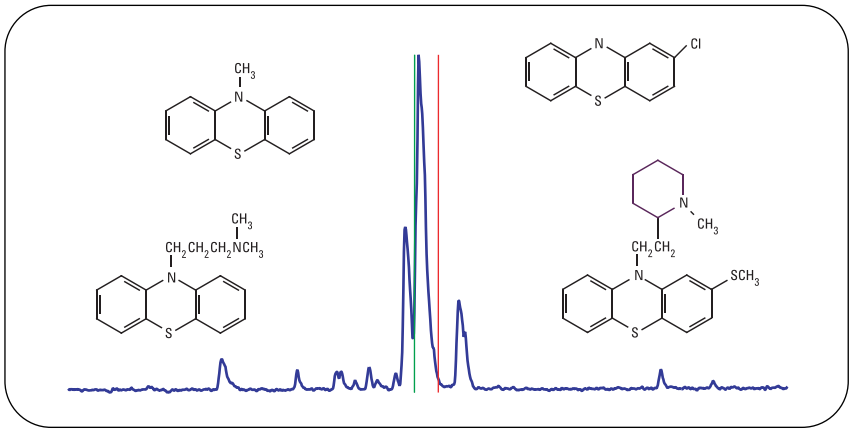
Mass-based fraction collection of compound libraries using the Agilent 1100 Series purification system

Application

Udo Huber



Abstract

Discovery of biologically active compounds is still an important empirical process in the pharmaceutical industry. Compound libraries generated by combinatorial chemistry offer a wide variety of related structures that can subsequently be screened for their capability as potential drug candidates. As combinatorial chemistry produces a wide range of products in parallel an efficient purification system is indispensable. The Agilent 1100 Series purification system keeps pace with high-speed synthesis in the pharmaceutical industry. Fully automated mass-based fraction collection controlled by an elaborated software package facilitates fast purification of target compounds from impurities and by-products. In this Application Note we show that mass-based fraction collection with the Agilent 1100 Series purification platform is an excellent one-vendor solution for time- and resource-effective purification of compound libraries.

Agilent Technologies

Introduction

Today, proteomics plays a key role in the continuos identification of new pharmacological targets. Once a target has been discovered only extensive compound libraries that are produced by combinatorial chemistry can cover the highly increasing demand for drugs. Such libraries consist of structural analogues that need to be screened for their biological activity. However, although combinatorial chemistry simplifies the synthesis process in comparison to conventional synthesis chemistry, compounds still have to be purified from impurities and reaction by-products. Since drug discovery in a high-throughput manner is of general interest, compound purification should not represent the bottleneck in the discovery process. In this Application Note we demonstrate how the Agilent 1100 Series purification platform can be efficiently applied for fast purification of a compound library by triggering fraction collection at specific masses.

Equipment

The Agilent 1100 Series purification platform used for mass-based fraction collection comprised the modules listed below. Figure 1 shows the instrumental set-up.
Analytical scale system:

- Agilent 1100 Series binary pump
- Agilent 1100 Series isocratic pump
- Agilent 1100 Series autosampler
- Agilent 1100 Series thermostatted column compartment
- Agilent 1100 Series diode-array detector
- Agilent active splitter
- Agilent 1100 Series high performance LC/MSD SL with API-electrospray source

