Development of a compound purification strategy for a medicinal chemistry group

Application

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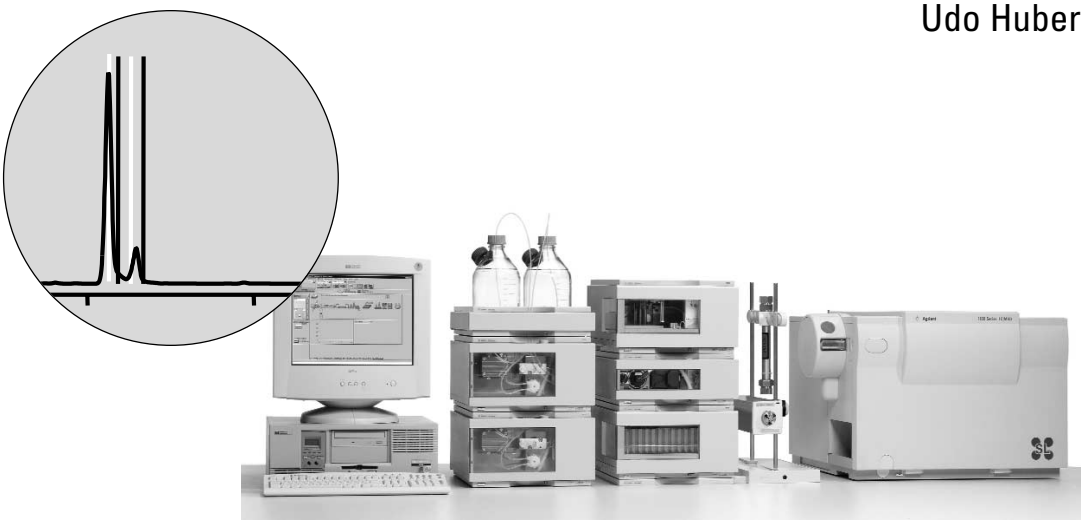

Abstract

This Application Note shows the development of a purification strategy for a medicinal chemistry group at Boehringer Ingelheim in Vienna, Austria, using the Agilent 1100 Series purification system[1,2]. The goal was to develop a simple purification process, which could be easily used by all chemists. Further challenges were to develop a set of pre-defined methods to purify not only simple samples but also problematic samples, such as insoluble samples, polar samples and samples that show poor ionization.

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Introduction

Preparative HPLC is the method of choice for high- and low-throughput compound purification in drug discovery. In this Application Note we describe the development of a purification strategy for a medicinal chemistry group. Prior to purification the crude reaction mixtures were analyzed on an analytical Agilent 1100 Series system in a walk-up fashion. The entire group uses a single generic method for the analysis of samples, with new samples being added to a running sequence. The results are automatically transferred to each individual chemist's office computer for viewing.

The goals for the development of the purification strategy were:

- a simple, walk-up workflow,
- a set of guidelines for sample preparation and method selection for the chemists,
- purification of problematic samples - insoluble samples, polar samples and samples that show poor ionization,
- one standard method suitable for 80 - 90 % of the daily samples,
- a small set of generic purification methods.

Equipment

The experiments were performed on an Agilent 1100 Series purification system containing the following modules:

- two Agilent 1100 Series preparative pumps
- Agilent 1100 Series preparative autosampler
- Agilent 1100 Series column organizer
- Agilent 1100 Series multi-wavelength detector
- two Agilent 1100 Series fraction collectors PS
- Agilent 1100 Series 12-position/ 13-port valve
- Agilent 1100 Series mass-selective detector
- Agilent 1100 Series isocratic pump (as make-up pump)
- Agilent active splitter

The system was controlled using the Agilent ChemStation (rev. A.10.01)

Results and discussion

Workflow

As the chemists were familiar with the ChemStation software for analytical HPLC-MS and usually submit small batches of 1–15 samples for purification at a time, the decision was made to use the ChemStation software for purification without the Agilent Purification software. The workflow was designed similar to the analytical workflow mentioned above – the chemists enter their own samples in a sequence, select a preparative method from a small list of methods and the result files are sent automatically to their office computers. The only differences between the preparative and analytical runs are:

- a fraction start location can be entered optionally (if no position is entered, the next free position in the fraction collector is automatically used),
- if the sample volume to be injected differs from the default sample volume (500 µL), it must be entered.

