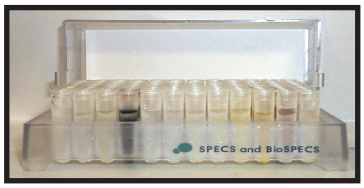
A Combinatorial Chemistry Library Analysis by LC/MS



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W. Van Scheppingen, Specs and BioSpecs, The Netherlands
B. Kenney, Waters Corporation, USA

Goals

- Fast check of compound confirmation by LC/UV/MS
- Minimize carryover
- Confirmation of quality of MS spectra
- Estimation of impurities

Sample Features

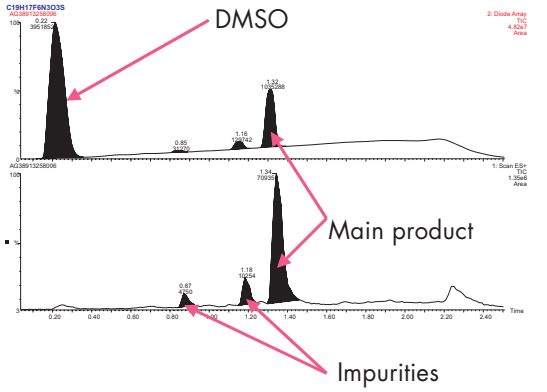
- 96 well plate containing 2 mL test tubes
- 2 µg/uL in DMSO
- Highly differentiated compounds
- Sample name and expected molecular weight list imported directly from spreadsheet

Analytical Conditions

- LC/MS
  - 2790 Separations Module
  - Fast Gradient
    - A = 0.1% HCOOH B= CH_3CN + 0.1% HCOOH
    - 95/5 to 0/100 in 1.5 minutes at 1.0 mL/min
    - Rapid equilibration with just-in-time gradient
  - Sample Injection
    - 2 µL, with 2 µL air gaps
    - Wash = 90/10 MeOH/H_2O, (two cycles)
  - XTerra™ C_{18} MS Column
    - 3.5 µm, 2.1 x 50 mm
    - Temperature = 60 °C
  - 996 Photodiode Array Detector
    - Scan from 210 to 400 nm
  - ZMD Mass Spectrometer
    - Scan ESI+ from 200 to 800 amu in 1 sec in centroid mode
    - Cone voltage = 40 volts
    - Split 1/5
  - OpenLynx™ Software Report
  - 3.5 min, Total Cycle Time

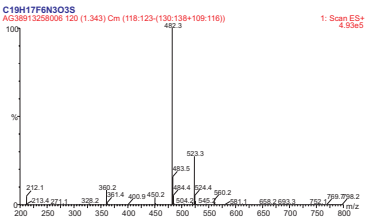
Chromatogram and Spectra

- Why perform a separation?
  - Eliminate potential ion suppression from DMSO
  - Eliminate potential co-elution with DMSO
  - Enable impurity spectra investigation
  - Enable estimation of purity

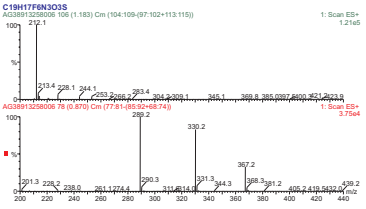


3.5 min, Total Cycle Time

Main product
[M+H]+ = 482.3



Impurities
[M+H]+ = 212.2
[M+H]+ = 289.2



Injection Carryover

- Why be concerned?
  - Carryover risks confusion in a series of similar compounds
  - 4 µg in DMSO were injected on-column

% Carryover (MS)	% of runs
0.00%	54.00%
< 0.1%	63.00%
< 0.25%	84.00%
< 0.5 %	96.00%

The carry over is calculated from MS chromatograms

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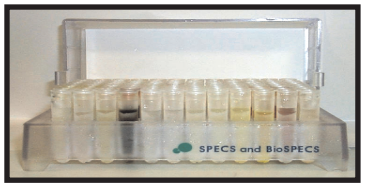

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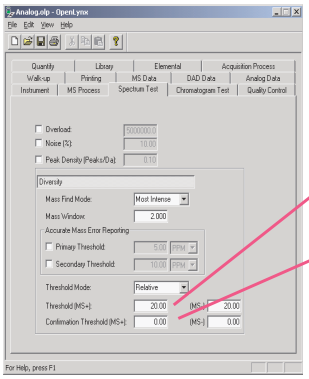
OpenLynx™ Compound Presence Decision Criteria

Two Thresholds



Differentiation from noise
% of base peak intensity

Molecular weight confirmation
% of base peak intensity

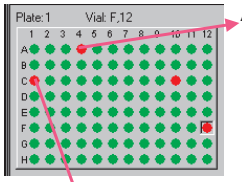
MS Spectrum Purity

$$SP(\%) = \frac{\text{Intensity of the expected fragment}}{\text{Base peak intensity}} \times 100$$

OpenLynx Plate Graphic

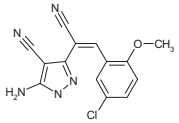
SP (%) < Threshold	**Red Well**
Threshold < SP (%) < Confirmation Threshold	**Yellow Well**
Confirmation Threshold < SP (%)	**Green Well**

Why Four Red Wells?

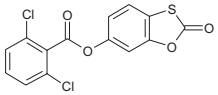


A4: formula doesn't correspond to the structure

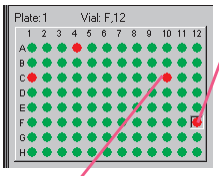
C14H10ClN5O ?



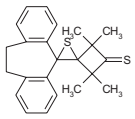
Structure of C1:



No or poor ionisation in positive ESI?
Wrong product ?

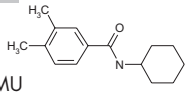


Structure of F12:

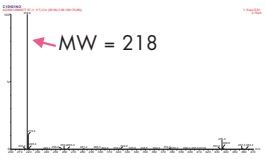


No elution
or
No product

Structure of C10:



Given MW: 230 AMU



Wrong formula ?
Wrong product ?

Conclusions

- Fast check of compound confirmation by LC/UV/MS
  - 3.5 min. cycle time
- Minimize carryover
  - Carryover in LC/MS <1.0% for all analyses
- Confirmation of quality of MS spectra
  - Separation removes DMSO from analytes
  - Noise differentiation and confirmation thresholds provide confidence in compound presence/absence
- Estimation of impurities
  - Impurity chromatogram/spectra available for examination