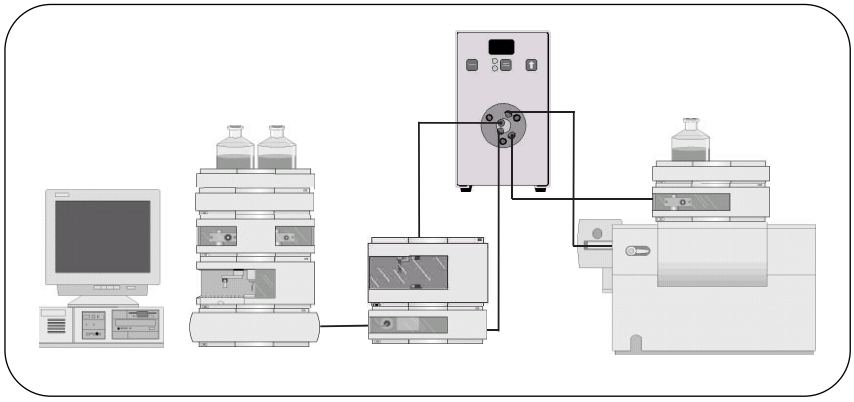


Figure 1
Instrumental set-up of the Agilent 1100 Series purification system for mass-based fraction collection.

- Agilent 1100 Series fraction collector AS

The system was controlled using the Agilent ChemStation (rev. A.09.01) and the Purification/HighThruput software (rev. A.01.01) with a license for mass-based fraction collection add-on software.

The instrumental set-up comprises two flow paths. The main flow leads from the binary pump to the autosampler, the thermostatted column compartment, the diode-array detector and the active splitter before reaching the fraction collector. Since the mass selective detector (MSD) is a destructive detector and the flow rate of the main flow is too high to route it directly into the electrospray source, a make-up flow is sustained by the isocratic pump. This make-up flow leads from the isocratic pump to the active splitter before reaching the MSD. In order to facilitate mass detection the active splitter transports an aliquot of the main flow into the make-up flow which carries it into the MSD. For further information about the active splitter, refer to literature reference[1].

In order to facilitate proper peak collection the delay times between the detectors (sources of peak trigger) and fraction collector have to be determined. This is conveniently done by the unique delay volume calibration feature of the Agilent 1100 Series fraction collectors[2]. The delay time between MSD and fraction collector depends on the adjusted flow rates of the main flow and make-up flow.
On this account the delay volume calibration has to be repeated whenever one of these flows is changed.

Results and Discussion

Sample Preparation

To demonstrate mass-based fraction collection with the Agilent 1100 Series purification platform we generated a library comprising 17 related compounds that are all derivatives of phenothiazine (figure 2). All compounds

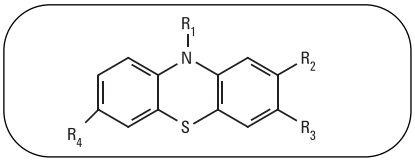


Figure 2
Structure of phenothiazine derivatives.

used are listed in table 1. R1 to R4 refer to the substituents attached to phenotiazine (vial 3, R1 to R4 = H). Fraction collection was triggered on the exact masses of the compounds as given in the last column of the table. All crude compounds were dissolved in AcN/H_2O (50 vol %, 1 mg/mL) solution.

Vial	Name	R1	R2	R3	R4	Exact mass
1	2-Chlorophenothiazine	H	Cl	H	H	233.01
2	Methotrimeprazine	$C_6H_{14}N$	CH_3O	H	H	328.16
3	Phenothiazine	H	H	H	H	199.05
4	Promethazine	$C_5H_{12}N$	H	H	H	284.13
5	Thionin	H	H	NH_2	NH_2	403.15
6	Perphenazine	$C_9H_{19}N_2O$	Cl	H	H	403.15
7	Triflupromazine	$C_5H_{12}N$	CF_3	H	H	407.16
8	Chlorpromazine	$C_5H_{12}N$	Cl	H	H	318.10
9	2-(Trifluoromethyl)phenothiazine)	H	CF_3	H	H	267.03
10	Fluphenazine	$C_9H_{19}N_2O$	H	H	H	437.17
11	Trifluoperazine	$C_8H_{17}N_2$	CF_3	H	H	407.16
12	Acetopromazine	H	CF_3	H	H	326.15
13	Ethopropazine	$C_7H_{16}N$	H	H	H	312.17
14	2-Acetylphenothiazine	H	CCH_3O	H	H	241.06
15	10-Methylphenothiazine	H	CH_3	H	H	213.06
16	Thioridazine	$C_8H_{16}N$	CH_3S	H	H	370.15
17	Promazine	$C_5H_{12}N$	H	H	H	284.13

Table 1
Assignment of phenothiazine derivatives.