Sample preparation

Since the system is operated using a 21.2-mm i.d. column at a flow rate of 20 mL/min the maximum sample load should be no more than 50 mg per injection.
The sample should be dissolved in the following solvent mixtures:

- 500 µL DMSO/acetonitrile 50:50 (v/v) for standard samples,
- 500 µL DMSO for insoluble samples,and
- 500 µL DMSO/water 50:50 (v/v) for polar samples.

The resulting samples are then filtered into the sample vial prior to injection. For complete sample injection Agilent high recovery vials (Agilent part number 5183-4510) were used.

Injection of insoluble compounds

For the injection of insoluble compounds into the mobile phase at the gradient starting conditions, two injection techniques are possible: organic-phase injection or sandwich injection[3]. The idea of the sandwich injection is to protect the sample from precipitating with two plugs of pure solvent to avoid mixing of the mobile phase with the sample (figure 1). This can be easily achieved by setting up an injector program in the ChemStation. From a vial containing pure DMSO the plug is drawn before and after the sample. For standard samples (default sample injection volume 500 µL) 50 µL of DMSO are drawn for each plug. For insoluble samples, 100 µL of DMSO are drawn for each plug.

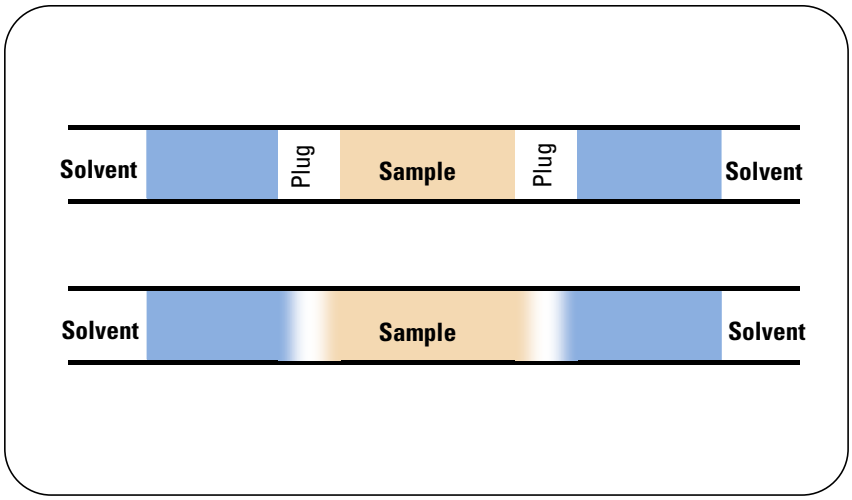

Figure 1
Sandwich injection

Purification of polar compounds

A dedicated method was set up for polar compounds. In order to avoid co-elution of polar compounds with the DMSO peak the following precautions were made:

- the gradient starting conditions were set to 5 % acetonitrile (10 % was used for the standard method)
- an initial hold phase for the gradient of 2.5 minutes was adopted
- gradient end conditions were set to 50 % acetonitrile (95 % was used for the standard method)

Set of methods

1. Standard method

Column:	Zorbax SB-C8, 21.2 x 100 mm, 5 µm
Mobile phases:	water + 0.1 % formic acid acetonitrile
Gradient:	at 0 min 10 % B at 2 min 10 % B at 10 min 95 % B at 12 min 95 % B at 13 min 10 % B
Stop time:	13 min
Flow:	20 mL/min
Inj. volume:	500 µL (plugs of 50 µL DMSO each)
Column temp.:	ambient
UV detector:	254 nm/8 (ref.: 360 nm/50) Prep. flow cell (path length 0.06 mm)
Mass-based fraction collection:	on threshold, 10000 counts

