Return of Investment in (U)HPLC method development systems

Rudy Sneyers, Jeroen Peeters, Luc Van Grieken and Gaby Török

Janssen Pharmaceutical Companies of Johnson & Johnson
Pharmaceutical Development & Manufacturing Sciences
Analytical Development - Small Molecules Method Development
Beerse, Belgium
THE PAST: Trial & Error

Method Development by Trial-and-Error

- Generally one single type of column is chosen at random
- Other chromatographic parameters are selected at random and adapted in several experiments (composition mobile phases, pH, buffer, column temperature, gradient)

Con’s

- Step by step optimization **without scientific background** resulting in a method which by definition is not the optimum separation
- Many chromatograms to evaluate manually - very complex - very time consuming
- Manual Raw Data evaluation
- No Peak Tracking
<table>
<thead>
<tr>
<th>No.</th>
<th>Methodology</th>
<th>Column</th>
<th>pH</th>
<th>OM</th>
<th>Gradient</th>
<th>Temperature</th>
<th>Development time</th>
<th>FTE time</th>
<th>FTE Costs</th>
<th>Investment</th>
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<td>≤ 3</td>
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</table>

**MATRIX**
Trial & Error - Process Output & Return

Development Time

FTE Cost

Investment
Method Development based on ‘SCIENCE’

- 8 preselected columns at 4 pH values (screening based on DoE)
- Final optimization of chromatographic parameters still adapted in several experiments by trial & error

Con’s

- Investment cost (4 x LC)
- Many chromatograms to evaluate manually which is very complex
- Still time consuming
- No peak tracking
Screening Experiments

**pH = 2.5**

1. Zorbax Extend C18
2. Zorbax Bonus RP
3. Waters XTerra MS C18
4. Waters XTerra RP18
5. Waters XTerra Phenyl
6. Waters Symmetry Shield
7. YMC Pro C18
8. YMC Pack C4

A: 10 mM NH₄OAc in water - CH₃CN (950/50 v/v) + 0.1%, v/v TFA
B: 10 mM NH₄OAc in water - CH₃CN (100/900 v/v) + 0.1%, v/v TFA

**pH = 4.8**

1. Zorbax Extend C18
2. Zorbax Bonus RP
3. Waters XTerra MS C18
4. Waters XTerra RP18
5. Waters XTerra Phenyl
6. Waters Symmetry Shield
7. YMC Pro C18
8. YMC Pack C4

A: 10 mM NH₄OAc in water - CH₃CN (950/50 v/v) + 0.05%, v/v CH₃COOH
B: 10 mM NH₄OAc in water - CH₃CN (100/900 v/v) + 0.05%, v/v CH₃COOH

**pH = 7**

1. Zorbax Extend C18
2. Zorbax Bonus RP
3. Waters XTerra MS C18
4. Waters XTerra RP18
5. Waters XTerra Phenyl
6. Waters Symmetry Shield
7. YMC Pro C18
8. YMC Pack C4

A: 10 mM NH₄OAc in water - CH₃CN (950/50, v/v)
B: 10 mM NH₄OAc in water - CH₃CN (100/900, v/v)

**pH = 9**

1. Zorbax Extend C18
2. Zorbax Bonus RP
3. Waters XTerra MS C18
4. Waters XTerra RP18
5. Waters XTerra Phenyl
6. Waters Symmetry Shield
7. YMC Pro C18
8. YMC Pack C4

A: 10 mM (NH₄)₂CO₃ in water - CH₃CN (950/50 v/v)
B: 10 mM (NH₄)₂CO₃ in water - CH₃CN (100/900 v/v)
<table>
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<th>≤ 3</th>
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Screenings module [LC-UV] - Process Output & Return

Development Time

FTE Cost

Investment
Method Development based on ‘SCIENCE’
- 5 preselected columns at 4 pH values (screening based on DoE)
- **Introduction of peak tracking with MS**
- Final optimization of chromatographic parameters still adapted in several experiments by trial & error

Con’s
- Investment cost (MS)
- Many chromatograms to evaluate manually
  - very complex (MS)
  - very time consuming
- MUX interface reduces S/N in MS experiments (loss of sensitivity)
- Optimization is done step by step
Peak Tracking

YMC Pro C$_{18}$ pH 2.5

XTerra RP$_{18}$ pH 7
Screenings module [LC-UV-MS]
Screenings module [LC-UV-MS]
## Screenings module [LC-UV-MS]

