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Technical Report

Data Quality Enhancement using Software-controlled Acquisition Strategies in MALDI-TOF MS

Simona Salivo[1]

Abstract:

In the typical MALDI process, the sample is mixed with a UV-energy absorbent MALDI matrix to aid ionisation. Upon solvent evaporation, the analyte molecules are embedded into the matrix forming a so-called co-crystallized structure. Even with an optimal matrix-to-analyte ratio, some crystals may contain optimal/non-optimal matrix-to-analyte ratios or no sample at all. This will lead to inconsistent ionisation efficiency ultimately affecting the spectral quality. Software-controlled strategies such as the 'data quality' feature in the Shimadzu MALDI software can help maximise the quality of the spectral data by only accumulating profile spectra which meet certain user-defined criteria. Here, we show examples of the use and benefits of the data quality feature e.g., for offset/non-centred sample spots, resolution and sensitivity enhancement and microbial applications.

Keywords: MALDI-TOF MS, data quality, MALDI Solutions

Introduction

Matrix assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry has emerged as a fundamental tool in many fields of application thanks to its robustness, speed of analysis, simple operation and high sensitivity. In a typical MALDI process, the sample containing the molecule(s) of interest is mixed with a UV energy-absorbent organic compound (the matrix), the latter aiding ionisation upon irradiation by a UV laser. During sample preparation, upon solvent evaporation the analyte molecules are embedded into the matrix forming a so-called co-crystallized structure. The matrix-to-analyte (M:A) ratio is a crucial factor as this largely influences the ionisation efficiency. Even with an optimal matrix-to-analyte ratio, crystallisation heterogeneity may be present so that crystals may contain optimal, non-optimal M:A ratios or no sample at all (Figure 1). This leads to inconsistencies in the ionisation efficiency and spot-to-spot variations, ultimately affecting the spectral quality in terms of mass resolution, mass accuracy and signal-to-noise (S/N). For example, if the M:A ratio is too low, poorer S/N or no signal at all may be observed. On the other hand, if M:A is too high, suppression of the analyte signal may occur, especially of low mass species. One way to overcome sample heterogeneity difficulties is to use software-controlled strategies that consist of only accumulating profile spectra that meet certain user-defined criteria during acquisition. The Shimadzu MALDI software provide the 'data quality' functionality to maximise the quality of the spectral data, which allows the user to choose among two strategies i.e., 'find sweet spots' and 'exhaust raster points', as well as defining pass-criteria e.g., minimum intensity, minimum resolution and minimum S/N. Here, we show examples of the use and benefits of the two data quality strategies. The data quality functionality is available for the *MALDI Solutions™ Data Acquisition*, *AuraSolution™* and *QC Reporter™* software used with the MALDI-8000 benchtop series. It is also in the *MALDI-MS* software used with Axima instruments.

Offset/non-centred sample spots – 'find sweet spots' and 'exhaust raster points' strategies

In this example, peptide samples were used to demonstrate the benefits of data quality in scenarios where samples are spotted off-centre due to inconsistencies with e.g., manual or robotic handling techniques (Figure 2A). Glu1-Fibrinopeptide B (GluFib) peptide prepared in CHCA matrix was used to show the advantage of the 'find sweet spots' strategy over the 'exhaust raster point' one in terms of speed of analysis and signal yield. Table I summarises the data quality criteria used, acquisition times, signal yields and resolution values.

