COMPOUND-ORIENTED PREPARATIVE HPLC PURIFICATION PLATFORM

www.inventivapharma.com

Nathalie Urban, Frédéric Bell, Cyrielle Couchoux

Analytical Sciences Group, Medicinal Chemistry, **INVENTIVA** 50 rue de Dijon, Daix. 21121- France

Presentation

Background

INVENTIVA is a fully integrated Drug Discovery company based in Dijon (France) dedicated to the finding of novel drug development candidates. The chemistry team (>30 Scientists) is providing an extensive panel of services encompassing medicinal chemistry, synthetic & parallel synthesis, computer-assisted-drug-design, and analytical & purification.
All compounds delivered to the Compound Management are checked by NMR and LC-MS. Purity criteria are ≥ 90% by UV in UPLC/UV/MS.

Our Purification Team

A team of 2 is dedicated to the purification of all final compounds synthesized by the parallel synthesis team. They also support the medicinal chemists for final or intermediate compounds that failed initial purification (flash-chromatography, precipitation, crystallization…)
The task of this team begins with the reception of the crude mixture together with the analytical LC-MS and is completed with the delivery of a bar-coded vial containing the fully characterized dried purified compound.

Purification challenges

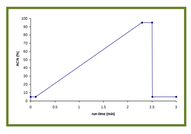
Our versatile team has to adapt to the specific requirements of each project :
- scale (from 10mg up to 5g)
- initial purity (UV purity range from 5 to 90%, chromatographic profiles…)
- single compound or library of compounds
- tailored purification conditions to fit the sample properties: chiral compounds, polarity, solubility, chemical stability, ion-pairing
- specific project requirements: very high purity (>98%), high recovery for "VIP" samples, very small samples

How to deal with these multiple situations ? → **have predefined strategies to gain in reactivity, efficiency and productivity**

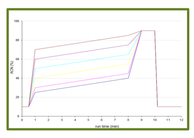
→ **use common sense, show professionalism and leverage technical expertise**

High throughput purifications

Operational criteria :
- only one injection per crude sample (max. 130mg)
- mass-triggered fraction
- injections with ACD technique (At Column Dilution)
- injections with acidic modifier (HCOOH)
- utilization of narrow [2] (also called focused) gradients



Direct transfer



Analytical gradient :

Acquity UPLC BEH C18, 50 x 2.1mm x1.7µm,
H_2O +0.1% CH_3COOH, ACN +0.1% CH_3COOH

Preparative gradient :

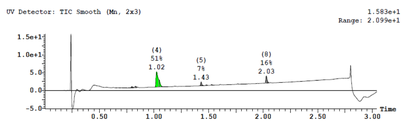
Kinetex C18 XB, AXIA,21,2x150mmx5µm
H_2O+0.1% HCOOH, ACN +0.1% HCOOH

Narrow gradient conditions are preferred to purify compound libraries [1]
This technique is also ideal in an open access environment

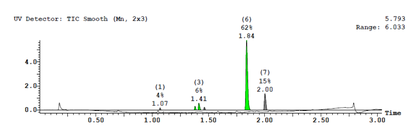
Complex separations

According to the properties of the compound (polarity, acid-base…), the purification in presence of acid modifier is not always appropriate.

Analytical run under **acidic** condition (0.1% AcOH)



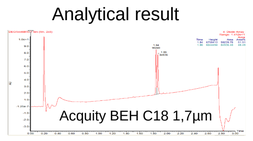
Analytical run under **basic** condition (0.1% NH_3)



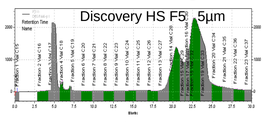
→ Best separation and loadability observed under **basic** condition

Separations of regio isomers …

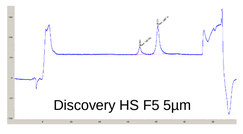
Analytical result

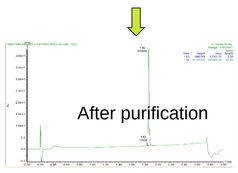


Crude : 152 mg



Preparative separation





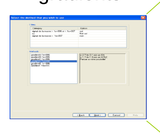
Quantity : 87mg

Other possibility of separation :
- in normal phase chromatography
- with other acidic modifier (TFA, CH_3COOH…)
- without any modifier….

