Isolation and purification of reaction byproducts using the Agilent 1100 Series purification system

Application

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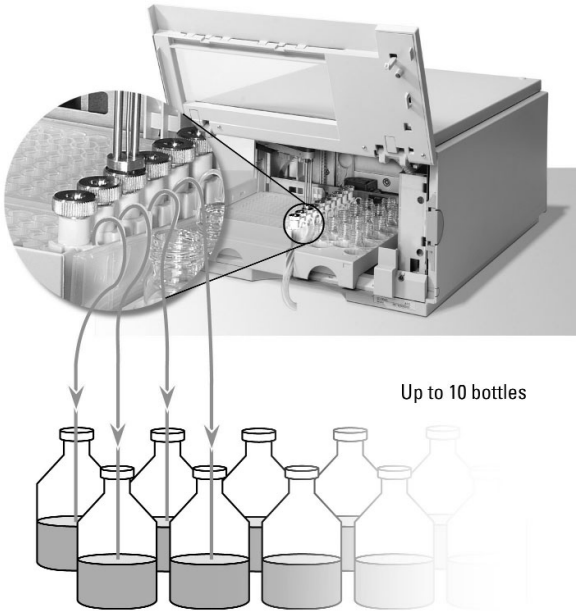

Abstract

Isolating reaction byproducts is a frequent and laborious task in the pharmaceutical industry. The Agilent 1100 Series purification systems AS (analytical scale) and PS (preparative scale)[1] can serve as excellent tools to automate purification of such compounds. In this Application Note we demonstrate that large-scale pooling can be elegantly facilitated by incorporating the funnel tray into the Agilent 1100 Series fraction collector AS.

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Introduction

Isolating low amounts of impurities from a sample is an important task in the pharmaceutical industry. These compounds have to be identified and kept as reference standards for production scale synthesis. Since column overloading is restricted by the major component of the sample, the minor component has to be collected from multiple injections of the same sample – a process usually referred to as "pooling".
In this Application Note we demonstrate how such a minor degradation product can be isolated successfully from a main product. A funnel tray, integrated into the Agilent 1100 Series fraction collector AS together with two Agilent 1100 Series preparative pumps, provides a reliable platform for pooling reaction byproducts in the mg-scale. The funnel tray overcomes the volume limitation of standard vials and allows fraction collection in almost any kind of vessel. Additionally, our solution demonstrates the high degree of flexibility the Agilent 1100 Series purification system offers. The fraction collector AS as well as other parts of the analytical scale purification system can be seamlessly integrated into the Agilent 1100 Series system.

Equipment

All experiments were performed on a preparative scale system using the following modules:

- Two Agilent 1100 Series preparative pumps
- Agilent 1100 Series preparative autosampler
- Agilent 1100 Series column organizer
- Agilent 1100 Series diode array detector
- Agilent 1100 Series fraction collector AS (firmware A.05.02)

The system was controlled using the Agilent ChemStation (rev. A.09.01) and the purfication/high thruput software (rev. A.01.01).

Results and Discussion

Sample Preparation

Famotidine is an inhibitor of histamine H2 receptors. Its primary, clinically-important pharmacologic activity is to inhibit gastric secretion. The occurrence of a byproduct during the industrial production process of famotidine is not unusual and has to be treated as outlined above. In order to simulate the formation of such a byproduct, degradation was initiated by adding an aliquot of 0.1 M HCl to a 2 mg/mL-famotidine solution. After about an hour the degradation process was stopped by neutralization with an aliqout of 0.1 M NaOH.

Analytical method development and scaling up

An analytical scale method was developed to separate the major and minor products. The chromatogram in figure 1 shows famotidine and its degradation product.

