

Direct coupling of non-reverse-phase liquid chromatography (LC) with mass spectrometry (MS) utilizing BioTrove's RapidFire technology

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Why RapidFire™ multidimensional chromatography?

We introduce the RapidFire™ multidimensional chromatography system (**2D RF-MS**), a novel solution to perform near-real-time qualitative mass spectrometry (**MS**) analysis directly on liquid chromatography (**LC**) eluates.

Many applications in arenas ranging from

- molecular biology (proteomics) to
- biopharma quality control (QC) to
- plasma fractionation

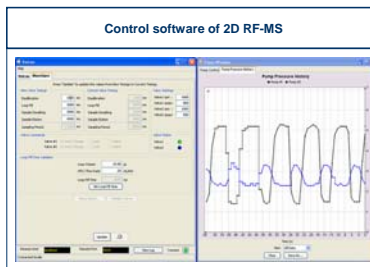
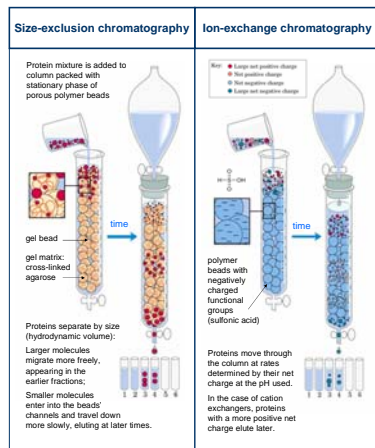
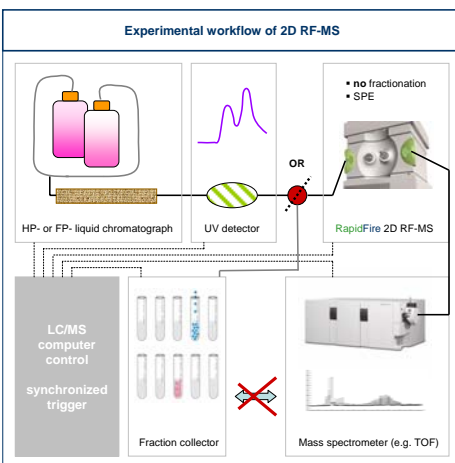
entail post-separation analysis of LC-isolated fractions.

MS has emerged as an excellent method to unambiguously identify compounds present in these samples.

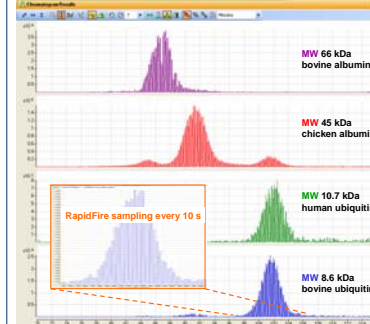
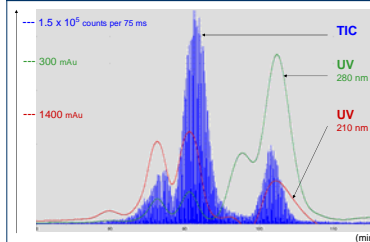
However, because MS is generally incompatible with solutions of high ionic strength, fractions collected from LC runs that involve the addition of salts or buffers to the mobile phase require extensive sample preparation prior to MS. These include:

- size-exclusion chromatography,
- cation exchange chromatography,
- anion exchange chromatography,
- reverse-phase chromatography where non-volatile salts and ion-pairing agents must be used.

2D RF-MS circumvents the rate-limiting step of offline fraction collection, purification and subsequent examination by providing a rapid in-line desalting technique that facilitates the direct interfacing of LC with MS.



Size-exclusion chromatography (SEC) directly coupled to MS

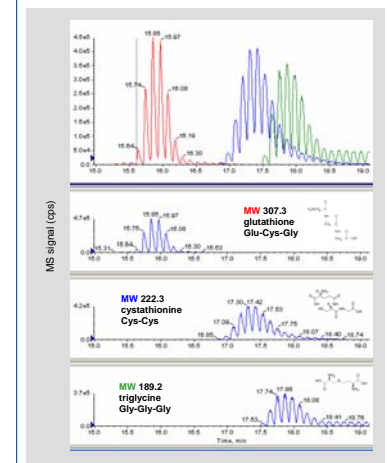


(top) Real-time MS (total ion chromatogram, or TIC) and UV data from a sample of proteins separated by sequential SEC and 2D RF-MS.
 (bottom) Individual real-time MS/MS traces for the four proteins in order of elution. An aliquot of the eluate of the chromatography system is analyzed by the 2D RF-MS instrument every 10 seconds (inset).

Assay conditions

- **Sample** SEC 50 µL of albumins (bovine 22 µM, chicken egg 43 µM) ubiquitins (bovine 10 µM, human 43 µM)
- **Pump** SEC 0.2 mL/min of PBS + 1 mM EDTA
IEC 0.2 mL/min of 100 mM NaH₂PO₄, pH 6.5
- **LC column** SEC Superose 6 10/300 GL (GE Healthcare)
IEC Zorbax 300-SCX (Agilent)
- **UV** SEC wavelengths of 210 nm and 280 nm
- **2D – RF-MS**
SPE SEC column "A" (C4 chemistry)
IEC column "D" (Hypercarb)
- **Buffer A** SEC A = water + acids **; B = 50% MeCN + acids ***
IEC A = water + acid ****; B = 0.01% MeCN + acid ****
acids ** = 0.09% FA + 0.01% TFA, acid *** = 0.1% TFA
flow rates of A = 1.5 mL/min, B = 1.0 mL/min
- **Period** SEC 10.0 s sampling rate
IEC 6.5 s sampling rate (longer wash)
- **Mass spectrometer, Agilent 6410 triple quadrupole**
Q1/Q3 (amu) SEC bovine alb. 1303.6 – 135.9 chicken alb. 952.9 – 109.9 human ub. 1071.7 – 260.0 bovine ub. 758.9 – 129.0
IEC Gly-Gly-Gly 190.1 – 132.6 Cys-Cys 223.1 – 134.1
Glu-Cys-Gly 308.3 – 178.6

Ion-exchange chromatography (IEC) directly coupled to MS



Real-time TIC and individual MS/MS data from a sample of peptides separated by IEC followed by 2D RF-MS. An aliquot of the eluate of the chromatography system is analyzed by the 2D RF-MS instrument every 6.5 seconds.



A fast solid-phase extraction (SPE) technology that enables direct interfacing of MS with ion-exchange and size-exclusion chromatography in real time

Summary of the features of 2D RapidFire™ chromatography

- **Near real-time MS detection:** highly selective mass spectrometry analysis avoids the confounding signal often encountered with chromatophore-based optical detection approaches;
 - Q/Q MRM's differentiate otherwise overlapping (co-eluting) LC effluents, e.g. human & bovine ubiquitins in SEC, Cys-Cys & Gly-Gly-Gly in IEC.
- **Integrated sample purification:** online desalting and concentration of direct liquid chromatography effluents by 2D RF-MS bypasses otherwise complex and inefficient sample collection and purification processes;
 - Fast SPE allows for LC mobile phases such as PBS in SEC or high-salt sodium phosphate in IEC.
- **Compatibility:** 2D RF-MS can be used upstream of all atmospheric pressure ionization (API) mass spectrometers including electrospray (ESI) and atmospheric pressure chemical ionizations (APCI);
- **Easy to use!** The stand-alone 2D RF-MS module is simple to insert into existing HPLC or FPLC systems where ensuring MS analysis is sought.