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<th>Investment</th>
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Screenings module [LC-UV-MS] - Process Output & Return

Development Time

FTE Cost

Investment
Automated Method Development

- 5 preselected columns at 4 pH values
- Introduction of peak tracking with MS
- Final chromatographic parameters optimised in 3 waves based on SCIENCE
- Custom Excel® software gives a quick overview of the best separation

Con’s

- Investment cost (2xMS)
- Compounds with no UV response but with mass response are covered
- Compounds with no mass response are not covered
- Many chromatograms to evaluate manually
  - very complex (MS)
  - very time consuming
Screening a matrix of all chromatographic parameters (full factorial DoE) takes too long
e.g. For 5 separation parameters
(column, pH, organic modifier, temperature, and gradient)
= (5 x 4 x 7 x 2) x (35 + 65) min = 28 000 min
= ± 467 hours or ± 20 days for one sub-sample

THEREFORE ...

The most important chromatographic parameters are optimized in multiple WAVES based on a scientific approach (DoE).

Chromatographic columns and pH are two of the most important parameters contributing to selectivity on reversed-phase HPLC. A wide pH range on each column is therefore screened first.

**WAVE 1:** Column screening and pH optimization
5 x 4 = 20 x 35 min = 700 min = 11h40
Screenings-module implemented in 1997

**WAVE 2:** Organic modifier optimization
7 x 35 min = 245 min = 4h05

**WAVE 3:** Temperature and Gradient optimization
(2 x 35 min) + (2 x 65 min) = 200 min = 3h20

TOTAL = ± 19 hours for one sub-sample
Twins [LC-UV-MS]

Liquid Chromatograph

Column Selector in Mistral Oven

Splitter

PDA 996

Waters ZQ™ Mass Detector

MassLynx™ Software, Version 4.0
20 experiments for each sub-sample are conducted. When all of the data are collected for each pH/column combination, the peaks are matched. A resolution map is generated for each column, giving a prediction for the optimal pH for each. The chromatograms are rated, and the optimal column and pH is chosen.
On the best column and with the optimized aqueous mobile phase from Wave 1, seven experiments (derived from the Snyder’s solvent triangle) with different organic modifiers are performed to predict the optimal organic mobile phase composition.
Wave 3: Gradient - Temperature Screening

<table>
<thead>
<tr>
<th>Column</th>
<th>XBridge C18 - 3.5 μm (150 x 4.6 mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent A</td>
<td>5 mM NH₄TFA + 10 mM NH₄AC + 0.1% TFA</td>
</tr>
<tr>
<td>Solvent B</td>
<td>CH₃CN - MeOH (50/50, v/v)</td>
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### Gradient

<table>
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<tr>
<th>Time</th>
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<th>25</th>
<th>27</th>
<th>35</th>
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<tbody>
<tr>
<td>% A</td>
<td>95</td>
<td>0</td>
<td>0</td>
<td>95</td>
<td>95</td>
</tr>
<tr>
<td>% B</td>
<td>5</td>
<td>100</td>
<td>100</td>
<td>5</td>
<td>5</td>
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<table>
<thead>
<tr>
<th>Flow</th>
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</thead>
<tbody>
<tr>
<td>Column temperature</td>
<td>30°C</td>
</tr>
</tbody>
</table>

On the best column and with the optimized aqueous mobile phase from Wave 1, and with the optimized organic modifier out of Wave 2, four experiments are conducted to predict the optimal gradient slope and column temperature.

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### Gradient

<table>
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<th>55</th>
<th>57</th>
<th>65</th>
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<tbody>
<tr>
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<td>95</td>
<td>0</td>
<td>0</td>
<td>95</td>
<td>95</td>
</tr>
<tr>
<td>% B</td>
<td>5</td>
<td>100</td>
<td>100</td>
<td>5</td>
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<td>Column temperature</td>
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</table>
Manual process for Data evaluation

- Make a stacked overlay of PDA (MaxPlot) and MS (TIC) signal
- Define the correlating mass for each PDA peak or vice versa
- Generate a component list (Excel® Component Table) of all masses found across all experiments with corresponding RT
- Reject false positives (Blank peaks, Ghost-peaks, baseline fluctuations, …)
- All labeled components are listed into one Component Table which can be transferred to commercial chromatographic experimental design software.
Stacked Overlay PDA - TIC

