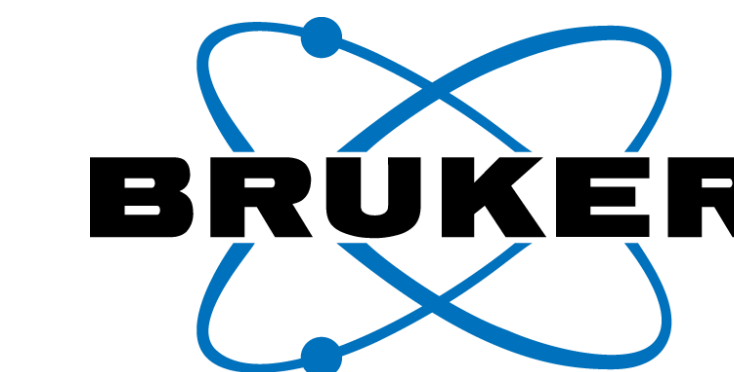


High sensitivity analysis of low molecular weight synthetic polymers using MALDI-Ion Mobility-TOF MS



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MALDI has large advantages for the analysis of synthetic polymers,

generating almost only singly charged ions and ionizing even insoluble samples. However, it is known that MALDI matrices cause strong interference signals in the low molecular weight region of acquired mass spectra. On the other hand, ion mobility is spreading its application, especially in bio science fields like proteomics or metabolomics, where its ion separation ability based on collisional cross section that can distinguish even isomers and provides increased selectivity of analysis and 'cleaner' spectra results. In this study, trapped ion mobility spectrometry was used for MALDI-MS analysis of low molecular weight synthetic polymers to remove background signals caused, mainly by matrix ions, thus resulting in spectra of increased selectivity and sensitivity.

