HIGH-THROUGHPUT PREPARATIVE HPLC-MS PURIFICATION WORKFLOW OF LIBRARIES WITHIN THE EUROPEAN LEAD FACTORY

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Introduction

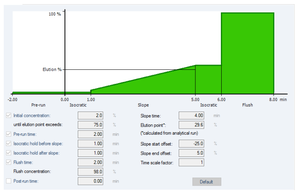
The European Lead Factory is a collaborative public-private partnership established in 2013 aiming to deliver over 500 000 of high-quality compounds and the opportunity to screen those compounds against potential drug targets to a broader community. Taros Chemicals, a privately owned CRO company, is leading the chemistry consortium and have contributed with more than 27.000 compounds into the Public Compound Collection (PCC) covering more than 40 scaffolds with a very high dissimilarity. The distinctive characteristic of the Taros' screening compounds is their high level of structural complexity and three-dimensionality which are further expanded by the decoration of a diverse in-house collection of final diversification reagents. The number of delivered compounds (ca 10000 per year) with a median purity of 97% (UHPLC) as well as the diversity between libraries and also within the compounds of the same library is making the purification process very challenging.

In this poster we present the purification results of four representative libraries designed validated and produced in Taros.

The Agilent OpenLAB CDS Automated Purification Software developed by Agilent Technologies in a close collaborative project with Taros was fully implemented creating automated purification workflows based on reversed phase high performance liquid chromatography (HPLC) and mass spectrometry (MS). The system is suitable for a smart combination of UV- and mass-based fraction triggering and the automated transfer of data between each single process streamlining the workflow for highest productivity as the purification of crude products is usually the most time consuming part of organic compound preparation.

A fine tuning has been made from the chemist, modifying the slope offsets due to the chromatographic diversity of the different compound classes. The slope offsets have been saved in template files. Different template files have been used to purify difficult batches of compounds.

The results and statistics collected from analytic and purification (retention time, reactivity, validation of chemistry and purification) have a high intrinsic value for the design, validation synthesis and production of new libraries.



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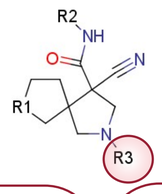
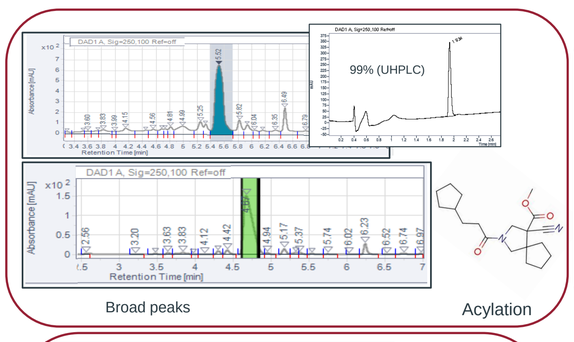
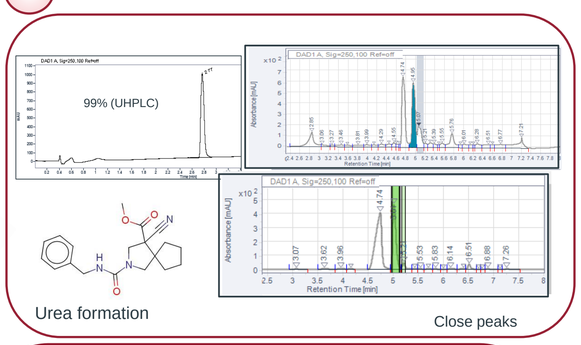
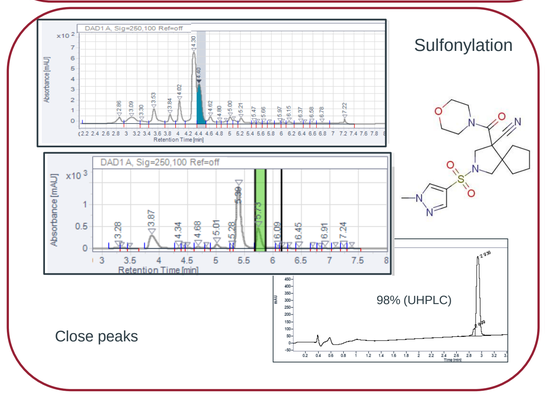
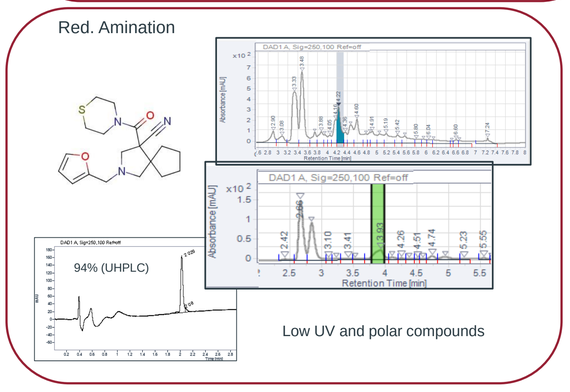