Mass-based fraction collection

In the Purification/HighThruput software for each of the 17 mixtures the target mass for fraction collection was set-up as listed in table 1. Fraction collection was triggered on the singly charged positive ion applying the chromatographic method shown on the next page. Figure 3 displays the results of the purification procedure as shown by the Purification/HighThruput software.

The left panel visualizes the positions of the vials in the autosampler and the right panel all collected fractions. Clicking on one of the sample positions in the left panel highlights the corresponding fractions in the right panel and vice versa. In this example all 17 target compounds could be collected – each in one fraction. In contrast to fraction triggering with a less specific detector such as a UV detector, fraction collection can be triggered on a specific mass with a MSD. Consequently only one fraction containing the target compound is collected in each run and no redundant fractions need to be sorted out which saves time and resources. The corresponding chromatograms to all of the collected fractions in the right panel can easily be displayed and

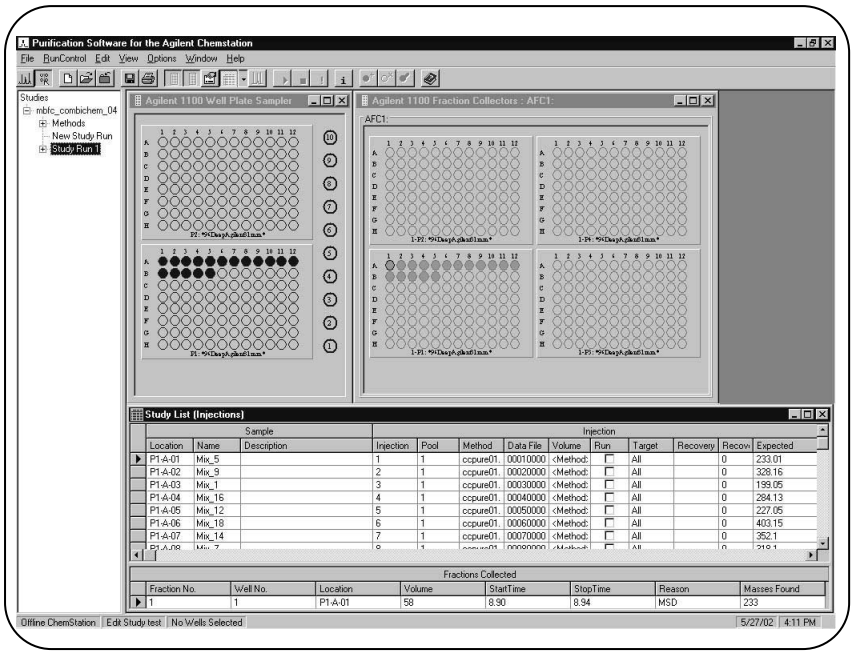


Figure 3
Screenshot of Purification/HighThruput software. The left panel shows the samples in the Agilent 1100 Series autosampler. The right panel displays the corresponding fractions for each sample. Additional information is given in the study list below.

