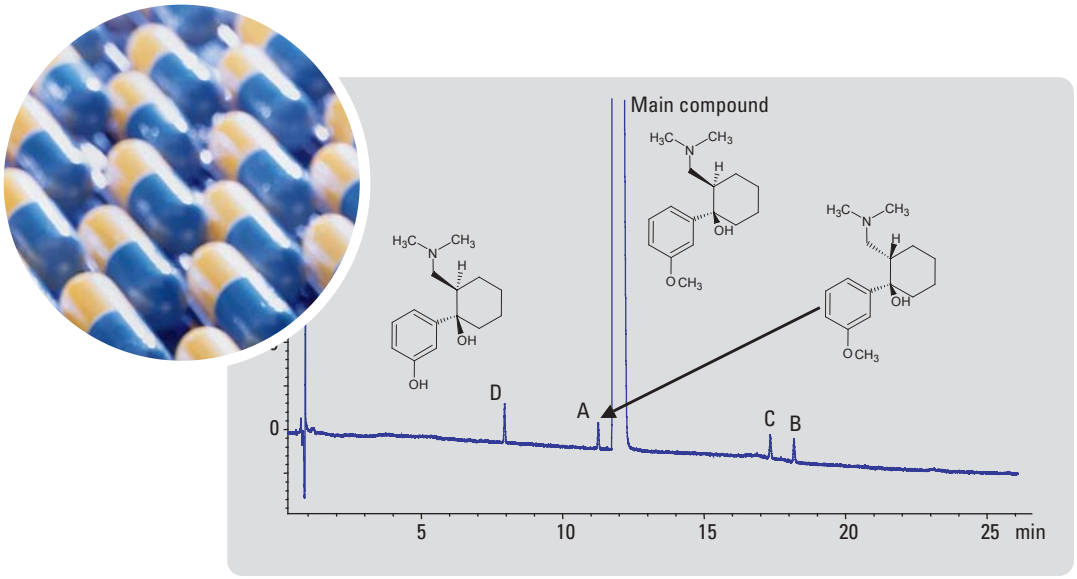
Impurity Profiling with the Agilent 1200 Series LC System

Part 2: Isolation of Impurities with Preparative HPLC

Application Note

Udo Huber



Abstract

The isolation and purification of synthesis impurities is an important task in the impurity profiling process. Ideally all impurities can be identified by hyphenated MS techniques, but usually some of them must be isolated and purified to elucidate the structure by NMR, for example. In this Application Note two impurities that could not be identified by MS are isolated from a crude sample. Starting with an analytical run using an Agilent 1200 Series Rapid Resolution LC (RRLC) system, the method was optimized regards to resolution of the critical impurity pair, and loading experiments were carried out. After scale-up to a preparative scale column, the impurities could be isolated in high purity and recovery. Sufficient amounts of each compound could be isolated after repetitive injections to elucidate the structures by NMR.

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Introduction

Impurity profiling describes a group of analytical activities aimed at the detection, identification, structure elucidation and quantitative determination of organic and inorganic impurities in drugs[1]. These activities are carried out across the entire drug discovery and development process, leading finally to a validated method used in QA/QC to assure the drug's safety for the patient. The first task in this process is the detection of all impurities. Various, orthogonal analytical methods such as gas chromatography, capillary electrophoresis and high-pressure liquid chromatography are used for this purpose. With the introduction of sub-2 µm particle columns for highest resolution, together with the Agilent 1200 Series RRLC system and hyphenated MS technologies such as Ion Trap and Time-of-Flight (TOF) instruments the chromatographer has at present strong tools to separate and identify the impurities in a relatively short time[2]. However, even with the most sophisticated MS instruments, the complete structure elucidation of all compounds is often impossible. These compounds have to be isolated and purified and are then characterized by ^{1}H- and ^{13}C-NMR. Since the impurities are present in the crude sample in low concentration, it is advantageous to use samples in which these compounds are enriched. This could be the mother liquor of a larger scale crystallization process, for example. The isolation and purification of a pair of impurities that could not be identified by MS is described in this Application Note.

Experimental

Equipment

The experiments were performed using an Agilent 1200 Series purification system containing the following modules:

- Two Agilent 1200 Series preparative pumps
- Agilent 1200 Series dual-loop autosampler PS (2000-µL loop)
- Agilent 1200 Series column organizer
- Agilent 1200 Series multi wavelength detector (flow cell: 3-mm path length)
- Agilent 1200 Series fraction collectors PS

The system was controlled using the Agilent ChemStation (rev. B.02.01. SR1).