Median Purity: 99% (UHPLC)
Purification Succes: 95,3%
Number of compounds: 674
Purification time: 9 days





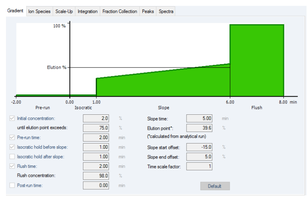




Crude sample
(96cp/day)

Analytical
Target
confirmation
HPLC-MS
Standard gradient
method (8 min)
96cp/day
Overnight

Scalability from
analytic
to purification
Selection of Template
Automated purification
(96cp/day purification
method 6-9min)



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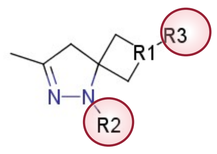
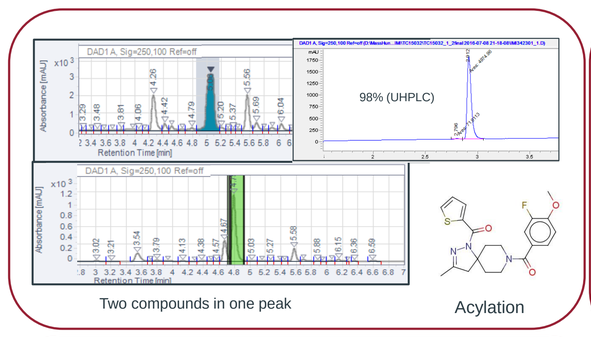
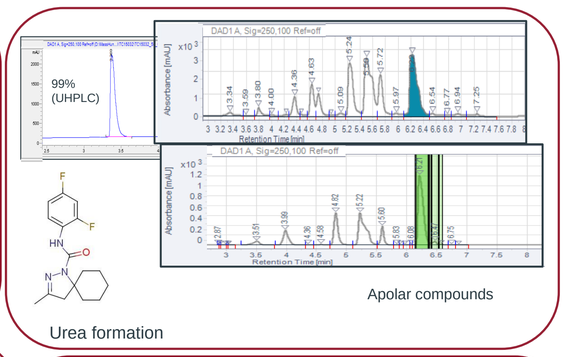
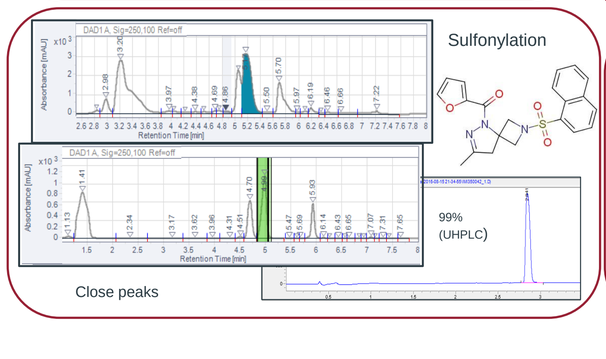
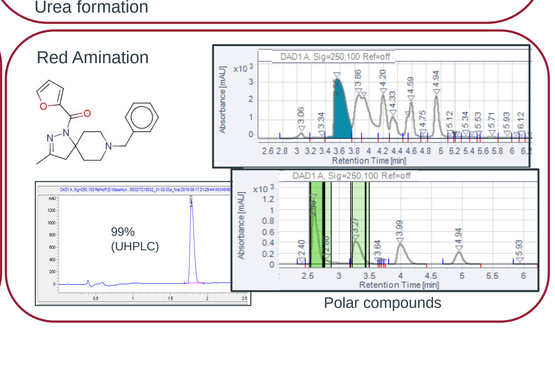