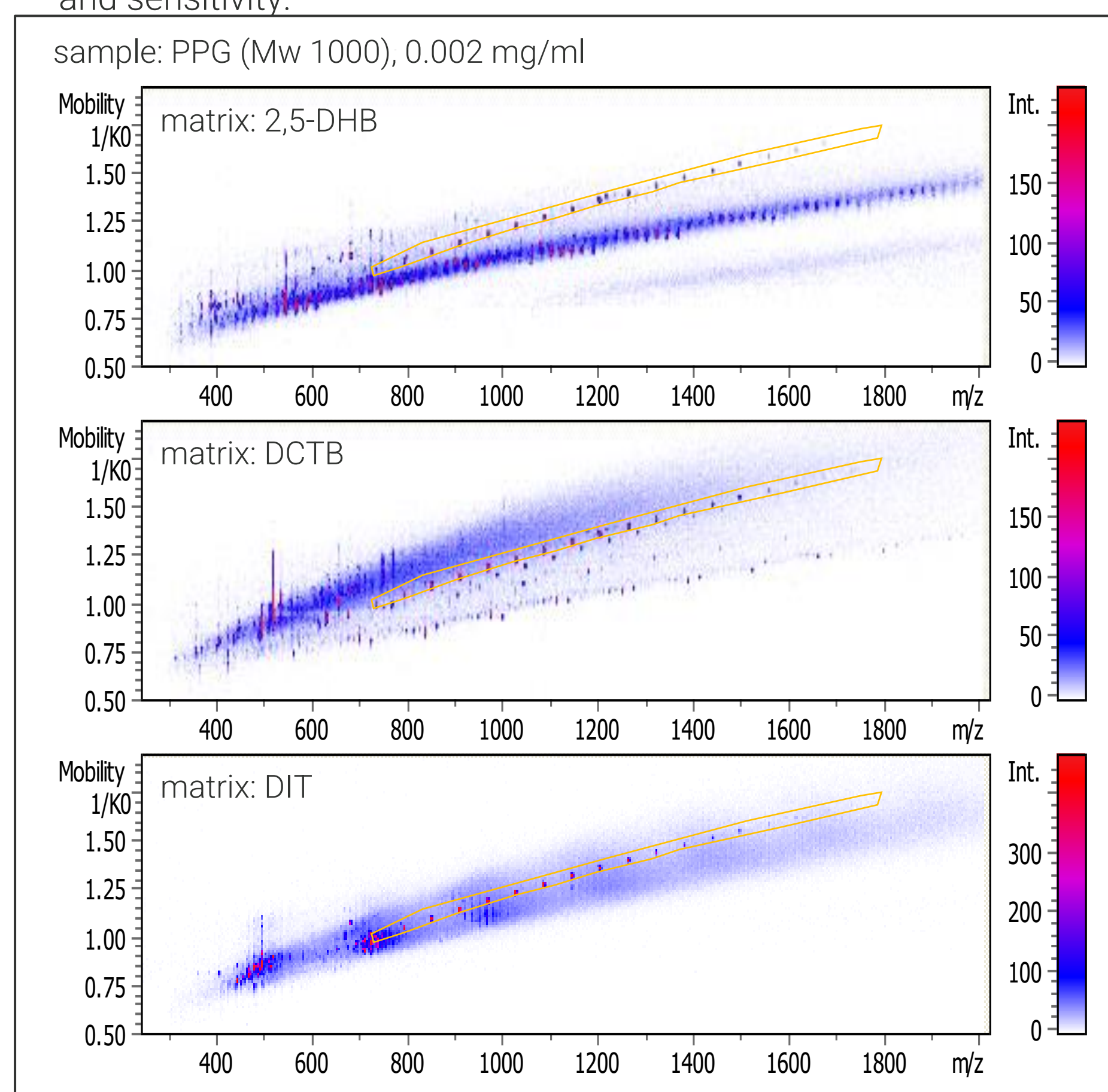


Fig. 1 The heatmap of tims-MS data taken from sample with 0.002 mg/ml concentration using 2,5-DHB, DIT and DCTB matrices. The yellow line shows the region of PPG signals to be extracted for background removal. Intensity is saturated against the color code for visibility of PPG signals.

Methods

A polypropyleneglycol (PPG, Mw: 1000 Da) standard was used as the sample and was dissolved in THF at different concentrations (20, 2, 0.2, 0.02 and 0.002 mg/ml). 2,5-dihydroxybenzoic acid (2,5-DHB), dithranol (DIT) and trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB) in THF at 20 mg/ml were used as matrix and sodium trifluoroacetate in THF at 2 mg/ml was used as salt. They were mixed at the ratio of 1:10:1, applied on a target plate and analyzed with MALDI-trapped ion mobility spectrometry (TIMS)-QTOF mass spectrometer (Bruker timsTOF flex). The data acquired in positive mode was processed with DataAnalysis (Bruker) and Polymerix (Sierra Analytics) software to calculate the ratio of PPG signal intensity against the total ion current (TIC). Then the efficiency of ion mobility separation to remove background signals mainly caused by matrices was calculated and evaluated from the ratio values.