Results and discussion

As described in a previous Application Note[2], the impurities A and D (chromatogram on cover page) could be identified by MS using an Ion Trap and TOF instrument. Impurities B and C, however, could not be characterized completely, and have therefore been isolated by preparative HPLC for further structure elucidation.

Method optimization

Since the sub-2 µm particle stationary phase used in the MS experiments is also available with 5-µm particle size as a preparative scale column, the method only had to be optimized for the resolution of impurities B and C and for shorter run times. The results for a sample enriched with impurities B and C are shown in figure 1.

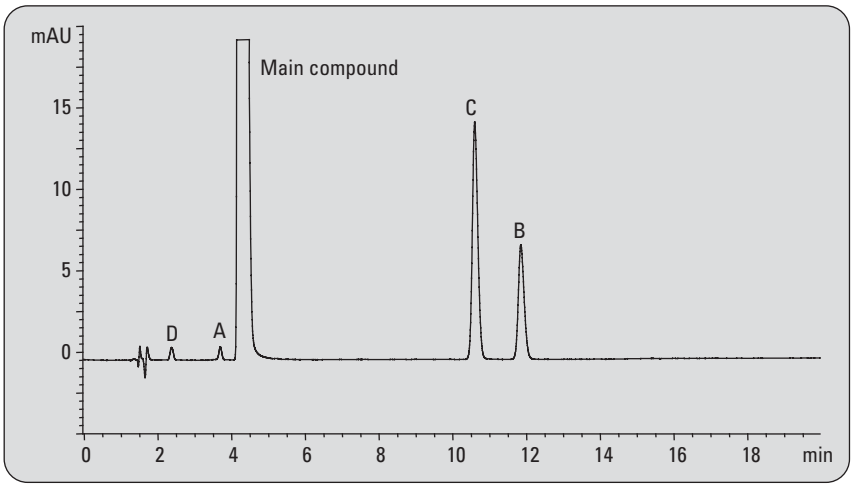

Figure 1
Optimized method for impurities C and B.

Chromatographic conditions:	
Column:	ZORBAX SB-C18 4.6 x 150 mm, 5 µm
Mobile phases:	Water + 0.1 % TFA = A Acetonitrile + 0.1 % TFA = B
Gradient:	at 0 min 25 % B at 14 min 32 % B at 16 min 50 % B
Stop time:	16 min
Post time:	5 min
Flow:	1 mL/min
Injection:	5 µL
Column temp.:	30 ° C
UV detector:	DAD 270 nm/30 (ref. 360 nm/100) Flow cell (10 mm path length)

Loading experiments

Before scaling-up the method for a preparative-scale column loading experiments were carried out on a analytical-scale column. In addition to the original sample, some diluted solutions as well as higher volumes were injected and the chromatograms monitored for the resolution of compounds C and B. The results of the overloading experiments are shown in figures 2a and 2b. Baseline separation of the impurities C and B could still be achieved with 1.0444 mg crude sample injected onto the column.

