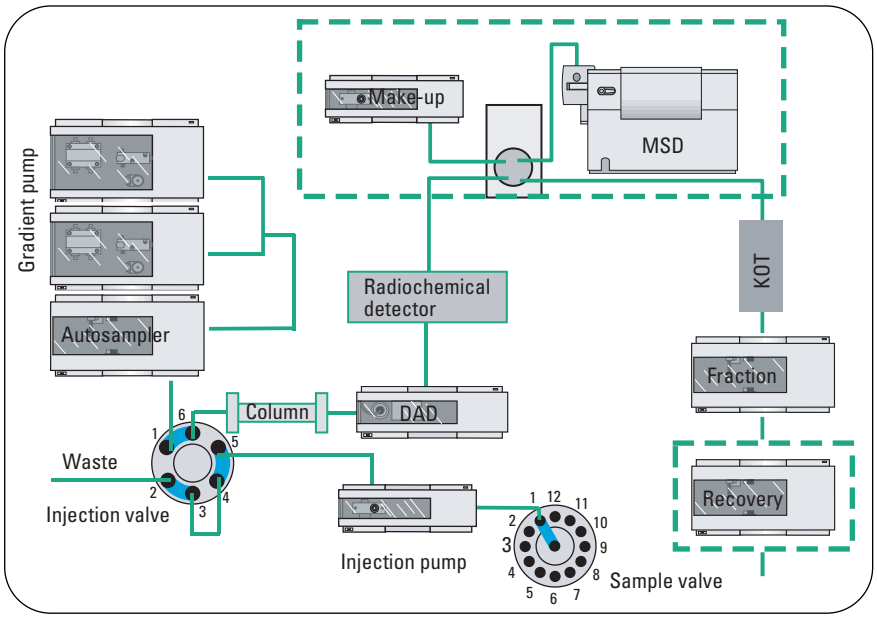
Isolation and purification of radiolabeled drug metabolites from pre-clinical urine samples

Application Note

Gordon Dear
Udo Huber



Abstract

This Application Note describes the configuration and set up of a purification system for the isolation of radiolabeled drug metabolites from biological samples applied by an injection pump system. Various fraction collection options, for example, time-based fraction collection monitored by a radiochemical detector or peak-based fraction collection on the radiochemical detector signal are described.

Agilent Technologies

Introduction

The identification of metabolites of novel pharmaceutical drug candidates is a fundamental part of the drug development process. It plays an important role in early drug discovery lead optimization, leading to drug candidates with more favorable pharmacokinetic and disposition characteristics. Later in drug development the identification of drug metabolites, in test animals and later in humans, is required by the regulatory authorities in support of safety testing. During drug development drug metabolism studies are usually conducted with the radiolabeled drug candidate and therefore the identification of drug-related metabolites is facilitated using radiochemical detection. Metabolites can often be present at low concentrations in very complex matrices, such as urine, bile and plasma, making it necessary to isolate and purify metabolites for unequivocal identification, by techniques such as nuclear magnetic resonance (NMR) spectroscopy. In this Application Note a configuration of the Agilent 1100 Series purification system[1] equipped with an injection pump system for the application of large sample volumes is described. Fractions are collected either by time-based fraction collection or by peak-based fraction collection on the signal from a radiochemical detector (RD). The monitoring of fraction collection using a mass-selective detector (MSD) is also described.

Equipment

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

- 2 Agilent 1100 Series preparative pumps
- Agilent 1100 Series dual-loop autosampler PS
- Agilent 1100 Series column organizer
- Agilent 1100 Series diode-array detector (flow cell: 3 mm path length)
- 2 Agilent 1100 Series fraction collectors PS
- Agilent 1100 Series isocratic pump as the injection pump
- Agilent 1100 Series 2-position/6-port valve
- Agilent 1100 Series 12-position/13-port valve
- Agilent 1100 Series MSD (G1956A)
- Agilent 1100 Series isocratic pump as the make-up pump
- Active splitter
- Agilent 1100 Series UIB
- HPLC Radioactivity detector LB 507 B (EG&G Berthold)

The system was controlled using the Agilent ChemStation (rev. B.01.01).

