

Performance of a New Sensitive LC-IMS-QTOF Platform for Proteomics Measurements

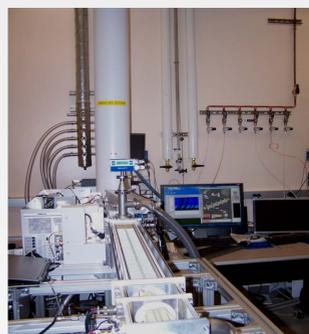
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Overview

- LC-IMS-QTOF platform was evaluated for proteomics measurements
- The new platform is seamlessly integrated with LC and QTOF.
- The platform was evaluated for sensitivity and robustness with tryptically digested depleted human plasma



LC-IMS-QTOF Platform for proteomics measurements

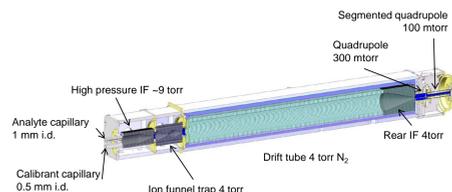
LC-IMS-QTOF MS Platform

LC system: Waters NanoAcquity, 60 min gradient, 100 μ m \times 10 cm, 1.7 μ m C18

IMS-QTOF platform: IMS coupled to Agilent 6538 UHD Accurate Mass QTOF

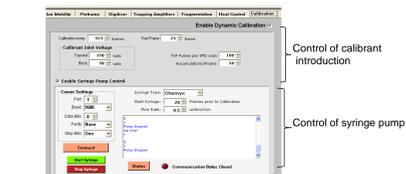
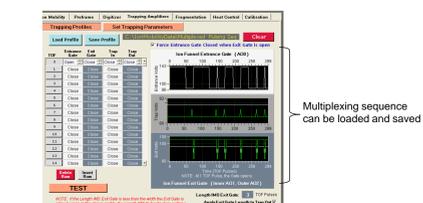
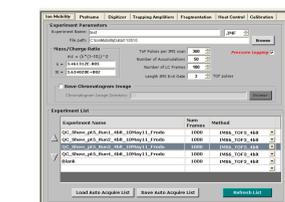
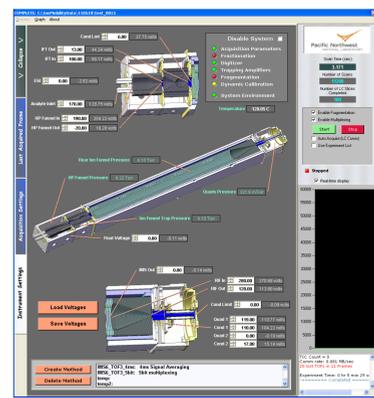
Source

- Dual-Funnel design featuring 1mm i.d. inlet capillary for maximum ESI sampling
- Separate calibrant inlet for tight mass measurement accuracy
- Calibrant introduction does not interrupt the actual data collection (only 200 ms)
- Double offset of the capillary and the two funnels for reduced contamination
- Funnel lenses are made of printed circuit boards (PCB)
- Efficient trapping at 4 torr on funnel trap
- Multiplexed injection of ion packets into drift tube (50 % duty cycle)



Control and visualization software

- Written in C#
- User friendly
- Logs all experimental parameters
- Communicates with LC and QTOF

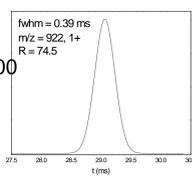


Drift tube

- 94 cm long drift tube
- 50 mm i.d. drift rings made of PCB lenses
- 4 torr pure nitrogen gas
- IMS resolution (for 1+): 75
- Optional high efficiency fragmentation (up to 70 % CID efficiency) in the segmented quadrupole