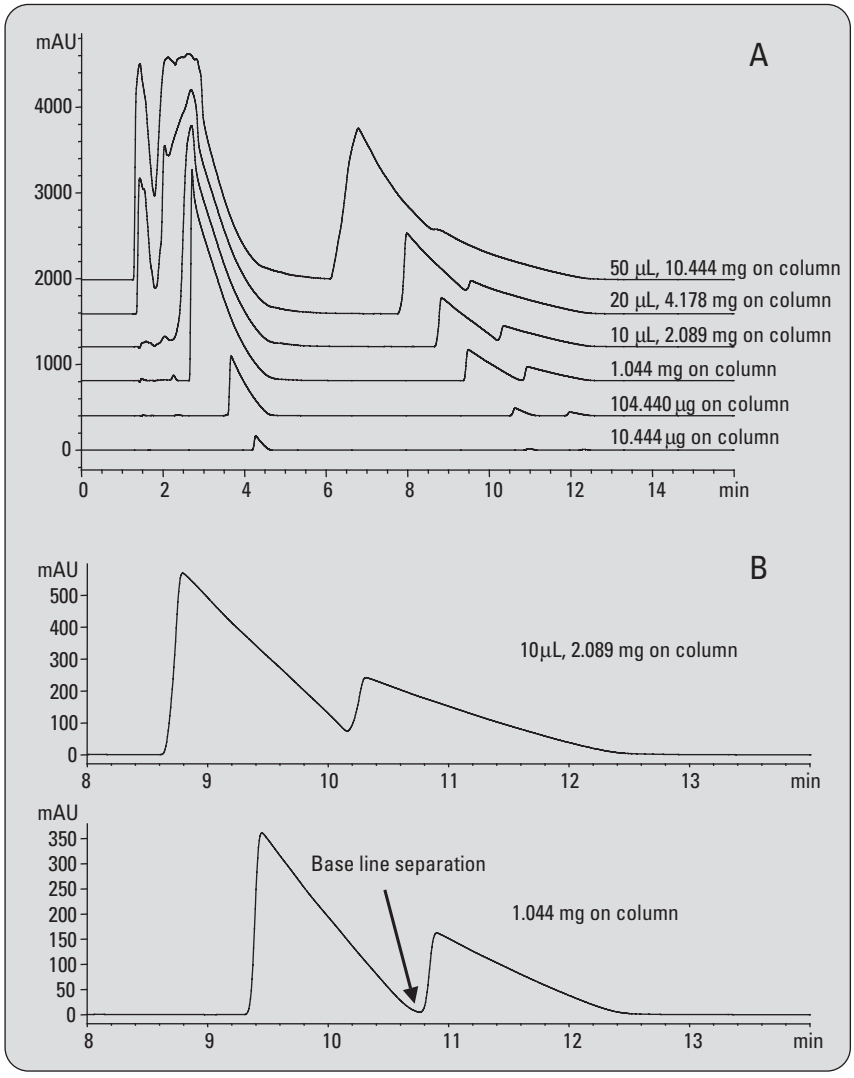

Figure 2
A) Loading experiments.
B) Baseline separation at 1.0444 mg crude sample on the column.

Scale-up calculations

The concentration of impurities C and B in the enriched sample was approximately 3 – 5 % of the main compound, which means that only about 0.03 – 0.05 mg of each impurity could have been isolated from a single injection. Therefore, the separation was scaled up to a preparative column with an inner diameter of 21.2 mm. Scale-up calculations have to be carried out for flow rate and loading as shown in figure 3. Since the stationary phase was the same as used in the first analytical run (cover page) and the loading experiments (figure 2a). The method transfer could be done without any changes in the elution order. With approximately 22 mg of crude sample applied, about 0.66 – 1.1 mg of each impurity could be isolated per injection. Therefore less than ten injections resulted in sufficient amounts for further NMR analysis.

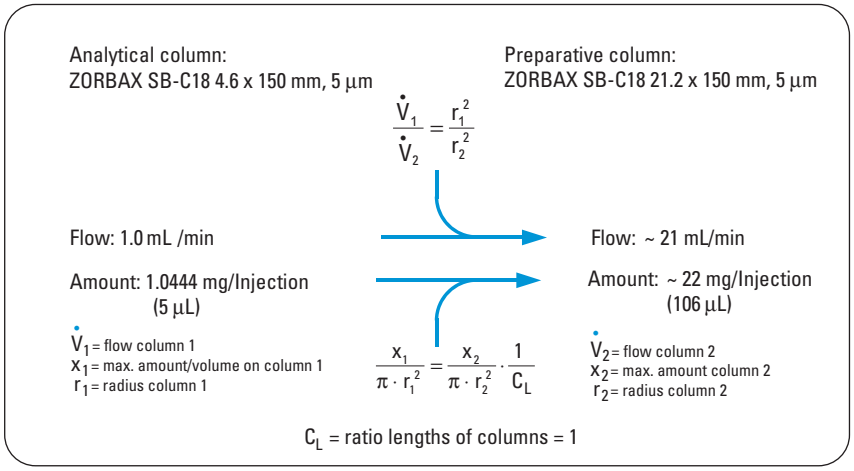

Figure 3
Scale-up calculations.