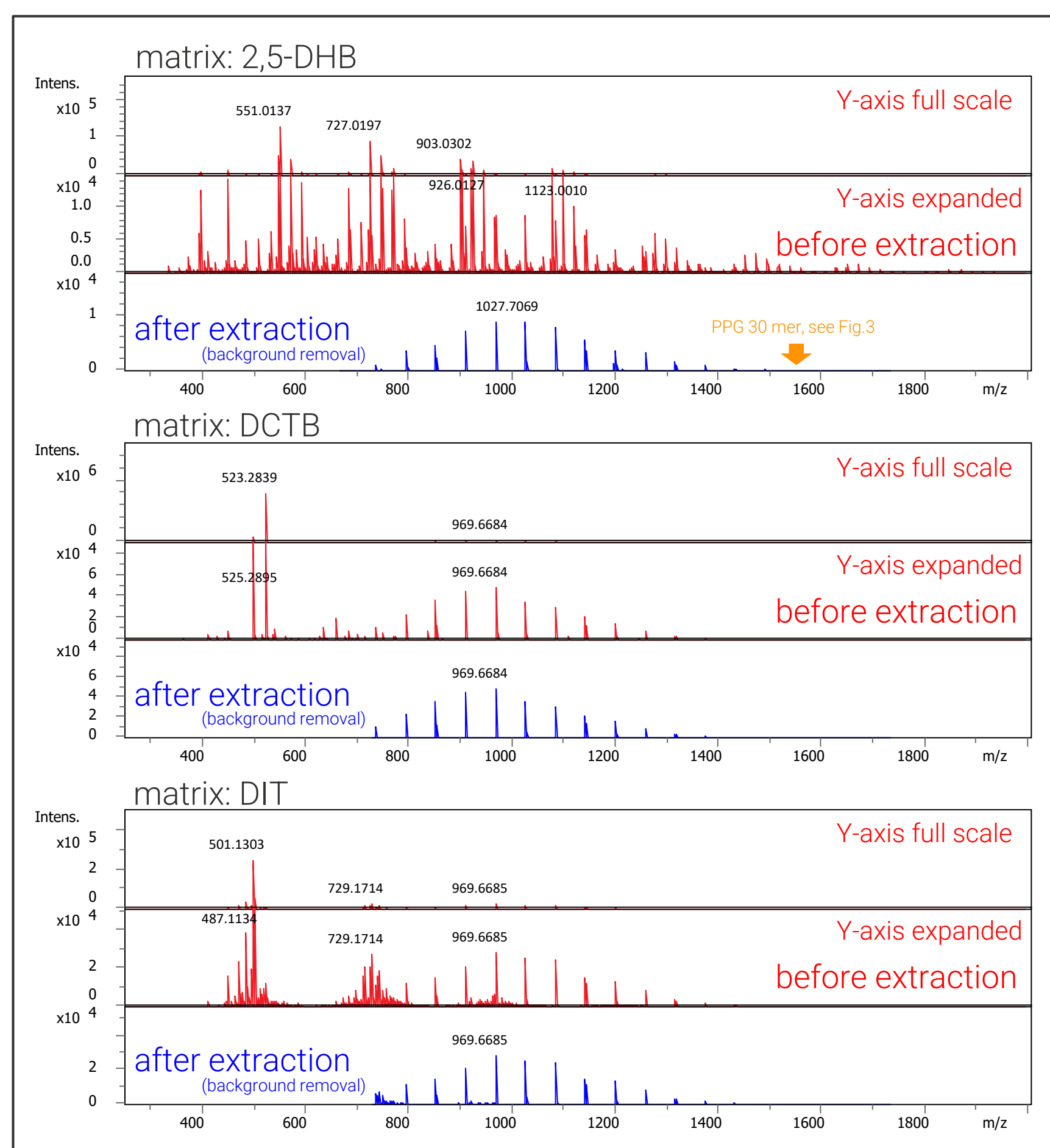


Fig. 2 Mass spectra of sample with 0.002 mg/ml concentration using 2,5-DHB, DCTB and DIT matrices, before and after extraction (background removal).

Results

The tims-MS data can be displayed in the heatmap format where X-axis shows m/z, Y-axis shows reciprocal number of ion mobility ($1/k_0$) and color shows signal intensity. Since higher molecular weight species generally have lower mobility, a heatmap typically shows distribution from left bottom to right top. However, different molecular classes show different tendency in the heatmap and this allows separation based on the molecular classes. On the other hand, typically, the low mass region of MALDI spectra is crowded by mixed signals from matrix and target analyte. Therefore, in this study, it was tried to remove background signals mainly caused by matrix using extracted heatmap based on m/z and $1/k_0$.

Figure 1 shows the heatmap of tims-MS data taken from 0.002 mg/ml sample with three matrices. PPG signals in the heatmap are easily recognized by finding a line in which signals are observed in a regular interval of 58 Da corresponding to the mass of monomer unit (C_3H_6O). And signals from matrices are detected like clouds rather than lines. In the case of data taken with 2,5-DHB matrix, the cloud caused by matrix is detected with $1/k_0$ value lower than PPG. DCTB shows cloud with higher $1/k_0$ value, and DIT shows the cloud overlapping the PPG line. Therefore, extraction of PPG signals from the heatmaps was easy for 2,5-DHB and DCTB data via simple manual operation as shown by the region defined by yellow line in the figure 1.

Figure 2 shows spectra of 0.002 mg/ml sample before and after the extraction of PPG signals, i.e. background removal.

matrix	concentration of sample (mg/ml)	Intensity % of PPG against TIC		BG removal efficiency	average
		before BG removal	after BG removal		
DHB	20	86.92	93.60	54.6%	88.9%
	2	49.96	94.90	94.6%	
	0.2	26.22	93.13	97.4%	
	0.02	4.22	87.14	99.3%	
DCTB	0.002	5.03	80.34	98.7%	83.3%
	20	90.01	95.08	53.4%	
	2	86.04	95.48	70.8%	
	0.2	68.73	97.56	94.5%	
DIT	0.02	21.49	95.54	98.7%	80.1%
	0.002	22.98	97.06	99.1%	
	20	93.98	96.36	41.0%	
	2	67.40	94.72	88.5%	
	0.2	26.82	85.83	93.9%	
	0.02	6.84	38.20	88.1%	
	0.002	7.14	40.55	88.7%	

Table 1 Intensity % values of PPG signals against TIC before and after background (BG) removal and its efficiency calculated from them.