2. Method for very insoluble samples

As for standard method

Gradient:	at 0 min 20 % B
	at 2 min 20 % B
	at 10 min 95 % B
	at 12 min 95 % B
	at 13 min 20 % B
Inj. volume:	500 µL (plugs of 100 µL DMSO each)

3. Method for polar compounds

As for standard method

Gradient:	at 0 min 5 % B
	at 2 min 5 % B
	at 10 min 50 % B
	at 12 min 50 % B
	at 13 min 5 % B
Inj. volume:	500 µL (no plugs)

4. Method for compounds that show poor ionization

As for standard method

Mass-based fraction collection: on threshold, 1000 counts

5. Method using the AND combination of the UV and MS signal

As for standard method

Fraction collection using AND combination of UV and MS signal

MS:	on threshold, 10000 counts
UV:	on slope only, up- and down slope 3 mAU/s

Setup of target mass for mass-based fraction collection

For mass-based fraction collection the mono-isotopic mass must be entered as the target mass. Using the Adducts from the *Fraction collection* setting of the MSD, the software calculates the trigger mass, e.g. the $[M+H]^+$ ion. In all five methods described before, M was selected from *Adducts*, which means the chemists can read the trigger mass directly from the MS spectrum of the analytical run and enter this mass as the target mass.

Applications

In this section the purification of a real-life crude reaction mixture is shown. The crude reaction mixture was analyzed on an Agilent 1100 Series analytical system, which showed that the desired product existed as a mixture of cis and trans isomers. The analytical chromatogram is shown in figure 2.

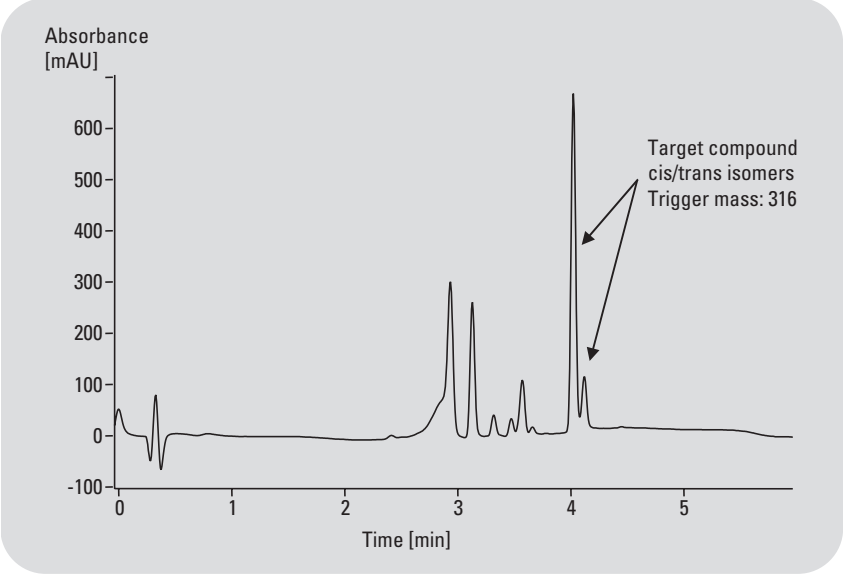

Figure 2
Analytical chromatogram of crude reaction mixture

The sample was purified on the preparative system using the standard method (method 1) and fractions were collected based on the target mass. Three fractions were collected as shown in figure 3.

The first two fractions contained compounds that also showed the trigger mass of 316 and fraction three contained the desired cis/trans isomers (see analytical run, figure 4).