Open access Prep

Login

Choice of focused gradients

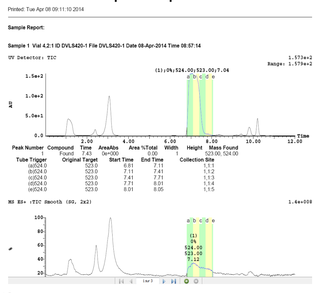


Solvent A: Water + 0.1 % formic acid
Solvent B: Acetonitrile + 0.1 % formic acid
Column Kinetex C18 XB 21,2*150mm*5µm
Run Time : 12 min

Gradient	OA5min	UPLC3min
gradient A 10-25-40-90	tr<1.9	tr<1.0
gradient B 10-30-45-90	1.9<tr<2.3	1.0<tr<1.4
gradient C 10-40-55-90	2.3<tr<2.7	1.4<tr<1.7
gradient D 10-50-65-90	2.7<tr<3.1	1.7<tr<1.9
gradient E 10-60-75-90	3.1<tr<3.8	1.9<tr<2.2
gradient F 10-70-85-90	tr >3.8	tr >2.2

New in 2014

Example of purification



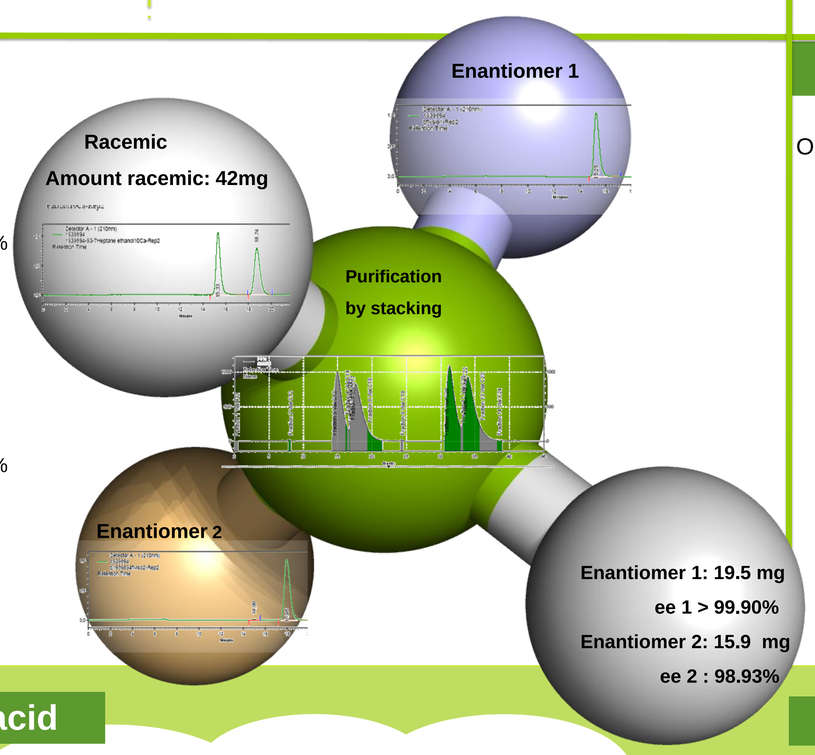
Automated Purification :
-System combines the performance of the focused gradients and automation of the preparative chromatography workflow.
-Collection is triggered by MS signals.

Available to the chemists 8 hours a day.

Chiral purifications

Analytical conditions :
- column : Chiralpak AD-H, 250*4.6mm
- Mobile phase : Heptan 93 % - Ethanol 7%
- Flow rate : 0.5ml/min
- T °c: 10°C

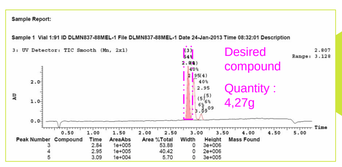
Preparative conditions :
- column : Chiralpak AD-H, 250*21.2mm
- Mobile phase : Heptan 93 % - Ethanol 7%
- Flow rate : 10ml/min
- T °c : 10°C
- Injection : stacking mode
- # 10mg per injection