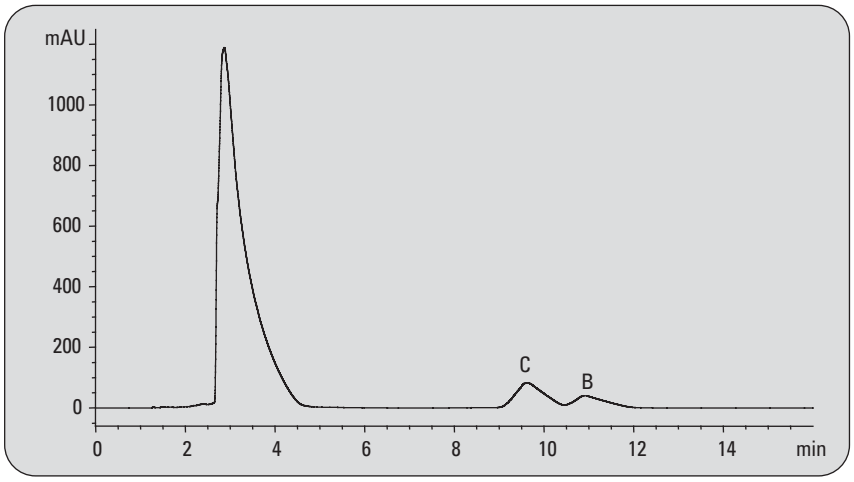

Figure 4
Run on preparative scale column according to the scale-up calculations

Chromatographic conditions:	
Column:	ZORBAX SB-C18 21.2 x 150 mm, 5 µm
Mobile phases:	Water + 0.1 % TFA = A Acetonitrile + 0.1 % TFA = B
Gradient:	at 0 min 25 % B at 14 min 32 % B at 16 min 50 % B
Stop time:	16 min
Post time:	5 min
Flow:	21 mL/min
Injection:	100 µL
Column temp.:	ambient
UV detector:	DAD 270 nm/30 (ref. 360 nm/100) Flow cell (3-mm path length)

Purification parameters

To optimize the fraction collection parameters the *Fraction Preview* of the ChemStation was used. As shown in figure 5, the chromatogram from the preparative run (figure 4) was loaded and the fraction collection parameters like threshold, up slope, down slope and upper threshold[3] were adjusted until the desired fraction collection performance was achieved. In this case a simple threshold-based collection within a time window (9 – 14 minutes) gave the best result. The result of the actual fraction collection run with the parameters optimized using *Fraction Preview* is shown in figure 6.

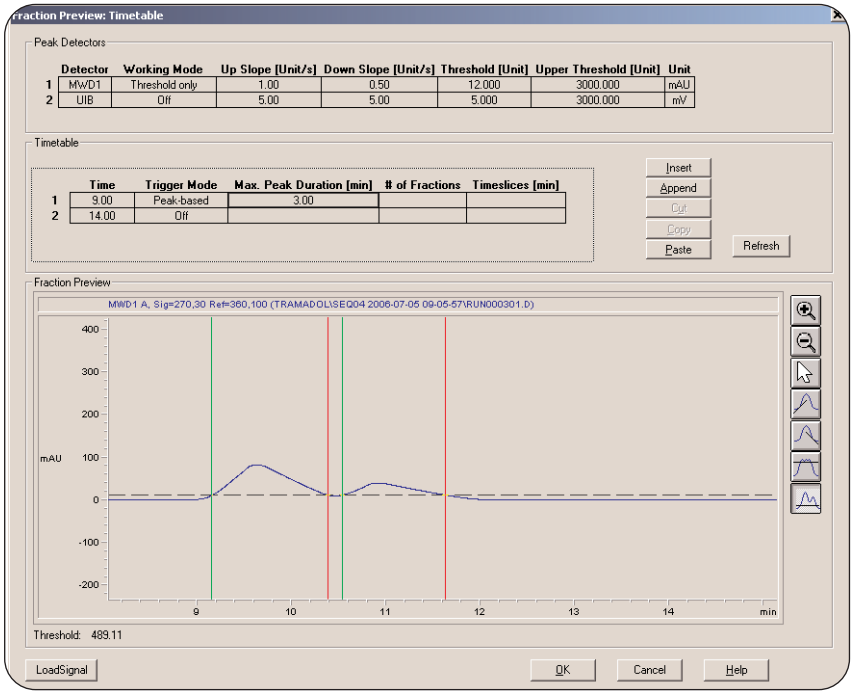

Figure 5
ChemStation *Fraction Preview*.