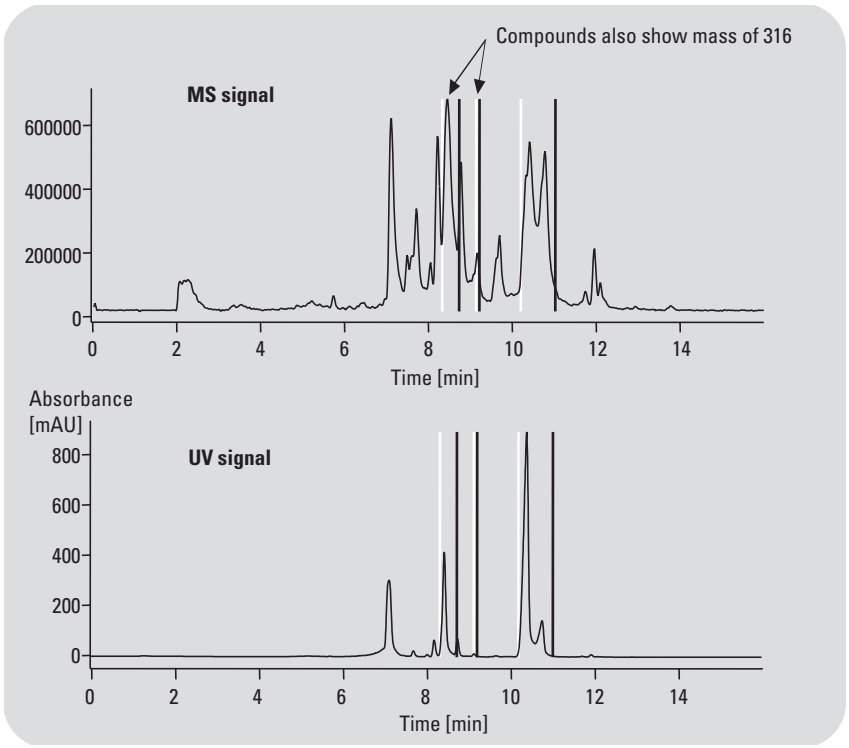

Figure 3
Purification run using the standard method

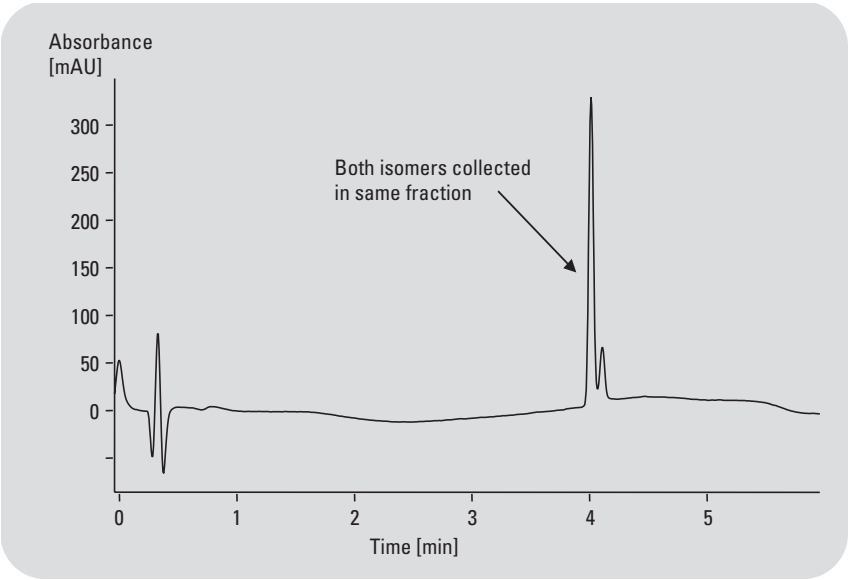

Figure 4
Analytical run of fraction three

In order to separate the two isomers, the crude product was purified again, this time using the *AND* combination of the UV and MS signal (method 5). As shown in figure 5, by using this method the isomers were successfully collected in two separate fractions.