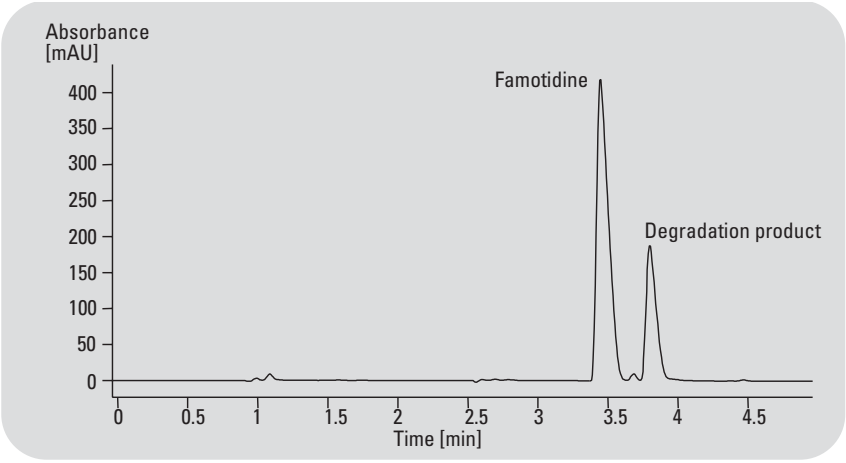

Figure 1
Analytical scale chromatogram of famotidine and its degradation product

Columns	Zorbax SB-C18 4.6 x 150 mm, 5 µm
Mobile phases:	A= water B= acetonitrile
Gradient:	0 % B to 20 % B in 4 min 20 % B to 100 % B in 0.1 min 100 % B for 0.9 min
Stop time:	5 min
Post time:	5 min
Flow:	1.4 mL/min
Injection:	20 µl
Column temp.;	40 °C
UV detector:	DAD: 254/4 nm (ref. 360/100 nm), standard flow cell (10 mm path length)

Volume overloading in preparative scale and fraction pooling

The analytical scale method was transferred to preparative scale for large scale isolation of the byproduct. After overloading the preparative column, the famotidine peak and the peak of the degradation product were collected from 40 consecutive runs. To facilitate collection of such large volumes, a funnel tray was integrated into the fraction collector AS, instead of using the standard vials. This funnel tray consists of a line of 10 funnels that serve as injector ports for the outlet needle of the fraction collector. Each of the funnels is connected to tubing, routing the collected fractions into the desired vessels (see schematic on front page). Figure 2 shows a chromatogram using the preparative column. The vertical lines mark start and end-points of fraction collection. The horizontal line gives the threshold value for fraction collection.

Figure 3 demonstrates the high reproducibility of the 40 consecutive chromatographic runs. Reliability of the system is prerequisite for pooling fractions over a longer period. Such reliable conditions are offered by the excellent pump performance of the Agilent 1100 Series preparative pumps, as well as by a highly homogeneous column packing and a sophisticated software package.

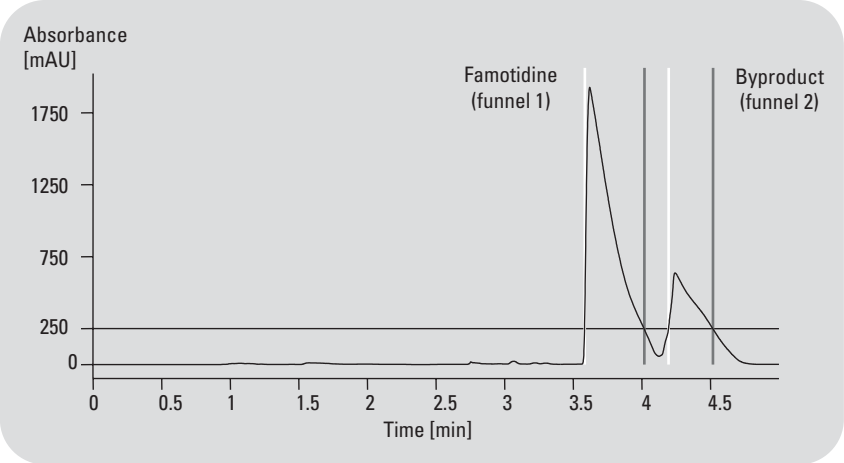

Figure 2
Preparative scale chromatogram

Columns	Zorbax SB-C18 9.4 x 150 mm, 5 µm
Mobile phases:	A= water B= acetonitrile
Gradient:	0 % B to 20 % B in 4 min 20 % B to 100 % B in 0.1 min 100 % B for 0.9 min
Stop time:	5 min
Post time:	5 min
Flow:	6.0 mL/min
Injection:	900 µl
Column temp.:	ambient
UV detector:	DAD: 254/4 nm (ref. 360/100 nm), standard flow cell (10 mm pathlength)
Fraction coll.:	peak-based, threshold only (250 mAU)