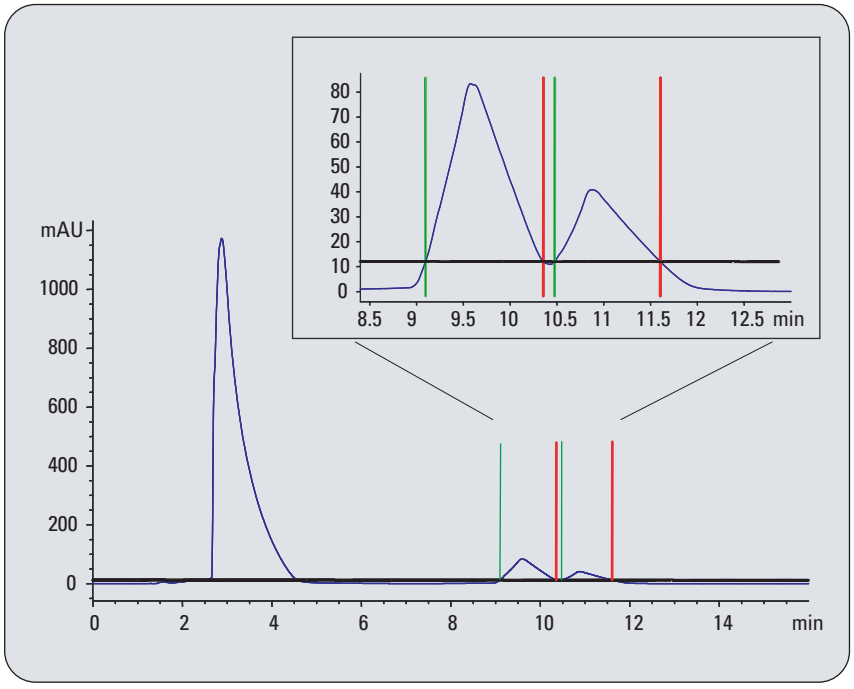

Figure 6
Result of fraction collection.

Since repetitive injections of the sample have to be done to acquire sufficient material for NMR analysis the pooling feature of the ChemStation could be used. With this feature the fractions of multiple injections are collected in the same fraction containers. In this case the volume of the fraction containers must be high enough to hold the combined volumes of all fraction collection runs. Therefore it is recommended to use the Agilent funnel tray for this application. The analysis (using the method shown in figure 1) of the combined fractions from ten consecutive purification runs is shown in figure 7. The structures as shown in figure 7could be elucidated by NMR analysis (data not shown) after collecting sufficient pure material of impurities C and B.

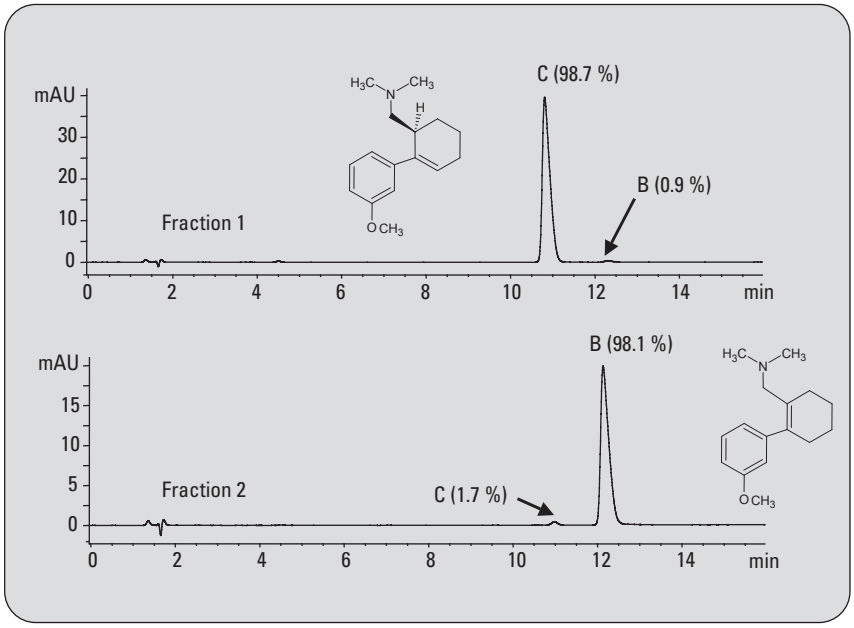

Figure 7
Identified impurities in the pharmaceutical drug synthesis of the main compound.

Conclusion

In this Application Note the isolation and purification of two impurities was illustrated, beginning with a high-resolution analytical method that was developed on an Agilent 1200 Series RRLC system. The transfer to a standard Agilent 1200 Series and to an Agilent 1200 Series preparative system was seamless because the stationary phase was available with sub-2 µm particles in analytical column size as well as in standard 5-µm particles in preparative column sizes. Therefore, the scale-up after the method optimization and loading experiments could be done directly and without any further method optimization on the preparative column. ChemStation features like the *Fraction Preview* and the automated pooling of fractions made it simple to achieve optimal purification results in a short time. The excellent purity and recovery of collected fractions made the structure elucidation of the impurities an easy task.

References

1.
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2.
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3.
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