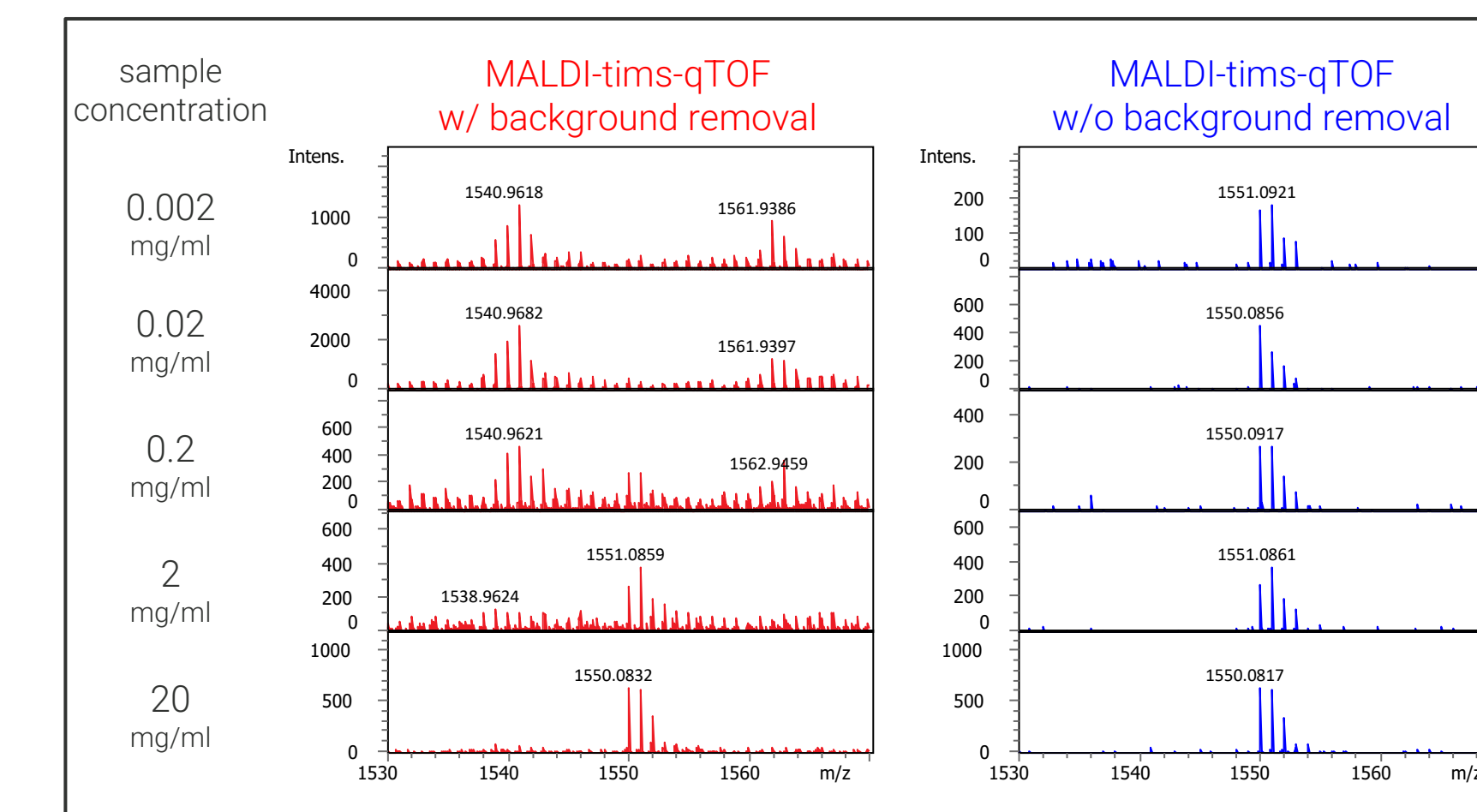


Fig. 3 Comparison of PPG 30 mer signals taken using 2,5-DHB matrix with and without background removal. The lower detection limit improves more than two orders of magnitude with background removal.

Table 1 shows intensity % values of PPG signals against TIC before and after background removal and its efficiency calculated from the values. The highest efficiency of matrix signal removal was observed in the case of 2,5-DHB matrix (88.9 % in average). Generally, higher efficiency was achieved at lower sample concentration.

Figure 3 shows the lower detection limit (LDL) of PPG 30 mer signal (m/z 1550) at the edge of the polymer distribution. The spectra without background removal shows the LDL is around 0.2 mg/ml. However, the spectra with background removal shows clear signal even at 0.002 mg/ml achieving high sensitivity analysis more than two orders of magnitude better.

Conclusion

- Trapped ion mobility was successfully used to remove background signals mainly caused by matrix from MALDI spectra of low molecular weight synthetic polymers.
- In the case of 2,5-DHB matrix, matrix removal efficiency was higher at lower samples concentration showing 99 % at maximum and 89 % in average.
- High sensitivity analysis more than two orders of magnitude was achieved after background removal.

MALDI-tims-qTOF MS