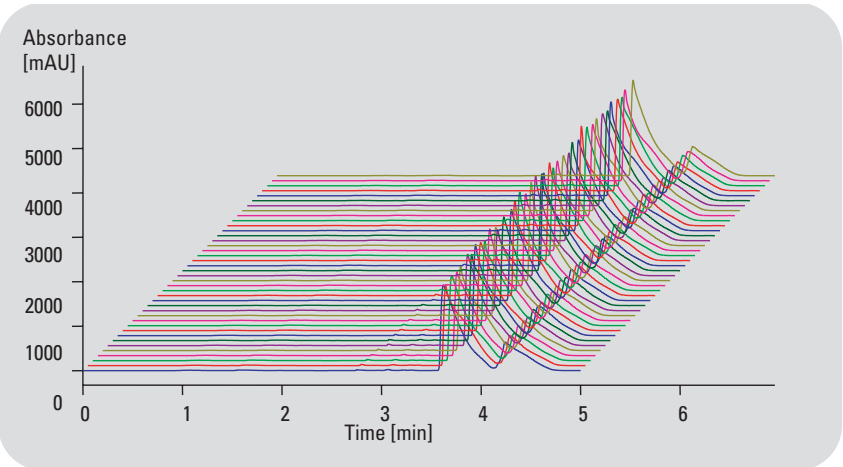

Figure 3
Chromatograms from 40 consecutive preparative scale runs

Reanalysis and Reproducibility

To assure purity of both collected fractions, each fraction was reanalyzed in analytical scale after 10, 20, 30 and 40 runs, applying the method described earlier. Figure 4 shows that both fractions are highly consistent during purification, which again demonstrates the reliability of the system.

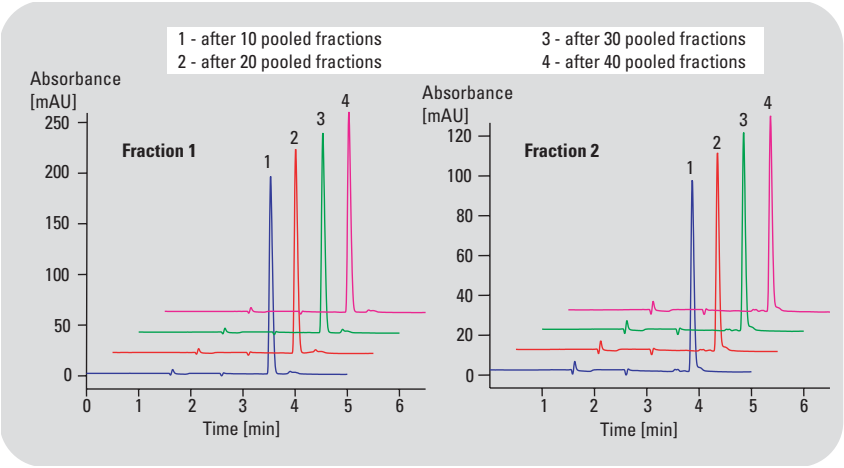

Figure 4
Fraction 1 and fraction 2 after 10, 20, 30 and 40 chromatographic runs

Conclusion

The Agilent 1100 Series purification system together with the funnel tray provides an elegant solution for pooling high-volume fractions after multiple injections of the same sample. The Agilent 1100 preparative pumps feature excellent performance up to 100 mL/min leading to highly reproducible running conditions. Such a platform is the right choice for the reliable purification of sample volumes that exceed the capacity of standard vials. Furthermore, this Application Note points out the versatility and flexibility of the Agilent 1100 Series AS modules, showing how PS components can be added to accomplish additional tasks.

References

1.
"New perspectives in purification with HPLC and HPLC/MS", *Agilent Technolgies Brochure*, **2001**, publication number 5988-3673EN

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