Acquisition system

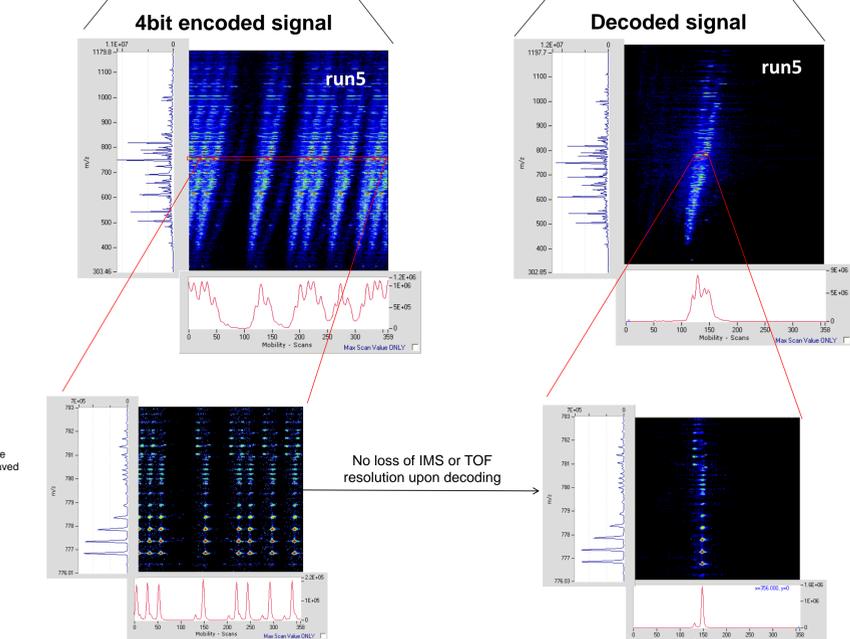
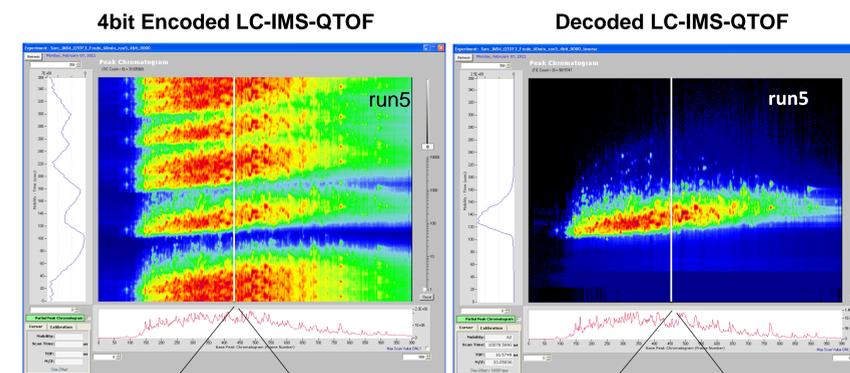
- QTOF resolution ~25,000 at 1500 m/z
- Acquiris 8 bit ADC
- 3 s IMS-QTOF frame



Samples: Human plasma, depleted of the 14 most abundant proteins and tryptically-digested

Results

Multiplexed IMS

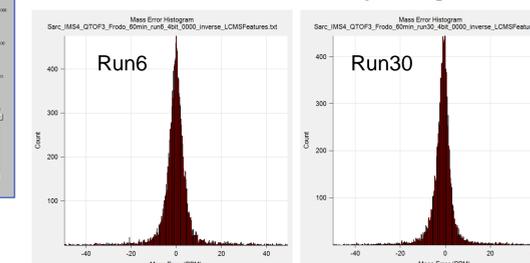


The 4bit multiplexing approach provides an extra 7-8 fold improvement in signal

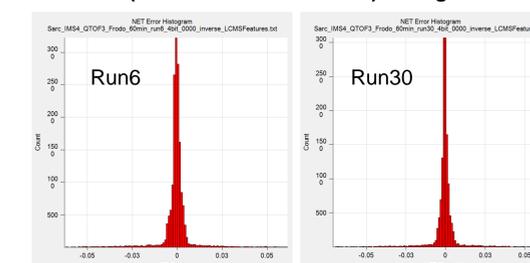
Clustering analysis

- Data from 30 LC-IMS runs were de-isotoped using Decon2LS
- Features found were clustered using the m/z , elution time and drift time information
- Run5 was used as reference dataset while the other 30 runs were aligned against it

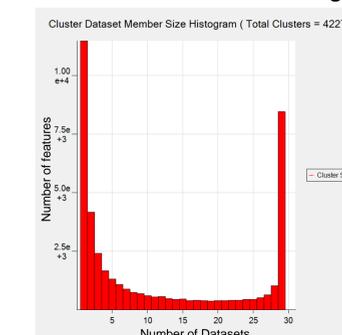
Mass measurement accuracy histograms



NET (normalized elution time) histograms



Cluster dataset member size histogram



This figure shows the number of features observed consistently across datasets. The high number of features in 30 datasets indicates excellent reproducibility.

Conclusions

- An LC-IMS-QTOF platform was evaluated for proteomics measurements.
- Sensitivity was increased by utilizing a dual funnel design and 1 mm i.d. inlet.
- 50 mm acceptance rear funnel to eliminate ions losses due to diffusion.
- Segmented quadrupole for efficient MS/MS.
- PCB lenses of the ion funnels and drift rings for ease of assembly and reduced cost of manufacturing.
- IMS resolution of 75 for singly charged ions.
- The new platform seamlessly integrated with LC and QTOF.
- The evaluation involved 30 LC-IMS-QTOF runs of a tryptically digested proteins of depleted human plasma.
- Excellent reproducibility across 40 runs.

Acknowledgements

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References

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