Evaluation of a New High-Capacity, High-Throughput, 96-Well Fraction Collector for Biotransformation Profiling

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Abstract

Microplate scintillation counters are utilized more and more commonly for the generation of metabolite radioprofiles from biological samples as well as in the isolation and purification of metabolites. The current technology is limited by the number of fractions that can be collected per injection as well as the potential for loss of sample as the fraction collector head moves from well to well. In addition, collection plates must be manually exchanged between each injection. A new automated fraction collector is being co-developed with LEAP Technologies to address these issues. Operation of the system is completely controlled with software, and up to 24 (96-well) fraction collection plates can be loaded in a completely automated run. The fraction collector employs a zero-loss design and has sub-ambient temperature control. The precision of the system to deliver a specific volume per well was determined gravimetrically and by counting the amount of [14C]-radiolabeled mobile phase dispensed into a 96-well plate. The reproducibility of the system for generating a radioprofile from an authentic [14C]buspirone metabolism sample was also evaluated and compared to the current technology. This new and innovative fraction collector should provide a major improvement in capacity and throughput for biotransformation profiling experiments.

Introduction

In the Biotransformation Department at Bristol-Myers Squibb, metabolite profiles of low-level radioactivity samples are generated by collecting fractions of the HPLC eluent into Deepwell Luma-96 or PE Wallac plates and counting the plates on a TopCount® or Microbeta radioactivity counter. In the current process, a conventional fraction collector (Gilson Model FC 204), is utilized to collect the HPLC eluent (at a rate of ~10-15 sec/well). This fraction collector is designed with a tray that can hold a maximum of 4 plates for each HPLC run. The collection can be triggered manually or by contact closure. At the end of each run, the plates must be manually removed from the tray and exchanged with new plates in preparation for the next injection. While producing acceptable results, this process is labor-intensive. In the search for a more efficient system, a group of scientists at Bristol-Myers Squibb collaborated with the engineers at LEAP Technologies to develop the CollectPAL, an automated high-throughput, high-capacity fraction collector (Figure 1). The system is fully controlled by LEAP Shell software (Figure 2) and consists of a zero-loss collection head and 12 drawers contained in two Peltier stacks, which can accommodate up to 24 plates. A typical system configuration for the analysis of samples from drug metabolism studies is shown in Figure 3. Prior to loading the plates in the Peltier stacks, plates must be covered with TopSeal S film using a heated sealer (MicroMate 496, Perkin Elmer). The HPLC eluent is delivered to the collection plates through a syringe needle which punctures the film cover. After sample collection, the plates can remain in the Peltier stack and be stored at temperatures as low as 4°C to minimize solvent evaporation prior to processing.

Figure 1. Schematic Diagram of the LEAP CollectPAL

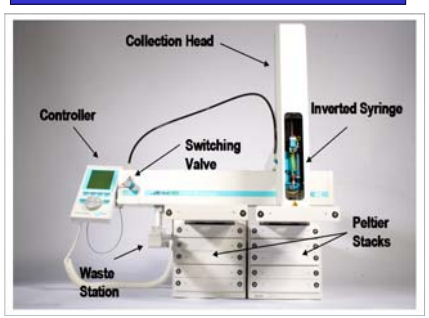


Figure 2. LEAP Shell Software

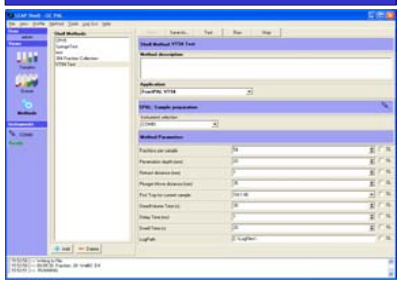
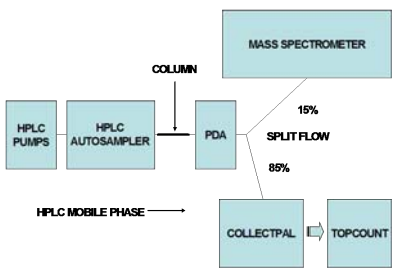


Figure 3. Schematic Typical System Configuration for Analysis of Drug Metabolism Samples



Equipment/Materials

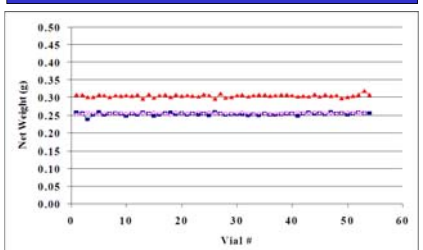
HPLC: Shimadzu Pumps, Model LC-10AD VP
Injector: LEAP Technologies HTC PAL
Plates: DeepWell Luma-96 (Perkin Elmer)
Fraction Collectors: Gilson FC 204, LEAP Technologies CollectPAL
Radioactivity Detection: TopCount (Perkin Elmer)
Column: Agilent Eclipse XDB-C18, 3.5 µ, 4.6x150 mm
Mobile Phases: 50/50 (v/v) acetonitrile/water
A: 10 mM ammonium acetate; B: acetonitrile
Flow rate: 1 mL/min;
Metabolism Sample: [14C]buspirone was incubated in Human Liver Microsomes for 1 h. The resulting incubation mixture was then spiked into blank bile to generate a sample for the evaluation.