Results and discussion

System configuration

The system was configured and set up as shown on the cover page. The MSD, including the active splitter and the make-up pump, and the recovery collector are optional as indicated by the dotted boxes. Due

to the slow response of the RD, delay tubing was required regardless of whether an MSD was configured into the system or not. To minimize the sample dispersion, a knitted open tubular (KOT) reactor from SeQuant was used as delay tubing, which was connected into the flow path between the active splitter and the fraction collector if the MSD was configured into the system. The delay calibrant was spiked with a radiolabeled compound to provide a response from the diode array detector (DAD), RD and MSD for the delay calibration.

System configuration – injection pump

Since the injection volume for the metabolite samples can vary between 1 and 500 mL the system was equipped with an Agilent 1100 Series dual-loop autosampler[2] and an injection pump system[3] as shown in figure 1. The injection valve is switched to guide the flow from the injection pump directly to the column when injecting with the pump. After the complete sample is applied the injection valve switches back to the position shown in figure 1 and the gradient pump starts to deliver the gradient

Column:	ZORBAX SB-C18 9.4 x 150 mm, 5 µm
Mobile phase:	A = water + 50 mM Ammonium acetate B = acetonitrile
Gradient:	at 0 min 1 % B at 10 min 1% B at 52 min 60 % B at 53 min 95 % B at 55 min 95 % B
Flow rate:	4 mL/min
Stop time:	55 min
Post time:	7 min
Inj.vol.:	10 mL
Column temp.:	ambient
DAD:	254 nm /4 (ref. off) Flow cell (3 mm path length)

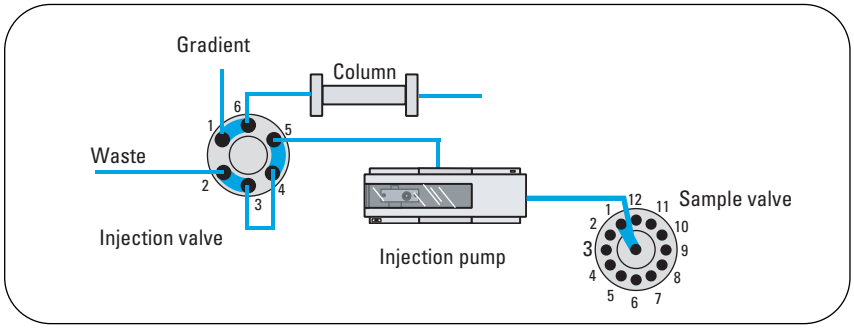


Figure 1
Injection pump system.

to the column. In parallel the injection pump and the valves are rinsed with an appropriate solvent and then re-equilibrated to the gradient starting conditions. This is achieved by connecting containers with the mobile phase, sample, wash solvent and again mobile phase to the sample valve in the order the different solvents are required during the injection cycle. Since water was the sample matrix of the metabolites as well as the mobile phase at starting conditions, water can also be used as the rinse solvent for the injection pump system.

Injection of a non-labeled standard of a GSK drug candidate

To test the chromatography system before the injection of a valuable biological sample an authentic standard of the drug candidate was injected from 10 mL of water (~1 µg/mL) using the injection pump. Using the method below the retention time of the parent compound was 36.3 min as shown in figure 2.