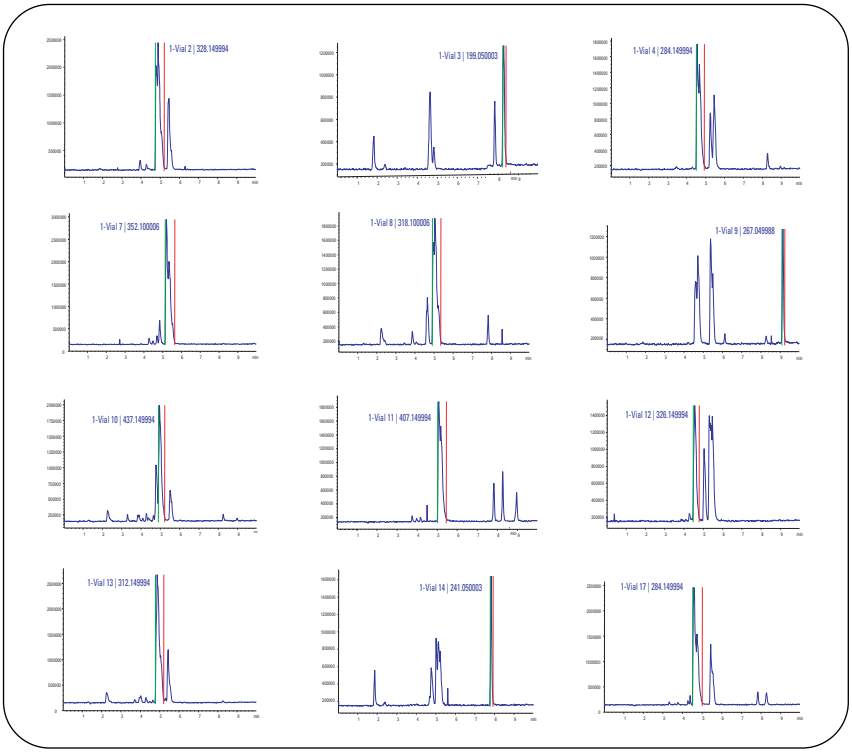


Figure 4
12 selected TIC chromatograms. The displayed tick marks show beginning and end of fraction collection.

evaluated in the ChemStation. Additionally to the chromatograms tick marks are displayed that visualize beginning and end of fraction collection. Supplementary, the target masses and fraction positions are indicated.
Figure 4 shows 12 selected total ion current (TIC) chromatograms of the purification process.

Conclusion

In this Application Note we demonstrated a solution for automated mass-based purification of a compound library with the Agilent 1100 Series purification platform. Fraction collection triggered by predefined masses is advantageous over conventional, less specific detectors. When applying this technique in each run, only the compound of interest is collected. It is not necessary to pick target compounds out of a series of fractions collected during chromatographic runs. Furthermore, Agilent's patented fraction collection delay calibration[3] ensures a reliable sample recovery. Altogether, the Agilent 1100 Series offers a time-and resource-efficient purification platform for mass-based fraction collection.

References

1. "Agilent 1100 Series Purification Platform" *Agilent Brochure* **(2001)**, publication number 5988-3673EN

2. "Agilent 1100 Series User Guide" **(2001)**, Agilent part number G2262-90001

3. Patent US6106710 A1

Chromatographic conditions

Column:	ZORBAX Eclipse XDB-C8, 4.6 x 150 mm, 5 µm
Mobile phases:	A = H_2O, 0.1 % formic acid B = ACN, 0.1 % formic acid
Gradient:	20 % B to 100 % B in 8 min 100 % B for 2 min
Stop time:	10 min
Post time:	5 min
Main flow:	1 mL/min
Make-up flow:	0.2 mL/min (75 % H_2O, 25 % ACN, 0.1 % formic acid)
Injection:	20 µL
Column temp.:	25 °C
UV detector:	DAD 254/16 nm (ref. 360/100 nm), standard flow cell (10 mm)
Active splitter:	split-ratio 100:1 (1.667 Hz, 100 nL)

MS conditions

Ionization mode:	API-ES positive
Nebulizer pressure:	20 psig
Drying gas temperature:	350 °C
Drying gas flow:	10 L/min
V_{cap}:	3000 V
Fragmentor:	70 V
Scan range m/z:	120 – 500

Udo Huber is Senior Application Chemist at Agilent Technologies Waldbronn, Germany.

www.agilent.com/chem/purification

Published September 1, 2004
Publication Number 5989-1656EN

Agilent Technologies