AUTOMATED HPLC SCREENING AND METHOD DEVELOPMENT SYSTEM

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DRUG EVALUATION - ANALYTICAL DEVELOPMENT
For the development of selective HPLC methods to be used for quantitative determination of impurities, degradants, excipients and active in the drug substance as well as in the drug product a screening concept is currently used. The concept is based on a matrix screening between different pH and stationary phases.

The screening module consist of 4 HPLC systems each operating with a different MS compatible mobile phase at a different pH. Each HPLC system is connected with a valve switcher to permit automated selection between 8 columns with different selectivity.

After screening 32 chromatograms for each sample have to be evaluated. The best separation is selected based on pH and column selectivity.
### Screening Experiments

#### pH = 2.5

<p>| | | | | | | | |</p>
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<tr>
<td>A : 10 mM NH₄OAc in water - CH₃CN (950/50 v/v) + 0.1%, v/v TFA</td>
<td>A : 10 mM NH₄OAc in water - CH₃CN (950/50 v/v) + 0.1%, v/v TFA</td>
<td>B : 10 mM NH₄OAc in water - CH₃CN (100/900 v/v) + 0.1%, v/v TFA</td>
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#### pH = 4.8

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#### pH = 7

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#### pH = 9

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</tr>
</tbody>
</table>
32 chromatograms for each sample

1. Zorbax Extend C18

2. Zorbax Bonus RP

3. Waters XTerra MS C18

4. Waters XTerra RP18

5. YMC Pack C18

6. Waters Symmetry Shield RP18

7. YMC Pro C18

8. Alltech Platinum EPS C18

stress, pH=6, 70 °C, OS, 2 dagen

stress, pH=4, 8h daglicht

stress, pH=2, 8h daglicht

R208176: JFHE_0105_077_1

T002474 + T002475

min0 2.5 5 7.5 10 12.5 15 17.5 20 22.5
Selected pH and column

stres, pH=2, 8h daglicht
stres, pH=4, 8h daglicht
stres, pH=6, 70 °C, OS, 2 dagen

mAU

0 2.5 5 7.5 10 12.5 15 17.5 20 22.5 min
Once a suitable pH and column is selected generally other appropriate chromatographic parameters (gradient, organic modifiers, temperature, ionic strength .....) are optimized step by step.
Major Roadblocks with currently used process

- Many chromatograms to evaluate manually
  - very complex
  - very time consuming
- The main problem with this manual selection is the peak tracking between the obtained chromatograms. Currently this peak tracking is performed based on peak heights, peak shapes and PDA spectra.
- Optimization is done step by step
The new approach is the on-line combination of four HPLC systems and one mass spectrometer with a computer modelling and simulation system. The mass spectrometer is installed with a MUX interface. In this way the related compounds can be immediately identified based on molecular mass.

Summary reports will be automatically generated and transferred to a chromatographic method development software (DryLab or ACD).

All important chromatographic parameters can be optimized with the assistance of the above described Method Development System.
Automated Screening and Method Optimization System

MassLynx™ Software, Version 4.0

OpenLynx™
- Integrate MaxPlot (BPI)
- Define correlating MS base peak
- Generate a list of all masses found
- Tracking of all masses found with corresponding RT

Visual Basic
- Summary Reports
- Reject false positives

AMD Resolution Map

ACD/LC Simulator
Installation
MUX Source on Waters ZQ

scan time: 0.13 sec
inter - scan delay: 0.1 sec
Waves
Method Variables

**WAVE 1**
(screening)

- Buffer Concentration
  - pH 2.5 (NH₄OAc + TFA)
  - pH 4.8 (NH₄OAc + CH₃COOH)
  - pH 7.0 (NH₄OAc)
  - pH 9.0 (NH₄OAc + DEA)

- 10 mM
- 25 mM

- Columns
  - Zorbax Extend C₁₈
  - Zorbax Bonus RP
  - X Terra MS
  - X Terra RP₁₈
  - X Terra Phenyl
  - Symmetry Shield RP₁₈
  - YMC Pro C₁₈
  - YMC Buthyl C₄