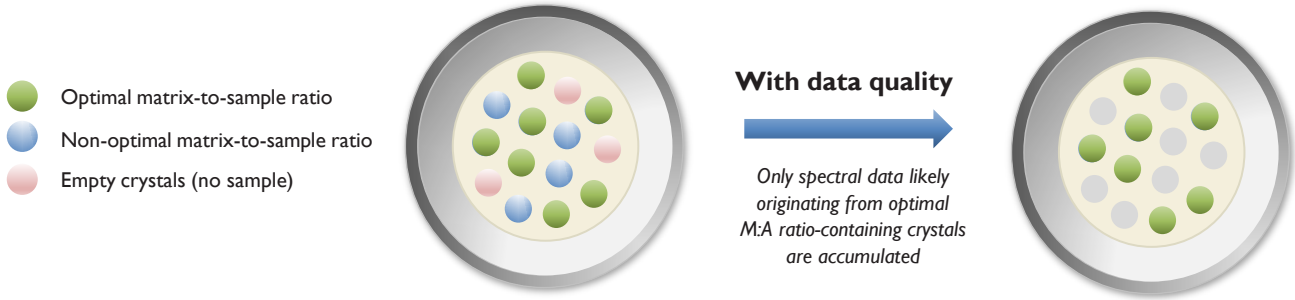


Figure 1. Left: during MALDI sample preparation, crystallisation heterogeneity may occur causing some crystals to contain optimal, non-optimal matrix-to-analyte ratios or no sample at all. The result of non-optimal matrix-to-analyte ratios is often a poor ionisation efficiency leading to poor spectral quality. Right: software-controlled strategies such as the 'data quality' in the Shimadzu MALDI software can improve the quality of the mass spectral data by rejecting profile spectra which do not meet certain user-defined criteria.

[1] Shimadzu Manchester, UK

Table 1. Data quality settings, acquisition times and spectral quality performance of the GluFib peptide sample from off-centred spots.

Data quality settings	Acquisition time	No. acquired profiles	SUM mV (*m/z* 1570)	Resolution (*m/z* 1570)
No data quality	~15 sec	109/109	703	4095
Strategy: **find sweet spots** Monitor mass range: 1560-1590 Da Minimum resolution: 2000 Minimum signal (mV)[a]: 10 Maximum consecutive failed profiles[b]: 109 Minimum accepted profiles per point (%)[c]: 30	~30 sec	54/109	1731	3913
Strategy: **exhaust raster points** Monitor mass range: 1560-1590 Da Minimum resolution: 2000 Minimum signal (mV): 10 Maximum consecutive failed profiles[d]: 4	~1.5 min	47/109	1542	3979

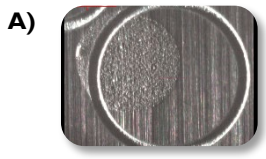
a: of the processed profile spectra.

b: the number of profile failures that are tolerated before the current sample spot is abandoned and moving on to the next one.

c: minimum percentage of good profiles accepted per raster point. Only applicable with 'find sweet spot' strategy.

d: the number of profile failures that are tolerated before the current raster point is abandoned and moving on to the next one.

As it can be seen, the acquisition time was longer with the 'exhaust raster points' strategy as, by design, the software will aim to acquire as many consecutive good profiles on a given raster point before moving onto the next one (~1.5 min with 'exhaust raster points' *vs.* ~30 sec with 'find sweet spots'). In contrast to the 'exhaust raster points' strategy, the 'find sweet spots' method first acquires a single profile from each available raster point. If the requested number of profiles cannot be achieved in the first pass, the software attempts to acquire the remaining profiles but only from raster positions which met the criteria in the previous pass. In the case of an offset sample spot, the software would identify the areas containing the sample spot in the first pass using the 'find sweet spots' method and focus subsequent passes on these areas whereas with the 'exhaust raster points' strategy, the software would have to sequentially fire on positions not containing sample until the sample is detected. Hence, in this scenario the acquisition time with 'exhaust raster points' would be longer. The signal was also higher with the 'find sweet spots' strategy (1731 (find sweet spots) *vs.* 1542 SUM mV (exhaust raster points)). To support the choice of the data quality settings (minimum resolution and signal (mV)), data were previously acquired with no data quality and all individual profiles stored to assess spectral quality in each profile spectra. Figure 2B shows three example MALDI-MS individual profiles of the GluFib (no data quality) where: *i)* profile No. 2 is from an area of the spot with no sample showing no signal from the GluFib; *ii)* profile No. 69 is an example of good quality spectrum from an area of the GluFib dried spot where the signal amount (14.3 mV) was good enough to produce good isotopic resolution; *iii)* profile No. 72 is an example of exceptionally good profile spectrum of GluFib with very good S/N, signal amount (44.7 mV) and isotopic resolution. Figure 2C shows three example individual MALDI-MS profiles of the GluFib acquired with the 'find sweet spots' strategy. As it can be seen, all three profile spectra meet the data quality criteria of minimum resolution (2000) and signal (10 mV).