W1_COL4_25_S1

100%

-10%

W1_COL4_25_S1

1: Scan ES+
TIC
5.50e7

2: Diode Array
TIC
6.71e7

Time

4.00 5.00 6.00 7.00 8.00 9.00 10.00 11.00 12.00 13.00

Janssen
Define correlating MH\(^+\) and RT

Column 4 pH 2.5

Generate a list of all masses found with corresponding RT
Co-elution

Column 4 pH 2.5

In case of co-elution, it can be expected that within 20 experiments all peaks are likely to be resolved at least once and the corresponding masses added to the Component Table
Co-elution

Column 1 pH 2.5

Column 3 pH 9.0
The Component Table can be transferred to advanced experimental design software (LC Simulator) where the experiments are modelled and a resolution map is generated, giving a prediction for the optimum for each examined chromatographic variable.
## Twins [LC-UV-MS]

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<td>88.7</td>
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</table>
Twins [LC-UV-MS] - Process Output & Return

Development Time

FTE Cost

Investment
To avoid all the previous disadvantages, the **COSMOS** process was developed.

**Computer Organized Screening and Method Optimization System**

This innovative Screening and Method Development system is based on 3 principles:

- the use of a **scientific strategy** (DoE)
- the introduction of a single quadrupole **mass spectrometer** beside the PDA detector (LC-MS)
- a **special designed software** for automated peak tracking and data evaluation
Rigorous method development performed with composite samples and multiple chromatographic detectors results in a tremendous amount of hyphenated data files. This amount of data makes it cumbersome to see the whole story that the data is telling.

One way to organize these data is the collection of the relevant peak data in one single location. The Method Development Console (AutoChrom) is designed to summarize these data while retaining full links back to the hyphenated raw data.

After data acquisition, the detector signals are directly imported to an AutoChrom workspace, then UV and MS Peak matching are applied to label the peaks in all experiments. Compounds with either UV or MS response are being picked up.

All labeled components are listed automatically into one Component Table which can be passed to LC Simulator for modeling and prediction.
AutoChrom Method Development Cycle

1. Acquire data
2. Automatically track peaks between injections
3. Separation acceptable?
   - YES: Final Method
   - NO: Go back to Step 1

Final Method

Create peak table
AutoChrom Method Development Cycle

1. Acquire data
2. Automatically track peaks between injections
3. Create peak table
4. Model separations
5. Create resolution map

Start

Select next experiment

Separation acceptable?

End

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N

Separation acceptable?

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Separation acceptable?

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Separation acceptable?
AutoChrom Method Development Cycle

1. Acquire data
2. Automatically track peaks between injections
3. Create peak table
4. Model separations
5. Create resolution map

Separation acceptable?

Start

Select next experiment

End

NO
AutoChrom Method Development Cycle

1. Acquire data
2. Automatically track peaks between injections
3. Separation acceptable?
   - YES
   - End
4. Final Method
5. Create peak table
IntelliXtract: baseline correction & peak tracking

2: Diode Array

-230

3.20e5%

1: Scan ES+

TIC

5.88e7%

2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00

Time

10.00 12.00 14.00 16.00 18.00
MS Peak Matching done

MS Peak Matching 100% accurate
### UV Peak Matching done

#### UV Peak Matching ± 60% accurate

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<th>C_2</th>
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Reconciliation
After the UV- and MS-MAP algorithms are executed, the results (tables of masses and corresponding retention times) are directly imported into the AutoChrom workspace. After completion of all sub-samples from the entire workflow, a component table can be generated.
Simulated Composite chromatogram after reconciliation
### LC Simulator

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**Experiments Summary**

1st: pH = 2.5  
2nd: pH = 4.8  
3rd: pH = 7  
4th: pH = 9

---

**Janssen**
Wave 1: Resolution Map

A quadratic polynomial model based on the 4 measurements is constructed in order to predict retention times as a function of the pH. The predicted retention times at intermediate pH-values with their corresponding peak widths (considered constant since gradient elution) are sorted from high to low, and finally the resolutions between successive peaks are calculated and the $R_s_{\text{min}}$ can be predicted for each pH value and the optimal pH can be determined.
Two strategies were applied to predict from the seven experiments, derived from a Snyder’s solvent triangle, at which mobile phase composition, i.e., at which fractions of organic modifiers, the highest $R_{s_{\text{min}}}$ values (i.e., the best separation for the worst separated peak pairs) can be reached. The first one explores the three sides of the solvent triangle one by one, whilst the second method investigates the complete triangle. Both strategies first predict the retention times and peak widths of each compound at every considered mobile phase composition by using a mathematical model, then retention times with their corresponding widths are sorted from high to low, and finally the resolutions between successive peaks are calculated. In that way, the $R_{s_{\text{min}}}$ can be predicted for each mobile phase composition and the optimal mobile phase can be determined.
Wave 3: Resolution Map