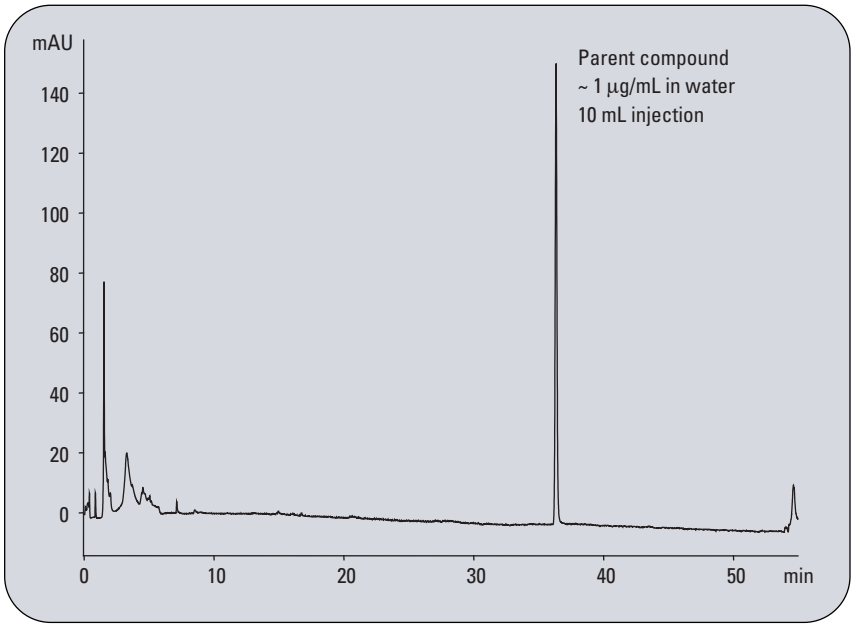


Figure 2
Injection of standard.

Injection of a dog urine sample

Figure 3 shows the RD and DAD trace following the injection of 10 mL dog urine sample containing radiolabeled drug candidate and its metabolites, which can easily be identified in the RD signal. To isolate the metabolites either time-based fraction collection using the RD as monitor or peak-based fraction collection based on the RD signal could be performed.

Time-based fraction collection monitored by RD

In the first purification experiment the sample was fractionated using time-based fraction collection collecting a fraction every 0.4 min into a 96-deepwell-plate (1.8 mL well volume). To minimize the fraction loss the fraction Collection order was set to *Shortest path by row*, which collects fractions in a meandering order. Furthermore the *Collection mode* was set to *Continuous flow*, in which the fraction collection needle moves to the next fraction position without switching the flow to waste in between[4]. Since the fraction tick marks in each signal are aligned using the delay time for each detector the fractions containing the metabolites can be easily identified from the RD signal as shown in figure 4.

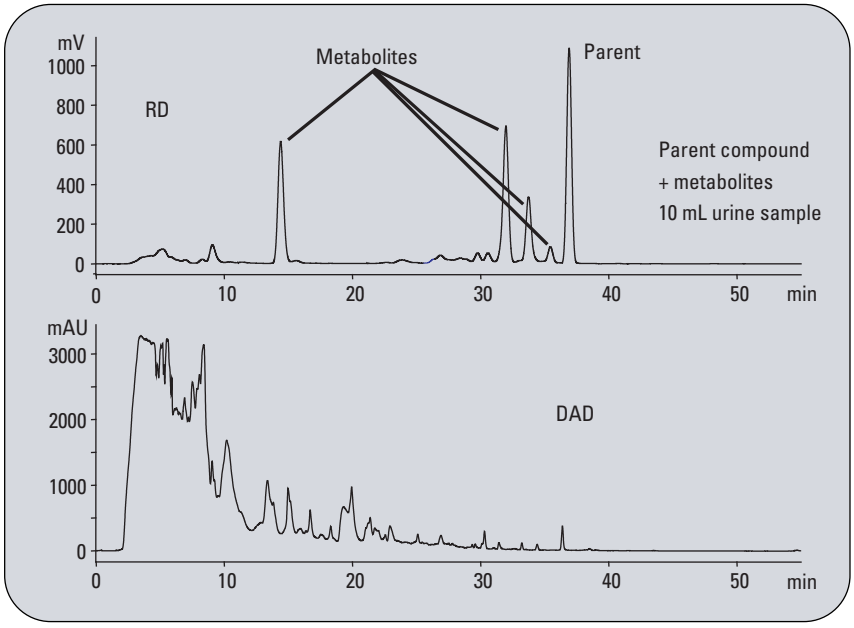


Figure 3
Dog urine sample.