Median Purity: 99% (UHPLC)
Purification Succes: 92,7%
Number of compounds: 734
Purification time: 10 days







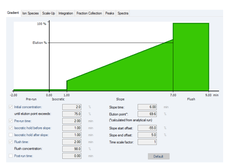


Purity evaluation
Fraction pooling
(Liquid handler
adapted format)

Final Purity
UHPLC-MS
5min
96cp/day
(overnight)

Packing and
Reformatting
final compound
(5-20µM)

Delivered for
Screening
ca. 80 cp/day
Median purity
97% (UHPLC)
> 5µM



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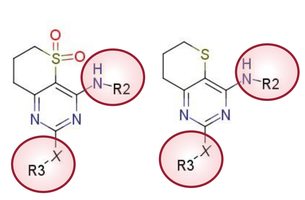
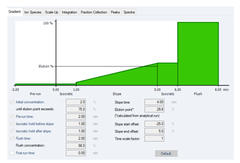
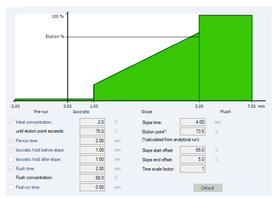
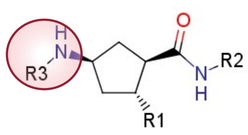


Median Purity: 99% (UHPLC)
Purification Succes: 94,89%
Number of compounds: 498
Purification time: 6 days

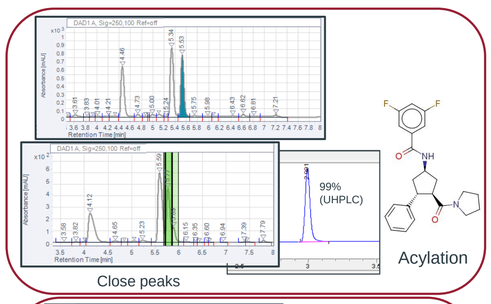


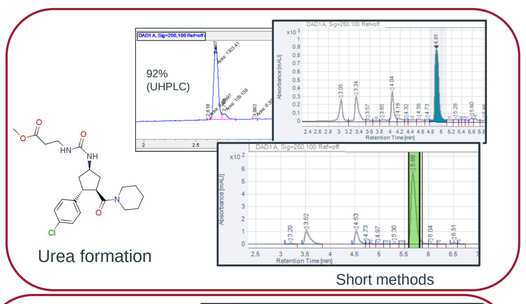
TC1318

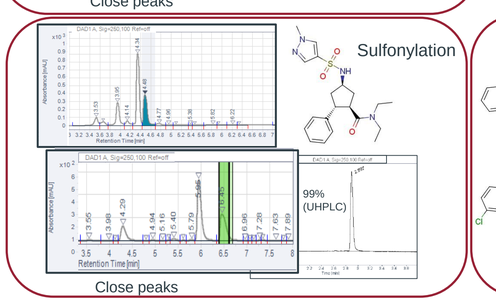


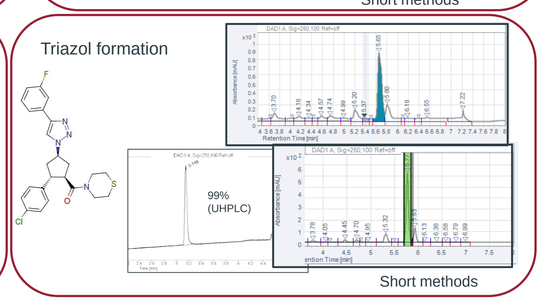


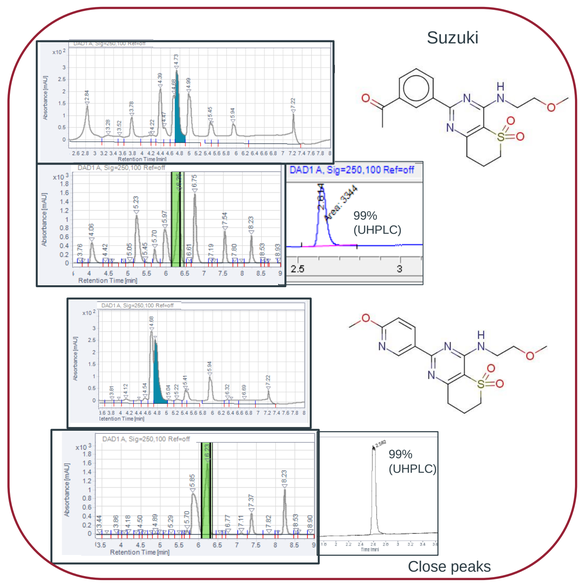
Median Purity: 99% (UHPLC)
Purification Succes: 94,9%
Number of compounds: 654
Purification time: 8 days