This wave is a 2-D optimization mode resulting in a 3-D resolution map. Small changes of the investigated parameters can influence the deterioration of the minimal resolution. The areas with good resolution are colored in red. A large area implicates a robust separation.

Method suitability is a function defining the quality of the proposed separation in terms of run time, robustness, resolution of the closest eluting peaks, and minimum retention factor. The optimal conditions with regard to resolution and suitability can be shown on the map (●).
Theoretical vs Practical
## COSMOS - Twins [LC-UV-MS]

### MATRIX

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<th>Temperature</th>
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COSMOS - Twins [LC-UV-MS] - Process Output & Return

Development Time

FTE Cost

Investment
By using 4 LC-MS systems it is possible to speedup method development or cover more space of the DoE by combining wave 1 and 2.

- On each column 4 pH’s and 3 organic modifiers can be screened.
- 4 columns x 4 pH’s x 3 OM = 48 experiments
  In a separated wave 1 and 2 only 22 experiments are performed
- Investment cost (4 x LC-MS)
Combined wave 1 and 2

**pH 2.5**

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<tbody>
<tr>
<td>Column 2</td>
<td>XBridge Shield RP18 - 3.5 µm (150 x 3.0 mm)</td>
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<tr>
<td>Column 3</td>
<td>XBridge Phenyl - 3.5 µm (150 x 3.0 mm)</td>
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<td>Column 4</td>
<td>XSelect Phenyl Hexyl - 3.5 µm (150 x 3.0 mm)</td>
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<td>Column 5</td>
<td>XSelect Fluoro Phenyl - 3.5 µm (150 x 3.0 mm)</td>
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<tr>
<td>Column 6</td>
<td>HSS T3 - 3.5 µm (150 x 3.0 mm)</td>
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Solvent A: 10 mM NH₄AC in water + 0.1% TFA
Solvent B: CH₃CN
Solvent C: CH₃CN - MeOH (50/50)
Solvent D: CH₃CN - IPA (50/50)

Gradient:

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Flow: 0.45 ml/min

Column temperature: 45°C

**pH 4.8**

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Solvent A: 10 mM NH₄AC in water + 0.05% CH₃COOH
Solvent B: CH₃CN
Solvent C: CH₃CN - MeOH (50/50)
Solvent D: CH₃CN - IPA (50/50)

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Flow: 0.45 ml/min

Column temperature: 45°C

**pH 7**

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Solvent A: 10 mM NH₄AC in water
Solvent B: CH₃CN
Solvent C: CH₃CN - MeOH (50/50)
Solvent D: CH₃CN - IPA (50/50)

Gradient:

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Flow: 0.45 ml/min

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**pH 9**

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Solvent A: 10 mM NH₄AC in water + 0.025% NH₄OH
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Solvent C: CH₃CN - MeOH (50/50)
Solvent D: CH₃CN - IPA (50/50)

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Flow: 0.45 ml/min

Column temperature: 45°C
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</tr>
</tbody>
</table>
COSMOS – Quads [LC-UV-MS] - Process Output & Return

Development Time

FTE Cost

Investment
By combining high resolution and speed of the UPLC chromatographic separation together with advanced MS detectors and software tools, it significantly improves the efficiency of method development.

Due to the analysis time reduction with UPLC it is possible to speedup method development or cover more space of the DoE by combining wave 1 and 2.

(1 UPLC-SQD vs 3 HPLC-ZQ’s).

On each column 4 pH’s and 3 organic modifiers are screened.