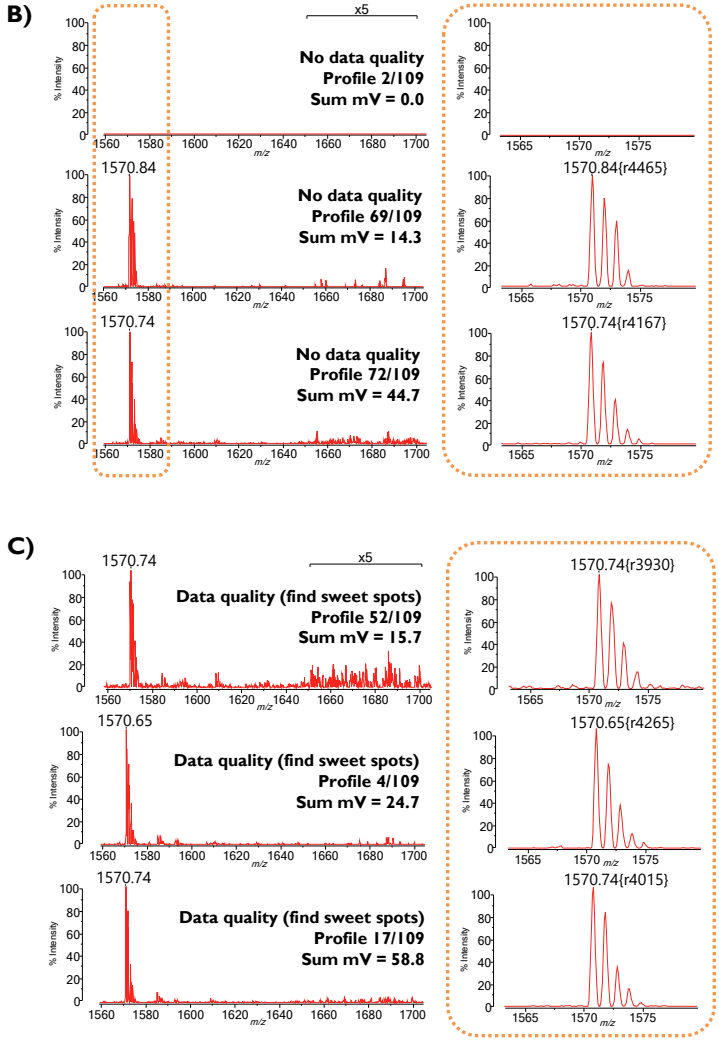


Figure 2. A) Off-centred spot of GluFib peptide. B) Example MALDI-MS individual profiles of GluFib acquired with no data quality to support the choice of the data quality settings. Profile No. 69 (middle) with 14.3 sum mV of the GluFib showed good isotopic resolution and quality. C) Example MALDI-MS individual profiles of GluFib acquired with data quality and 'find sweet spots', all showing excellent quality and isotopic resolution.

Resolution enhancement with 'find sweet spots' strategy

The possibility to improve the resolution of spectral data affected by poor topography using the data quality feature was explored. A 12-mer oligonucleotide example is provided in Figure 3. Oligos prepared with 3-Hydroxypicolinic acid (3-HPA) matrix produce a unique topography upon drying, with a raised edge and thin inner layer (Figure 3A). Due to the height difference between the raised edge and centre, the mass resolution may be degraded if the spectrum is acquired from all areas of the dried spot. Preliminary data acquired without data quality selectively from the edge and centre of the dried spot were used to determined the data quality settings (data not shown). The data quality settings are summarised in Table 2. Figure 3B shows the MALDI-MS spectra of the 12-mer oligo with no data quality (bottom red trace) and with data quality using 'find sweet spots' strategy (top blue trace). As it can be seen, the peak of the 12-mer oligo is notably narrower in the data acquired with data quality, exhibiting a resolution value which is nearly double that with no data quality (943 (data quality) *vs.* 492 (no data quality)). The minimum resolution value set to 850 allowed the software to exclude the areas of the raised edge of the sample spot which would affect the resolution and acquire mainly from the flatter/thinner central area.

Table 2. Data quality settings for the 12-mer oligo.

Data quality settings
Strategy: find sweet spots
Monitor mass range: 3600-3670 Da
Minimum resolution: 850
Maximum consecutive failed profiles: 109
Minimum accepted profiles per point (%): 30