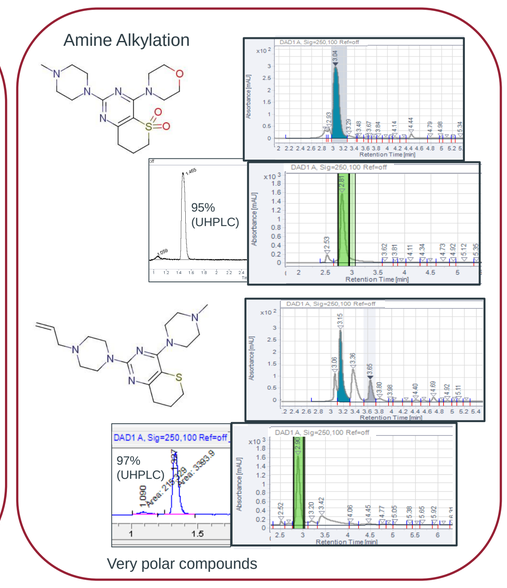












Experimental

Analytical: UHPLC Agilent 1260 Infinity LC/MS System: 1260 Infinity Binary Pump (G4220A), 1260 Infinity Auto Sampler (G4226A), 1260 Infinity Thermostated Column Compartment (G1316A), 1260 Infinity Diode Array detector (G1315C) with the Standard flow cell, Agilent 6120 Quadrupole Mass spectrometer (G6120B)

Preparative: Agilent 1260 Infinity preparative scale LC/MS Automated Purification System: 1260 Infinity Auto Sampler (G2258A), 1260 Infinity Preparative Pumps (G1361A), 1260 Infinity Diode Array detector (G1315D) with the standard flow cell, Agilent 1260 Infinity Fraction Collector PS (G1364B), Agilent 6120 Quadrupole Mass spectrometer (G6120B)

Columns: Waters ACquity UPLC®BEH C18, 2.1x50mm, 1.7µm, VanGuardTM Pre-Column 2.1x50mm; Waters XBridge® C18, 4.6x100mm, 3.5 µm, XBridge® C18, 3.5µm 4.6x20mm Guard Cartridge; Waters XBridge® C18, 19x100mm, 35µm, XBridge® C18, 5µm 19x10mm Guard Cartridge

Software: Agilent OpenLAB CDS ChemStation Edition for LC/MS, Rev. C.01.07 [110], Agilent Automated Purification Software Add-On, Rev. A.01.03 [42]

Solvents and samples: Purification mixture for analytical and preparative runs: drug-like samples, Solvent A: Water (HPLC-Grade) + 0.1% Formic Acid, Solvent B: Acetonitrile (HPLC-Grade) + 0.1% Formic Acid

Conclusions

Analytical-prep HPLC/MS is ideal for separation of complex synthetic mixtures:

- Efficient, high throughput analysis-purification set-up (>90% success, 30% time gained, 25% less solvent)
- High separation power even with difficult crudes (no workup necessary)
- Possibility of generation of method templates that can be applied to similar samples.
- Small modifications from the template will provide a high separation power even with difficult crudes (no workup necessary)
- Purification of low UV absorbance compounds by MS-triggered Protocols
- Possibility of collection of two compounds with different mass
- Possibility of collection of two/more compounds with the same mass (Diastereoisomers)

Automatic transfer of analytical results to purification systems eliminates manual work

- Minimal learning curve for untrained operators
- Straightforward implementation in work-flow
- Convenient export of fraction information to liquid handler from purification software (flexibility, time saving, avoiding errors)

Intrinsic value for the chemistry process

- Up to 40-50% chemistry time gained
- Possibility to back-calculate chemistry scale and library design
- Possibility to automatically collect reagent statistics for improved chemistry/ design success

The research leading to these results has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement n' 115489, resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7 / 2007-2013) and EFPIA companies' in-kind contribution.