4 columns x 4 pH’s x 3 OM = 48 experiments
In a separated wave 1 and 2 only 22 experiments are performed

Investment cost (2 x UPLC-MS)
**Combined wave 1 and 2**

**pH 2.5**

<table>
<thead>
<tr>
<th>Column 1</th>
<th>Acquity BEH C18 - 1.7 µm (150 x 2.1 mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column 2</td>
<td>Acquity CSH Fluoro Phenyl - 1.7 µm (150 x 2.1 mm)</td>
</tr>
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<td>Acquity CSH Phenyl Hexyl - 1.7 µm (150 x 2.1 mm)</td>
</tr>
<tr>
<td>Column 4</td>
<td>Acquity HSS T3 - 1.8 µm (150 x 2.1 mm)</td>
</tr>
</tbody>
</table>

**Solvent A**
10 mM NH₄AC in water + 0.1% TFA

**Solvent B**
CH₃CN

**Solvent C**
CH₃CN - MeOH (50/50)

**Solvent D**
CH₃CN - IPA (50/50)

**Gradient**

<table>
<thead>
<tr>
<th>Time</th>
<th>% A</th>
<th>% B-C-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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<td>5</td>
</tr>
<tr>
<td>17</td>
<td>95</td>
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**Flow**
0.35 ml/min

**Column temperature**
45°C

**pH 4.8**

<table>
<thead>
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<td>Acquity HSS T3 - 1.8 µm (150 x 2.1 mm)</td>
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</tbody>
</table>

**Solvent A**
10 mM NH₄AC in water + 0.05% CH₃COOH

**Solvent B**
CH₃CN

**Solvent C**
CH₃CN - MeOH (50/50)

**Solvent D**
CH₃CN - IPA (50/50)

**Gradient**

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**Flow**
0.35 ml/min

**Column temperature**
45°C

**pH 7**

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**Solvent A**
10 mM NH₄AC in water + 0.025% NH₄OH

**Solvent B**
CH₃CN

**Solvent C**
CH₃CN - MeOH (50/50)

**Solvent D**
CH₃CN - IPA (50/50)

**Gradient**

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**Flow**
0.35 ml/min

**Column temperature**
45°C

**pH 9**

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**Solvent A**
10 mM NH₄AC in water + 0.025% NH₄OH

**Solvent B**
CH₃CN

**Solvent C**
CH₃CN - MeOH (50/50)

**Solvent D**
CH₃CN - IPA (50/50)

**Gradient**

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**Flow**
0.35 ml/min

**Column temperature**
45°C
## AutoChrom UPLC - Twins [LC-UV-MS]

<table>
<thead>
<tr>
<th></th>
<th>Column</th>
<th>pH</th>
<th>OM</th>
<th>Gradient</th>
<th>Temperature</th>
<th>Development time</th>
<th>FTE time</th>
<th>FTE Costs</th>
<th>Investment</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Trial &amp; Error</td>
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<td>≤ 3</td>
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</tr>
</tbody>
</table>
AutoChrom UPLC - Twins [LC-UV-MS] - Process Output & Return

**Development Time**

**FTE Cost**

**Investment**
- How many chromatographic interactions are evaluated at the same time??
- How many variables are evaluated within each chromatographic parameter??
- How many DoE’s are executed??
- Use of MS and Evaluation software??

Evaluating 3 interactions at the same time will improve the quality of the method significantly!!

The contribution of MS and AutoChrom are important for the overall quality!!
Summary

Development Time

FTE Cost

Investment

Quality
Conclusions

- The use of mass spectrometry with dedicated evaluation and modeling software is an innovative way to perform method development based on scientific principles.

- COSMOS will significantly improve quality (reliability, robustness, and life time) of the methods, reduces development time and costs but also reduces loss of interpretation information and expensive retesting of samples.

- With the hyphenated data from modern instruments and the available computer capacity, AutoChrom is a tool for organizing, visualizing, and tracking the data retaining full links back to the original raw data.

- The “AutoChrom UPLC - Twins [LC-UV-MS]” approach is a QbD approach in chromatographic method development and will return the investments within a short time.
Acknowledgement and Contact Information

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Mike McBrien
Advanced Chemistry Development, Inc. (ACD/Labs)
Toronto, Ontario

M. Dumarey and Y. Vander Heyden
Vrije Universiteit Brussels (VUB)
Department of Analytical Chemistry and Pharmaceutical Technology
Jette, Belgium

CONTACT INFORMATION
Rudy Sneyers
rsneyers@its.jnj.com
☎ +32.14.60.29.83

Jeroen Peeters
jpeeterc@its.jnj.com
☎ +32.14.60.65.60