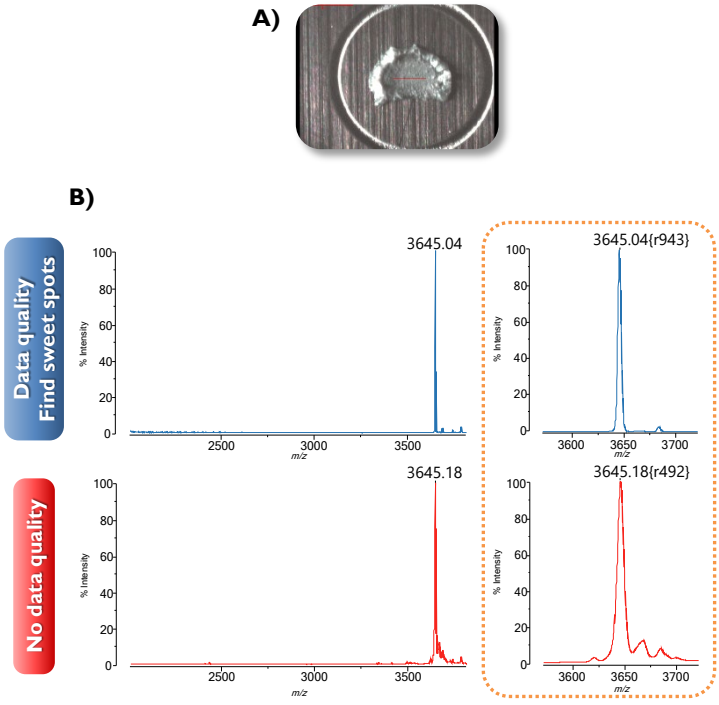


Figure 3. A) Spot of 12-mer oligo prepared with 3-HPA matrix. B) MALDI-MS spectra of 12-mer acquired without data quality (red trace) and with data quality/find sweet spots (blue trace). The peak of the 12-mer is visibly narrower when data quality was used, with the resolution value nearly double that with no data quality.

Another example of resolution enhancement using a peptide sample is provided in Figure 4. ACTH 7-38 fragment peptide was prepared at 1 pmol/µL and in CHCA matrix. Achieving isotopic resolution of this peptide can be challenging for a compact linear MALDI-TOF instrument and requires optimised sample preparation and analysis conditions. If the matrix-to-analyte ratio is not consistent across the sample spot, a degradation in the mass resolution may occur. Figure 4 shows the MALDI-MS spectra of ACTH 7-38 without and with data quality and 'find sweet spots' strategy (red and blue traces, respectively). The data quality settings are summarised in Table 3. As it can be seen, the peptide is not isotopically resolved without data quality. The data acquired with data quality shows how the peptide is fully isotopically resolved exhibiting resolution values exceeding the minimum value set (6000).

Table 3. Data quality settings for the ACTH 7-38 fragment peptide (1 pmol/µL).

Data quality settings
Strategy: find sweet spots
Monitor mass range: 3650-3675 Da
Minimum resolution: 6000
Minimum signal (mV): 10
Maximum consecutive failed profiles: 109
Minimum accepted profiles per point (%): 30

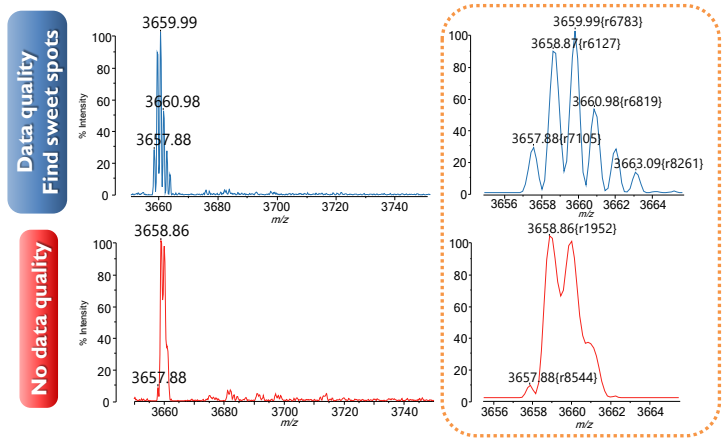


Figure 4. MALDI-MS spectra of ACTH 7-38 fragment peptide (1 pmol/µL). Bottom red trace: spectrum acquired without data quality. The peptide could not be isotopically resolved. Top blue trace: spectrum acquired with data quality and find sweet spots strategy. The peptide is fully isotopically resolved.

Sensitivity enhancement with 'find sweet spots' strategy

The use of data quality to improve the sensitivity on low concentrated samples is demonstrated. Bovine serum albumin (BSA) tryptic digest at 2 fmol/µL in CHCA matrix is used as an example (Figure 5). Table 4 summarises the data quality settings. The sample was acquired with no data quality (bottom red trace) and with data quality and 'find sweet spots' strategy (top blue trace), then submitted to a MASCOT PMF search. As it can be seen, the signal yield and S/N of the peptide digest peaks is improved when data quality was used. The spectrum acquired with no data quality could not be identified. On the contrary, the spectrum acquired with data quality led to a successful MASCOT PMF identification thanks to the improved signal yield.