Experimental

The reproducibility of the CollectPAL to deliver a constant volume of mobile phase to each fraction was assessed using several typical HPLC solvents (methanol, acetonitrile and a 50/50 (*v/v*) mixture of acetonitrile/water). Each mobile phase was pumped through the system at a rate of 1 mL/min and fractions were collected in 20 s intervals into 54 pre-weighed tubes. The collected fractions were weighed and the results were plotted (Figure 4). The ability of the system to deliver a 50/50 (*v/v*) acetonitrile/water mobile phase and the effect of leaving the plates in the Peltier stack before processing was also evaluated. A radioactive compound was added to the mobile phase which was then pumped at a rate of 1 mL/min. The mobile phase was collected at a collection interval of 12 sec/well into three 96-well plates. One plate was processed immediately; the other 2 plates were processed after 24 or 60 h of storage in the Peltier stack. The results were compared to the current technology (Table 1). To assess the quality and reproducibility of the CollectPAL for generating radioprofiles, a 5-µL aliquot of a metabolism sample was injected into the HPLC. The eluent was collected into DeepWell Luma-96 plates with either the CollectPAL or the current technology. The plates that were used for the CollectPAL were heat-sealed with TopSeal S film before the eluent was collected. Each system was evaluated with triplicate injections (Figures 5 and 6). The plates from all experiments were dried in a Savant SpeedVac (Thermo Fisher Scientific) at 45°C overnight and the radioactivity was determined using TopCount Microplate Scintillation and Luminescence Counter (Perkin-Elmer). The radioprofiles were plotted using Microsoft Excel. The advantages of the CollectPAL over the current technology are summarized in Table 2.

Results

Figure 4. Gravimetric Test for Reproducibility of Delivering Various HPLC Mobile Phases



Solvents	Average	SD	% CV
100% Acetonitrile	0.2532	0.004	1.4 %
100% Methanol	0.2552	0.002	0.6 %
50/50 (v/v) Acetonitrile:water	0.3045	0.004	1.2 %

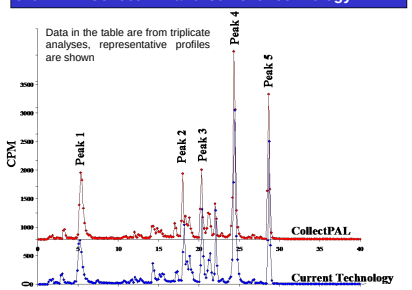
Table 1. Evaluation of Mixed Mobile Phase and Plate Storage

Fraction Collector	Storage Time (h)	CPM/well (n = 96)		
		Average	SD	%CV
CollectPAL	0	443	23	5.3
	24	453	20	4.4
	60	396	28	7.1
	Average	431	24	5.6
Current Technology	0	404	30	7.5
Spiked Plate	0	496	22	4.0

Note: For spiked plates, the same amount of the radioactive solution was manually added into each well.

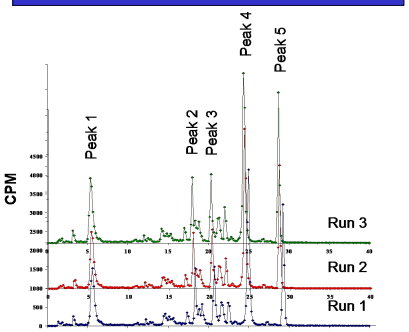
Figure 5. Comparison of Radioprofiles Generated by the LEAP CollectPAL and Current Technology

Data in the table are from triplicate analyses, representative profiles are shown



Peak #	% Sample Radioactivity					
	CollectPAL			Current Technology		
	Average	SD	CV	Average	SD	CV
1	13.44%	0.27%	2.0%	13.89%	2.30%	16.6%
2	6.18%	0.38%	6.2%	7.71%	0.40%	5.1%
3	7.71%	0.35%	4.6%	7.61%	0.21%	2.8%
4	24.10%	0.22%	0.9%	23.32%	0.77%	3.3%
5	14.24%	0.28%	2.0%	14.33%	0.40%	2.8%

Figure 6. Reproducibility of Radioprofiling using the LEAP CollectPAL



Peak #	% Sample Radioactivity					
	Run 1	Run 2	Run 3	Average	SD	CV
1	13.74%	13.38%	13.20%	13.44%	0.27%	2.0%
2	6.61%	5.88%	6.05%	6.18%	0.38%	6.2%
3	8.12%	7.57%	7.46%	7.71%	0.35%	4.6%
4	24.03%	24.35%	23.94%	24.10%	0.22%	0.9%
5	13.98%	14.20%	14.54%	14.24%	0.28%	2.0%

Table 2. Advantages of the LEAP CollectPAL Over Current Technology

	Current Technology	CollectPAL
Sample capacity	4 plates	24 plates
Injection handling capacity	1 injection	Multiple injections
Temperature control	No	Yes
Collection control	Manual	Programmable
Sample Collection	Open Plates	Sealed Plates

Summary and Conclusions

Results from gravimetric and radioactivity counting experiments demonstrated that the CollectPAL was capable of delivering a constant volume of mobile phase to each fraction. The delivery was consistent (within 7.1% CV) regardless of mobile phase composition, and was comparable to the current technology.
The chromatographic resolution of peaks in a metabolism sample was comparable between the CollectPAL system and the current technology. The between-run reproducibility of radioprofiles generated with the CollectPAL system was excellent (the variation in the % Sample Radioactivity was within 6.2% CV).
The CollectPAL can be fully automated to collect fractions in up to 24 plates from multiple injections without manual intervention. In addition, there was no significant impact on radioactivity determinations when plates were stored in the Peltier stack for up to 60 h prior to processing. Therefore, it can be run while unattended overnight or over the weekend to improve sample throughput and overall productivity.
An additional benefit of the CollectPAL process, which includes collection of the HPLC eluent into sealed plates and storage of the plates at sub-ambient temperature, is reduced exposure of scientists to volatile solvents or potent compounds.

References

[1]Zhu M, Zhao W, Vazquez N and Mitroka JG (2005) Analysis of low level radioactive metabolites in biological fluids using high-performance liquid chromatography with microplate scintillation counting: method validation and application. *J Pharm Biomed Anal.* **39**:233-245.