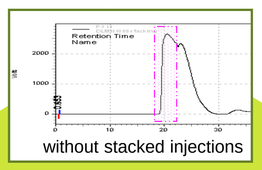
Stacking method

Concertina injections to avoid instrument downtime :
-Reduced cycle time
-Reduction of solvent consumption
-Currently used in chiral purification

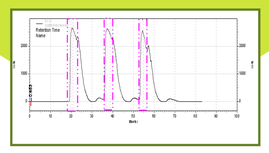
Operational criteria:
-separation of only 2 compounds
-isocratic mode
-more than in 1 injection is required



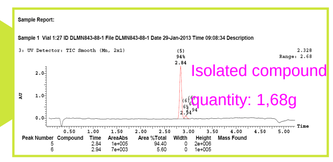
Crude sample



without stacked injections



with stacked injections



After purification

Purification time:
1 injection without stacking : 40 min
3 injections without stacking : 120min
3 injections stacking mode : 80 min → Time saving : 40min

Mobile phase used: (Flow rate 118ml/min)
1 injection without stacking : 4,72 L
3 injections without stacking : 14,2L
3 injections stacking mode : 9,44L → Solvent saving :4,72L

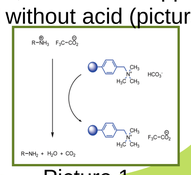
Removal of residual acid

Acidic modifiers are known to improve the chromatographic peak shape, but with acid sensitive compounds they need to be removed prior to the evaporation step

Principle : The solution percolates through the cartridge *, the acid is trapped by the stationary resin phase and the sample can be collected without acid (picture 1),

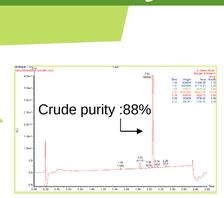
This method can :
- eliminate residual acids (present in the mobile phase)
- eliminate counter-ions such as trifluoroacetate.
- allow to isolate the free base

*StratoSpheres SPE PL-HCO3 from Varian (Agilent)

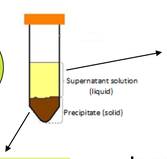


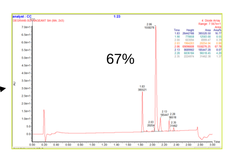
Picture 1

Precipitation / Crystallization



centrifugation





Insoluble samples ?
No need to force the solubilization, first centrifuge then check for purity

CONCLUSION

Combination of well designed generic strategies, optimal selection of columns/mobile phases and high level of competences allows us to purify almost all the submitted samples. A regular quality control of the instruments and a fine tuning of the process allow us to maintain a minimum recovery of 90%, secured by selective waste bottles. Statistical analysis during the last two year period highlighted an average purification yield of 78%. INVENTIVA has set up a versatile, comprehensive and robust platform supporting our team of chemists.

Purification team motto : you create, we isolate, together we invent !

Devices and columns

Main used columns :

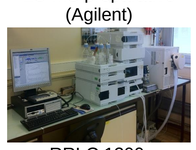
- Kinetex C18 XB, AXIA, 21,2x150mm,5µm[3] (Phenomenex)
- Sunfire C18 OBD (diam 19 or 30, 5 or 10µm) (Waters)
- XBridge C18 OBD 5µm (diam 19 or 30) (Waters)
- Luna Prep C18, 50X250mm, 10µm (Phenomenex)
- Discovery HS F5, 21.2X250mm,5µm (Phenomenex)
- Chiralpak AD-H, 21.2X250mm (Chiral Technologies)
- Uptisphere Si 21.2X250mm,5µm (Interchim)
- Flash cartridges….

Instruments and technologies

LC-MS open access (Waters)

LC-MS preparative (Agilent)

LC-UV preparative (VWR)



RRLC 1200 (Agilent)

Genevac (HT8,EZ2+….)

References & contacts

[1] High throughput purification platform in support drug discovery, M.Liu et al , Merck & Company, ACS Combinatorial Science, **2011**

[2] Preparative LC-MS purification : Improved Compound-Specific Method Optimization, K.F. Blom et al, Incyte Corporation, J. Comb. Chem , **2004**, 6, 874-833

[3] Evaluation and performance comparison of new Core-Shell media vs fully porous media for preparative Purifications, M.Jacob et al, Phenomenex Inc, **2013**, http://www.phenomenex.com

Contact : nathalie.urban@inventivapharma.com, frederic.bell@inventivapharma.com