Table 4. Data quality settings for the BSA digest (2 fmol/µL).

Data quality settings
Strategy: find sweet spots
Monitor mass range: 900-2600 Da
Minimum resolution: 1500
Minimum signal (mV): 5
Maximum consecutive failed profiles: 109
Minimum accepted profiles per point (%): 30

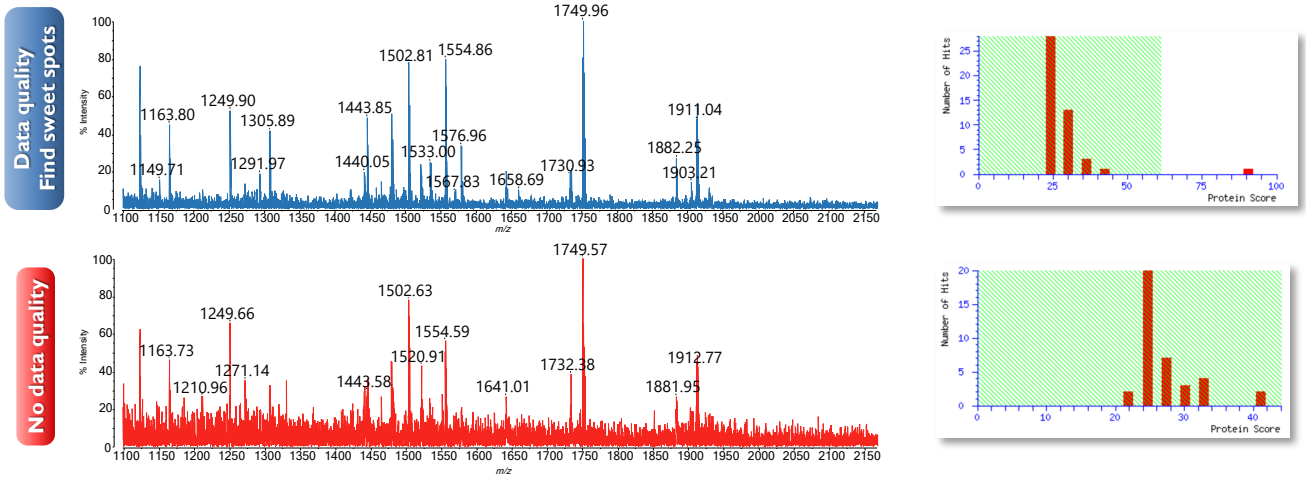


Figure 5. MALDI-MS spectra of a low concentrated BSA digest (2 fmol/µL). Bottom red trace: spectrum acquired without data quality. The digest could not be identified through a MASCOT PMF search. Top blue trace: spectrum acquired with data quality and find sweet spots strategy. The digest could be successfully identified through a MASCOT PMF search.

Use of data quality for microbial applications

MALDI-TOF MS is widely used for microbial analysis for its simplicity, minimal sample preparation and speed of analysis. However, depending on the matrix used and amount of bacterial material transferred onto the spot, the dried spots often present variations in topography and crystallisation. This often poses challenges in the obtainment of good signal yield during analysis. For this application, the use of data quality with the 'exhaust raster points' strategy can help maximising ion yield from each raster point by ensuring as many profile spectra that meet the criteria are accumulated. To understand why, a series of acquisitions were performed using *E.coli* smears prepared in CHCA matrix. Figure 6A shows the MALDI-MS spectra of *E.coli* acquired without data quality and from consecutive acquisitions performed by firing the laser on the same raster positions. With the consecutive acquisitions, the yield of high mass species is improved (*m/z* 10,000-13,000). This suggests that the consecutive runs performed on the same positions have ablated the surface of the spot supposedly from matrix and salt crystals, yielding cleaner profile spectra. This test simulates the behaviour of the 'exhaust raster points' strategy. To test this in an automated acquisition, data quality was used.

The data quality settings are summarised in Table 5. Figure 6B shows the MALDI-MS spectrum of *E.coli* acquired with data quality and the 'exhaust raster points' strategy. As it can be seen, the data quality feature was able to improve the detectability of the *E.coli* proteins, both at the low- and high-end of the mass spectrum, with good signal yields and S/N.

Table 5. Data quality settings for the *E.coli* smears in CHCA.

