Purification of a combinatorial chemistry library using the Agilent 1100 Series purification system

Application

Hiroki Kumagai
Udo Huber

Introduction

Today, combinatorial chemistry is often used in drug discovery to synthesize large numbers of compounds for high throughput screening. Since combinatorial chemistry is usually done with solid-phase synthesis the number and amount of byproducts is low compared to liquid phase synthesis. Due to the relatively high purity of a combinatorial chemistry library the fractionation of samples can be done by fraction collection based on peaks using an UV-visible detector such as the Agilent 1100 Series diode array detector (DAD). Since the structures and therefore the molecular masses of the library compounds are known from the library design, it is also possible to collect fractions based on masses using an Agilent 1100 Series mass selective detector (MSD).

In this Application Note we describe the peak-based purification of 15 samples, which model a part of a combinatorial chemistry library, using the Agilent 1100 Series purification system[1]. The samples were purified using a generic method with a gradient from 5 to 95 % organic phase in relatively short time. The re-analysis of the fractions was done on an analytical HPLC system to check the collected fractions for purity of the desired compounds.

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Equipment

The system included:

- two Agilent 1100 Series preparative pumps,
- an Agilent 1100 Series diode array detector,
- an Agilent 220 micro plate sampler, and
- Agilent ChemStation (rev. A.08.04) and micro plate sampling software (rev. A.03.02) for system control.

Results and Discussion

Peak-based fraction collection

Due to the way a combinatorial chemistry library is synthesized the number and amount of byproducts is usually low. Therefore, the model samples contained one major compound which had to be isolated and up to two byproducts which were of no interest. The goal was to separate the major compound with sufficient purity. To make sure all compounds elute from the column within a certain time a generic method was set up using a gradient from 5 to 95 % acetonitrile in eight minutes. The overlaid chromatograms for a few samples using this method are shown in figure 1.

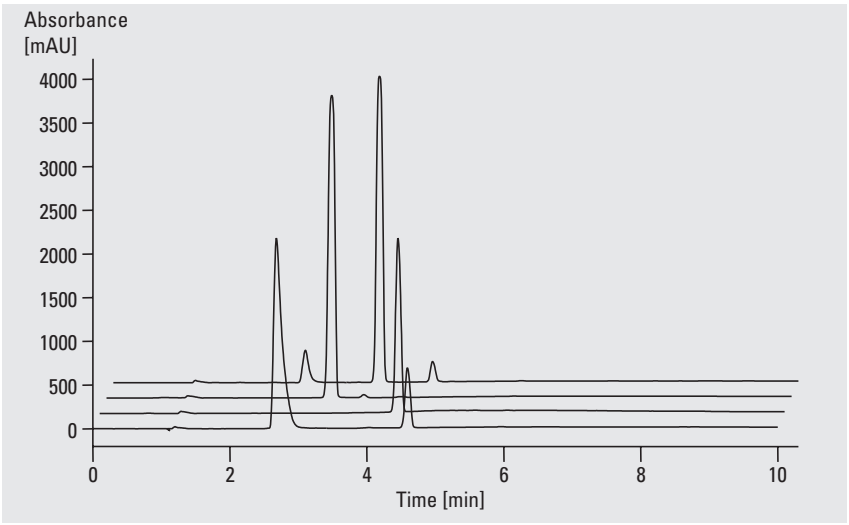

Figure 1
Chromatograms of samples from combinatorial chemistry library

Column:	Zorbax SB-C18 9.4 x 150mm, 5 µm
Mobile Phases:	water = A, acetonitrile = B
Gradient:	5 % B to 95 % B in 8 min 95 % B for 1 min 95 % B to 5 % B in 1 min
Stop time:	10 min
Post time:	7.5 min
Flow:	6 ml/min
Injection:	50 µl
Column temp.:	ambient
UV detector:	DAD 204 nm/8, (ref. off) preparative flow cell (3 mm)

To purify the samples, fractions were collected based on peaks in the UV-visible detector. Two example purification runs are shown in figure 2. The following peak detection criteria were set for the UV-visible detector:

Threshold:	150 mAU
Peak slope:	30 mAU/min
Max. fraction duration:	0.5 min

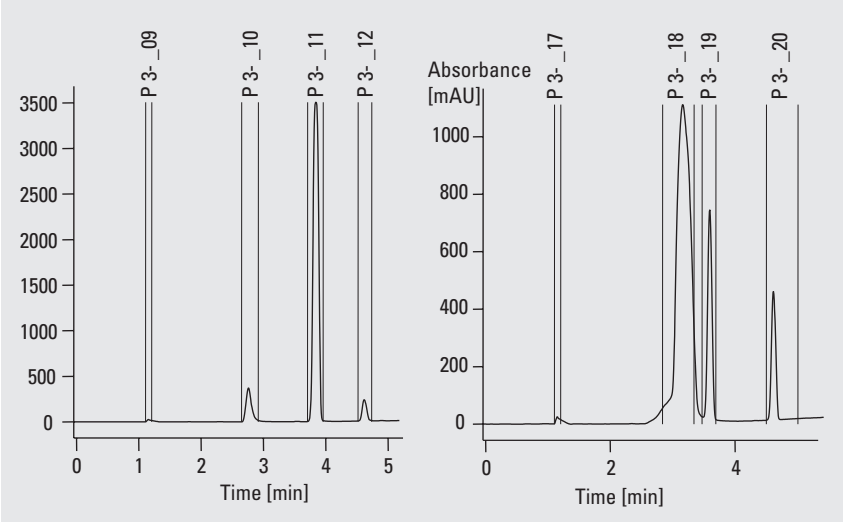

Figure 2
Examples of purification runs

Fraction re-analysis

To check if the main compound was collected with sufficient purity the collected fractions were analyzed on an analytical Agilent 1100 Series HPLC system. Some results are shown in figure 3.

Conclusion

In this Application Note we demonstrated the purification of compounds from a combinatorial chemistry library using a model scenario. Peak-based fraction collection was demonstrated and the fractions were re-analyzed to show the good performance of the fractionation. Due to the characteristics of a combinatorial chemistry library, known chemical structures and masses of the library compounds, mass-based fraction collection would also have been possible. Both types of fraction collection can be performed with the Agilent 1100 Series purification system. It is therefore an ideal tool, not only for the purification of a combinatorial chemistry library, but also for other purification needs in the pharmaceutical industry.

References

1.
"Optimized solutions for sample purification from µg to gram quantities", Agilent Technologies Brochure, **2000**, publication number 5980-2808EN

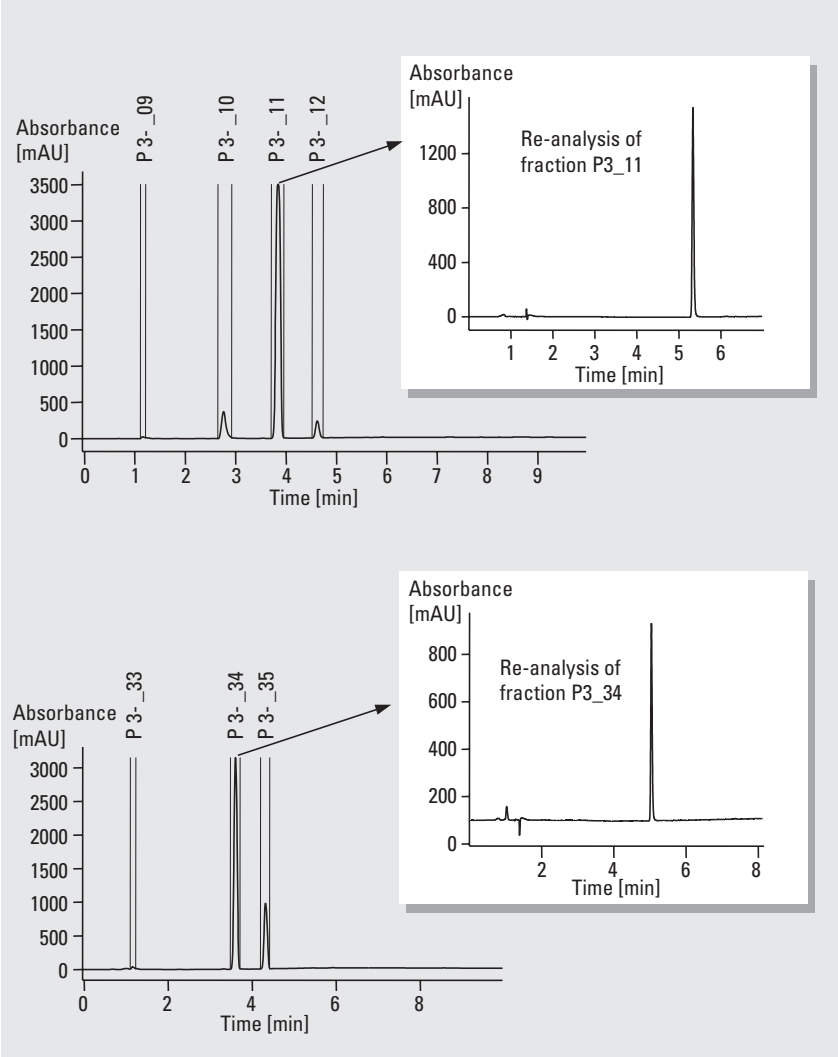

Figure 3
Fraction re-analysis for main compound

Hiroki Kumagai is application chemist at Agilent/Yokogawa Analytical Systems, Tokyo, Japan. Udo Huber is application chemist at Agilent Technologies, Waldbronn, Germany.

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