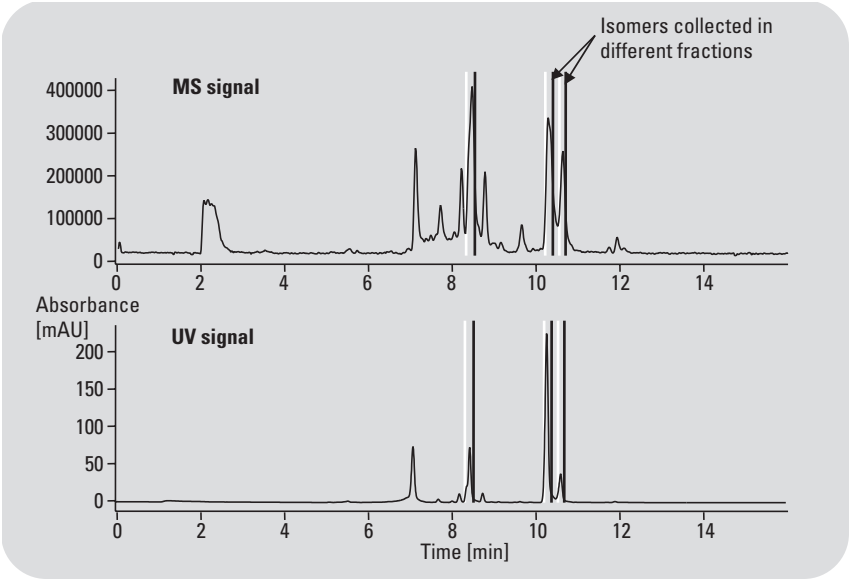

Figure 5
Fraction collection using the AND combination of UV and MS signal

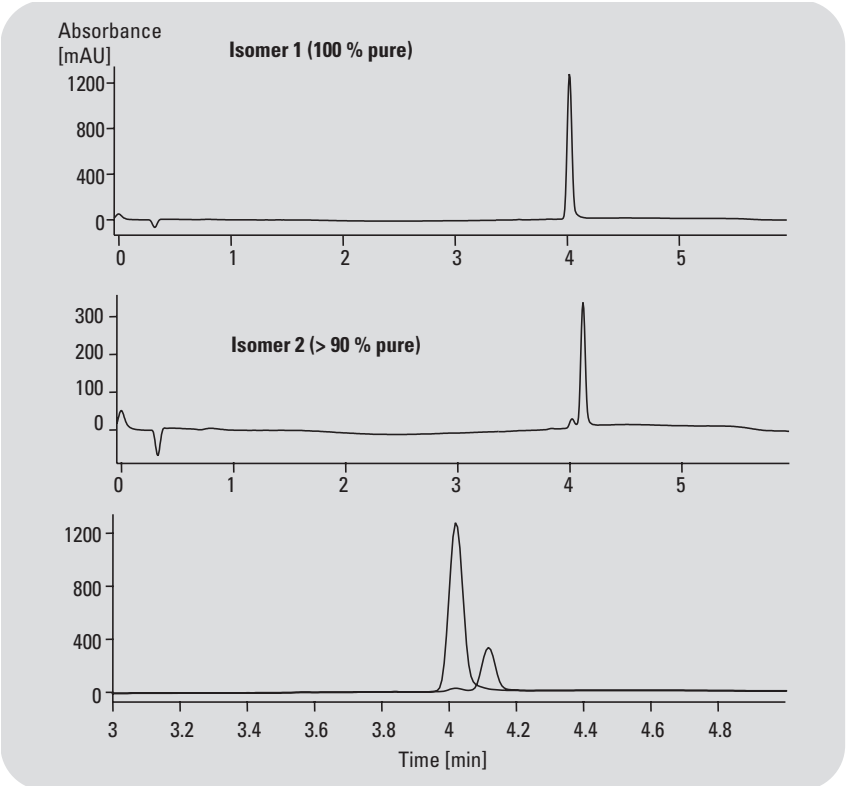

Figure 6
Analytical runs of the two fractions containing the isomers

Conclusion

In this Application Note we showed the development of a purification strategy for a medicinal chemistry group. A simple, walk-up workflow for preparative HPLC-MS purification was established. A set of five pre-defined methods were generated for different sample types, with a standard method suitable for 80–90 % of the daily samples. Further methods were set up for insoluble samples, polar samples and for samples that show poor ionization. An additional method was established using the AND combination of the UV and MS signal. Finally, a real-life application example including the separation of cis/trans isomers was shown using two of the pre-defined methods.

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