Data quality settings
Strategy: exhaust raster points
Monitor mass range: 3000-13000 Da
Minimum resolution: 300
Minimum signal (mV): 10
Maximum consecutive failed profiles: 100

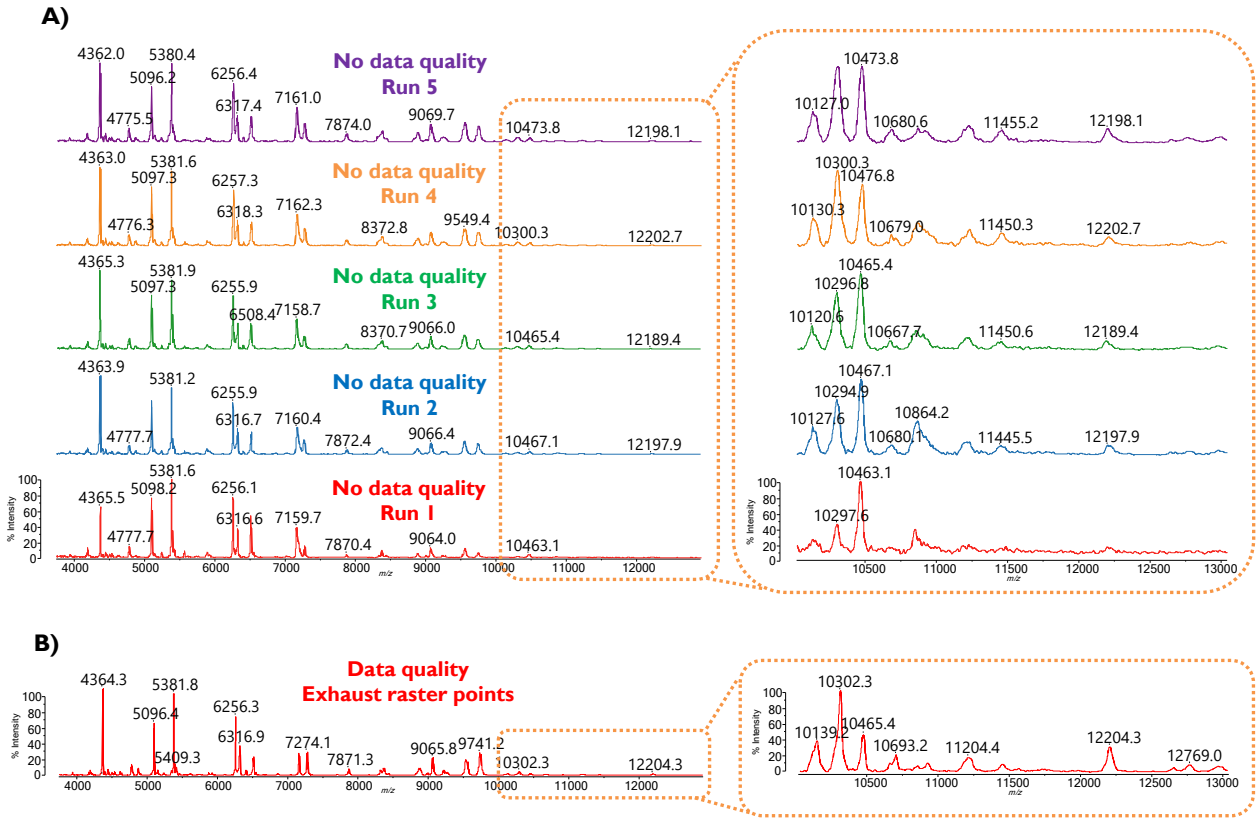


Figure 6. A) MALDI-MS spectra of *E. coli* smears in CHCA run without data quality, from consecutive analyses performed on the same raster positions. The yield of high mass proteins (*m/z* 10,000-13,000) is improved with the consecutive acquisitions. B) MALDI-MS spectrum of *E. coli* smears in CHCA run with data quality and 'exhaust raster points' strategy. The detectability of the *E.coli* proteins is improved, both at the low- and high-end of the mass spectrum.

Conclusion

In MALDI TOF MS, identifying optimal matrix-to-analyte ratios is critical to ensure best ionisation efficiency and mass spectral quality. However, some matrix/sample co-crystal heterogeneity may still be present leading to inconsistencies in the signal yield of the sample analytes. The 'data quality' feature in the Shimadzu MALDI acquisition software can help maximise the spectral quality by accumulating only profile spectra which meet certain user-defined criteria. Here, we showed a variety of examples demonstrating the usefulness of the data quality feature where improvements of the overall quality of the spectral data, signal yield, resolution and sensitivity were obtained.

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