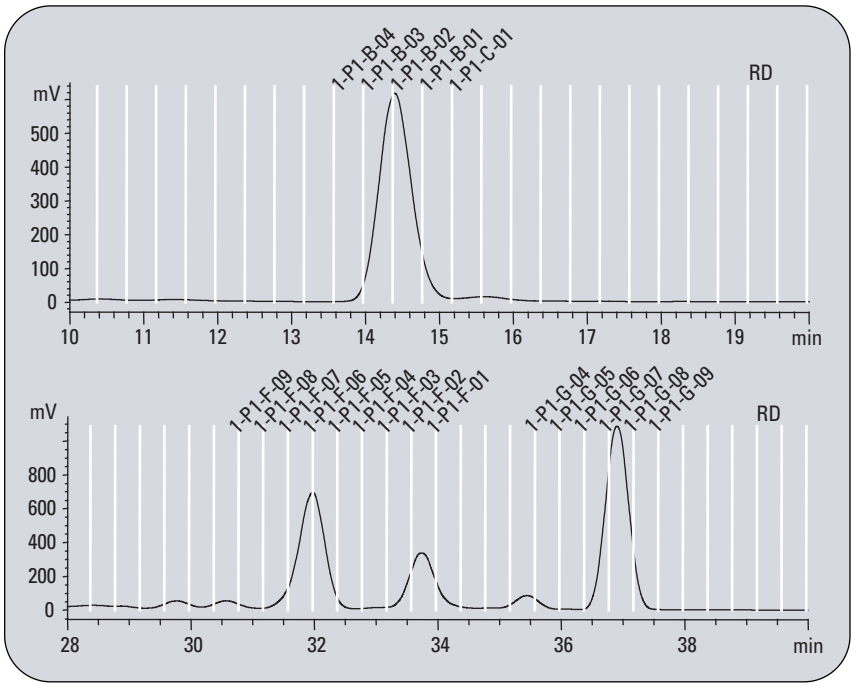


Figure 4
Identification of fractions containing drug metabolites.

Peak-based fraction collection on the RD signal

To be able to trigger on the signal coming from the RD the signal output was connected to the Universal Interface Box (UIB), which was connected to the CAN network of the Agilent 1100 Series modules[5]. To make sure no metabolite was lost even if it fails to trigger the fraction collector a recovery collector[6] was configured into the system. The same well-plates were used in the recovery collector to collect everything that was not collected as a fraction in a time-based manner. The result of the peak-based fraction collection is shown in figure 5.

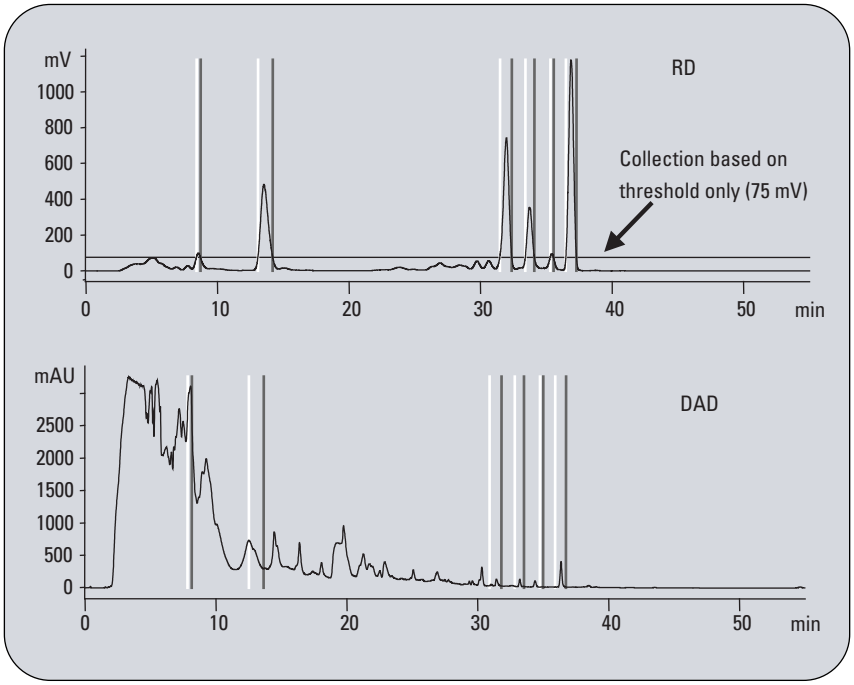


Figure 5
Peak-based fraction collection on the RD signal.