**Optimum pH**

**Best column**

**WAVE 2-3**
(optimization)

- Optimum buffer concentration
- Gradient
  - 25 min (35 min)
  - 55 min (65 min)

- Temperature
  - 15°C
  - 60°C

- Organic Modifier
  - CH₃CN
  - CH₃OH
  - IPA
  - THF

with and without additive

**12 experiments**

**64 experiments**
• Integrate PDA BPI (MaxPlot)
• Define correlating MS base peak for each integrated PDA peak
• Generate a list of all masses found
• Reject false positives based on missing $^{13}$C-isotope
• Tracking of all remaining masses with corresponding RT - Area - Peak Width
• Generate summary table
Integrate MaxPlot, define BPI and generate list masses found

Example 1: MH+ 459 (BPI) added to list
Condition: 10mM pH 2.5
Retention Time: 9.79

Example 2: co-elution
MH+ 477 (BPI) added to list
MH+ 462 not added to list

Example 2: MH+ 477 (BPI) added to list
Condition: 10mM pH 2.5
Retention Time: 12.50
Integrate MaxPlot, define BPI and generate list masses found

Example 3
Condition: 10mM  pH 7
Retention Time: 14.93

It is statistically significant that within 64 experiments all peaks are once separated and the corresponding masses are added to the list.
OpenLynx: Define All Masses

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Compound</th>
<th>Mass</th>
<th>Function</th>
<th>Time</th>
<th>Time</th>
<th>%Total Area</th>
<th>Height</th>
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<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td>MS ES+</td>
<td>3.97</td>
<td></td>
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<tr>
<td>2</td>
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<td>3</td>
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<td>MS ES+</td>
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</table>
Reject false positives

“Ghost-peaks, baseline fluctuations, blank peaks ... will also add a mass to the list but will be rejected afterwards based on the missing $^{13}$C-isotope (marked yellow in the list).

Example 4
Condition: 10mM pH 2.5
Retention Time: 3.97

Example 4:
MH$^+$ 214 (BPI) will be rejected
The list of all masses is reprocessed by OpenLynx to obtain for each mass the corresponding RT, area and peak width in each experiment. Co-elutors can now easily be tracked.
Tracking of all masses found with corresponding RT - Area - Peak Width

<table>
<thead>
<tr>
<th>M.H</th>
<th>pH 2.5 10 mM</th>
<th></th>
<th>pH 4.8 10 mM</th>
<th></th>
<th>pH 7 10 mM</th>
<th></th>
<th>pH 9 10 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RT</td>
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<td>0.21</td>
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</tr>
</tbody>
</table>

The list of masses is now extended to a Summary Table.
### Summary Table

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<thead>
<tr>
<th>M.H +</th>
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<td>0.22</td>
<td>0.15</td>
<td></td>
</tr>
</tbody>
</table>

If a particular mass is only found in a few experiments (number set by user), that mass is statistically not relevant and will be rejected. (marked green in the Summary Table)

If in the Summary Table two masses consistently co-elute over the whole number of experiments (retention time in two rows of the Summary Table match exactly), then the highest mass may be dropped. e.g. $^{13}$C-isotope, $^{81}$Br-isotope, $^{37}$Cl-isotope etc… (marked blue in the Summary Table)
The lean Summary Table will be transferred to a chromatographic method development software (DryLab or ACD) for prediction of the optimum in wave 1, 2 and 3.
Conclusions

This screening and method development system will improve significantly the quality (reliability, robustness and cost reduction) of the methods and reduce the development time.

Remarks

• Integrated peaks with no mass response are not covered.
• Compounds with no UV response but with mass response are not covered.
• Interference of non UV additives (cyclodextrines) can make the process of mass tracking more difficult.
• ZQ-MUX is exclusively used for peak tracking. The peaks not covered by the system can be investigated afterwards with high resolution LC-MS.
CONTACT INFORMATION
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☎ +32.14.60.29.83