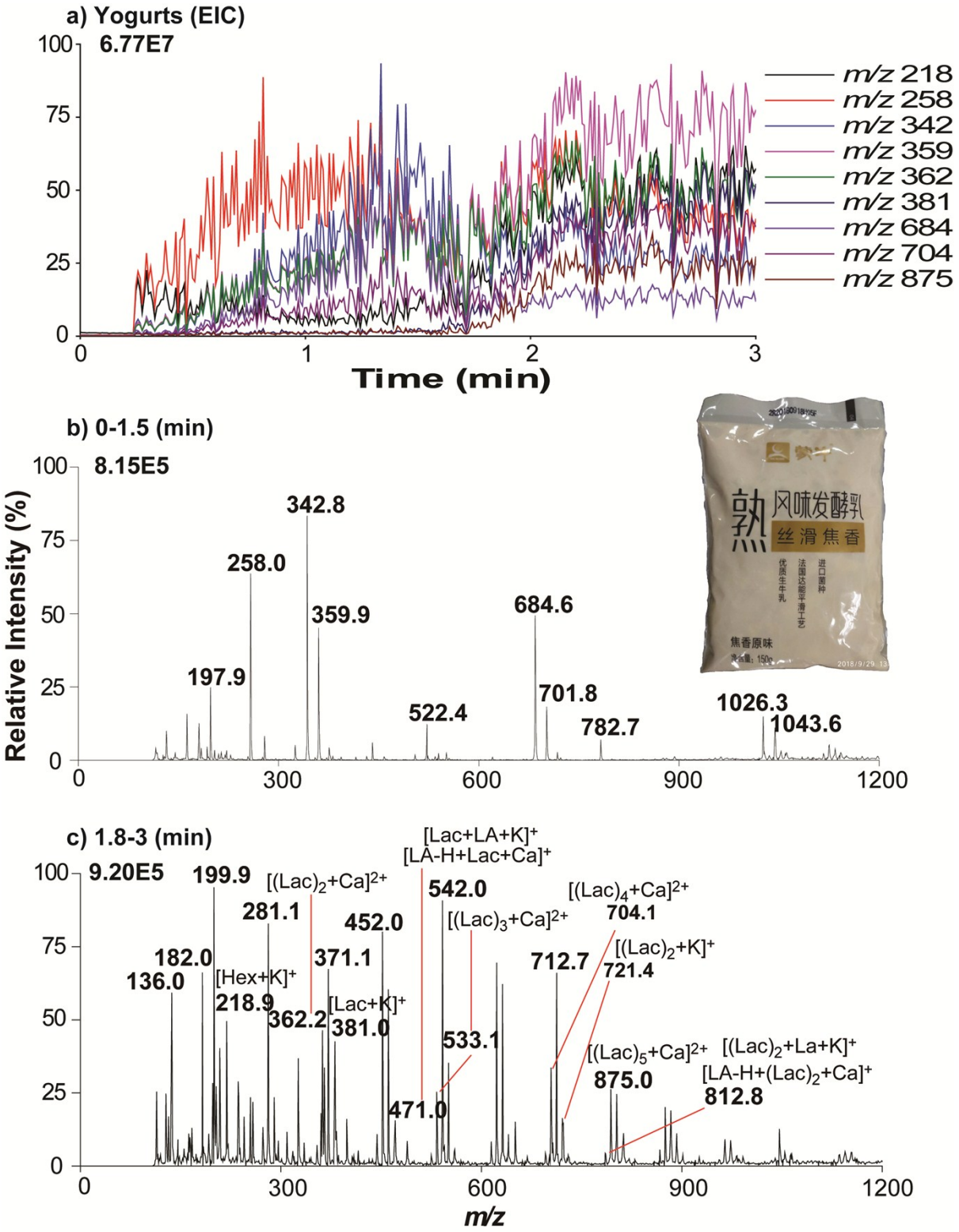

Fig. S10 Direct PR-R-nESI-MS analysis of untreated yogurts sample: a) EIC of yogurts b) 0-1.5 minute c) 1.8-3 minute. Lac and LA indicate lactose and lactic acid, correspondingly.

References

1 Md. M. Rahman, T. Jiang, Y. Tang and W. Xu, *Analytica Chimica Acta*, 2018, **1002**, 62–69.

2 Md. M. Rahman, D. Wu and K. Chingin, *J. Am. Soc. Mass Spectrom.*, , DOI:10.1007/s13361-019-02142-5.

3 M. M. Rahman and K. Chingin, *Anal. Methods*, 2019, **11**, 205–212.