Monitoring fraction collection results with an MSD

If an MSD is configured into the system as shown on the cover page additional information about the collected metabolites can be acquired "in-real" time during the purification run[4]. Since the MSD is only used to monitor the run but not to collect fractions it is important to disconnect the LC/MS fraction collection control cable (between MSD and UIB, G1968-60002). The identification of the parent compound and one metabolite by the MSD is shown in figure 6.

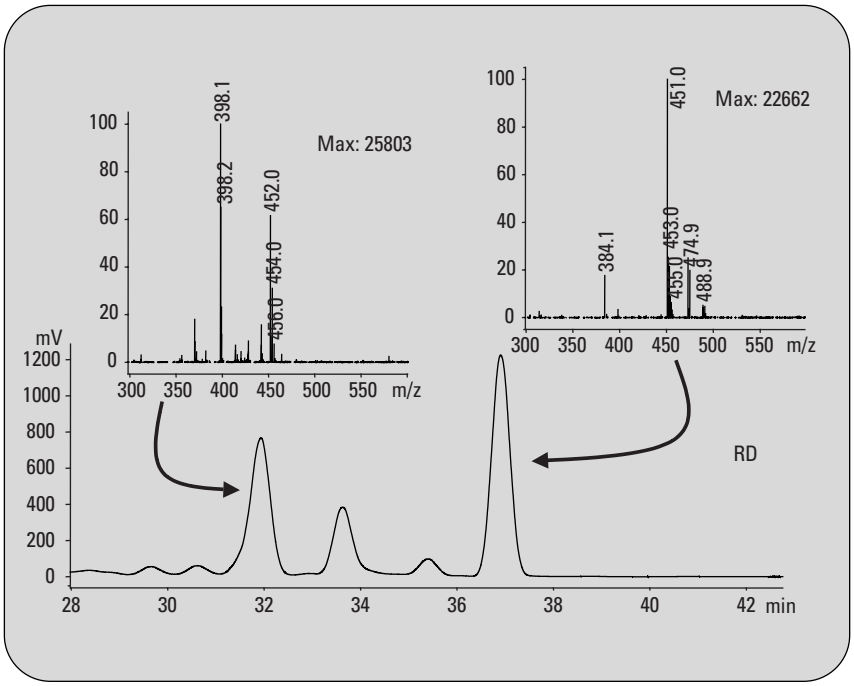


Figure 6
Identification of metabolites by MSD.

Conclusions

In this Application Note the set up and configuration of a purification system optimized for the isolation and purification of radiolabeled drug metabolites from typical DMPK samples, is described. The most important aspects of the configuration are the injection pump system enabling large injection volumes, and the possibility to identify the fractions containing the metabolites using a radiochemical detector. Furthermore, fraction collection strategies were shown using a real-life sample from a metabolism study carried out in a DMPK group at GlaxoSmithKline, Ware, UK.

References

1.
"Agilent 1100 Series purification system"; *Agilent Technologies Brochure, publication number 5989-1255EN*, **2004.**

2.
"The Agilent 1100 Series dual-loop automsampler PS - Optimum performance when injecting large sample volumes"; *Agilent Technologies Technical Overview, publication number 5989-1714EN*; **2004.**

3.
"Injection of large samples volumes using the Agilent 1100 Series purification system with an injection pump"; *Agilent Technologies Application Note, publication number 5989-0029EN*; **2004.**

4.
"Compound purification using the Agilent 1100 Series Purification system with a mass-selective detector"; *Agilent Technologies Application Note, publication number 5988-7830EN*; **2002.**

5.
"Peak-based fraction collection using an evaporative light scattering detector with the Agilent 1100 Series purification system"; *Agilent Technologies Application Note, publication number 5988-5816EN*; **2002.**

6.
"Recovery collection with the Agilent 1100 Series purification system"; *Agilent Technologies Application Note, publication number 5988-9650EN*; **2003.**

Gordon Dear is a Principal Investigator at DMPK, GlaxoSmithKline, Ware, UK
Udo Huber is a Senior Application Chemist at Agilent Technologies, Waldbronn, Germany.

www.agilent.com/chem

Published December 1, 2004
Publication Number 5989-2088EN

Agilent Technologies