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Development and evaluation of a multiple-plate fraction collector for sample processing: Application to radioprofiling in drug metabolism studies

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ABSTRACT

Microplate scintillation counters are utilized routinely in drug metabolism laboratories for the off-line radioanalysis of fractions collected during HPLC radioprofiling. In this process, the current fraction collection technology is limited by the number of plates that can be used per injection as well as the potential for sample loss due to dripping or spraying as the fraction collector head moves from well to well or between plates. More importantly, sample throughput is limited in the conventional process, since the collection plates must be manually exchanged after each injection. The Collect PAL, an innovative multiple-plate fraction collector, was developed to address these deficiencies and improve overall sample throughput. It employs a zero-loss design and has sub-ambient temperature control. Operation of the system is completely controlled with software and up to 24 (96- or 384-well) fraction collection plates can be loaded in a completely automated run. The system may also be configured for collection into various-sized tubes or vials. At flow rates of 0.5 or 1.0 mL/min and at collection times of 10 or 15 s, the system precisely delivered 83-μL fractions (within 4.1% CV) and 250-μL fractions (within 1.4% CV), respectively, of three different mobile phases into 12 mm × 32 mm vials. Similarly, at a flow rate of 1 mL/min and 10 s collection times, the system precisely dispensed mobile phase containing a [^{14}C]-radiolabeled compound across an entire 96-well plate (% CV was within 5.3%). Triplicate analyses of metabolism test samples containing [^{14}C]buspirone and its metabolites, derived from three different matrices (plasma, urine and bile), indicated that the Collect PAL produced radioprofiles that were reproducible and comparable to the current technology; the % CV for 9 selected peaks in the radioprofiles generated with the Collect PAL were within 9.3%. Radioprofiles generated by collecting into 96- and 384-well plates were qualitatively comparable; however, the peak resolution was greater in the profiles that were collected in 384-well plates due to the collection of a larger number of fractions per minute. In conclusion, this new and innovative fraction collector generated radioprofile results that were comparable to current technology and should provide a major improvement in capacity and throughput for radioprofiling studies.

1. Introduction

An evaluation of the metabolic profiles in plasma and other biological matrices from multiple species is an essential component of the development package for each drug candidate [1–3]. Traditionally, definitive metabolism studies are conducted by administering [^{14}C]- or [^{3}H]-radiolabeled drug to pertinent animal species and to humans. Biological matrices including plasma, bile, urine and feces are then collected and analyzed by LC/MS and HPLC with radiometric detection to generate metabolite profiles that provide both qualitative and quantitative data. This data includes but is not limited to the extent and magnitude of the biotransformation of the administered drug, potential metabolic pathways and estimates of any metabolite concentrations. For samples with low-levels of radioactivity, it is a common practice to utilize a post-column fraction collector to partition the HPLC eluate into 96-well Deepwell Luma or PE Wallac plates and count the radioactivity off-line with a TopCount [4] or Microbeta [5] radioactivity counter. Important metabolites identified in these radioprofiling experiments may then be isolated and characterized in further experiments.

Abbreviations: CPM, counts per minute; CPS, counts per second; DPM, disintegrations per minute; HLM, human liver microsomes; MIM, multiple ion monitoring.

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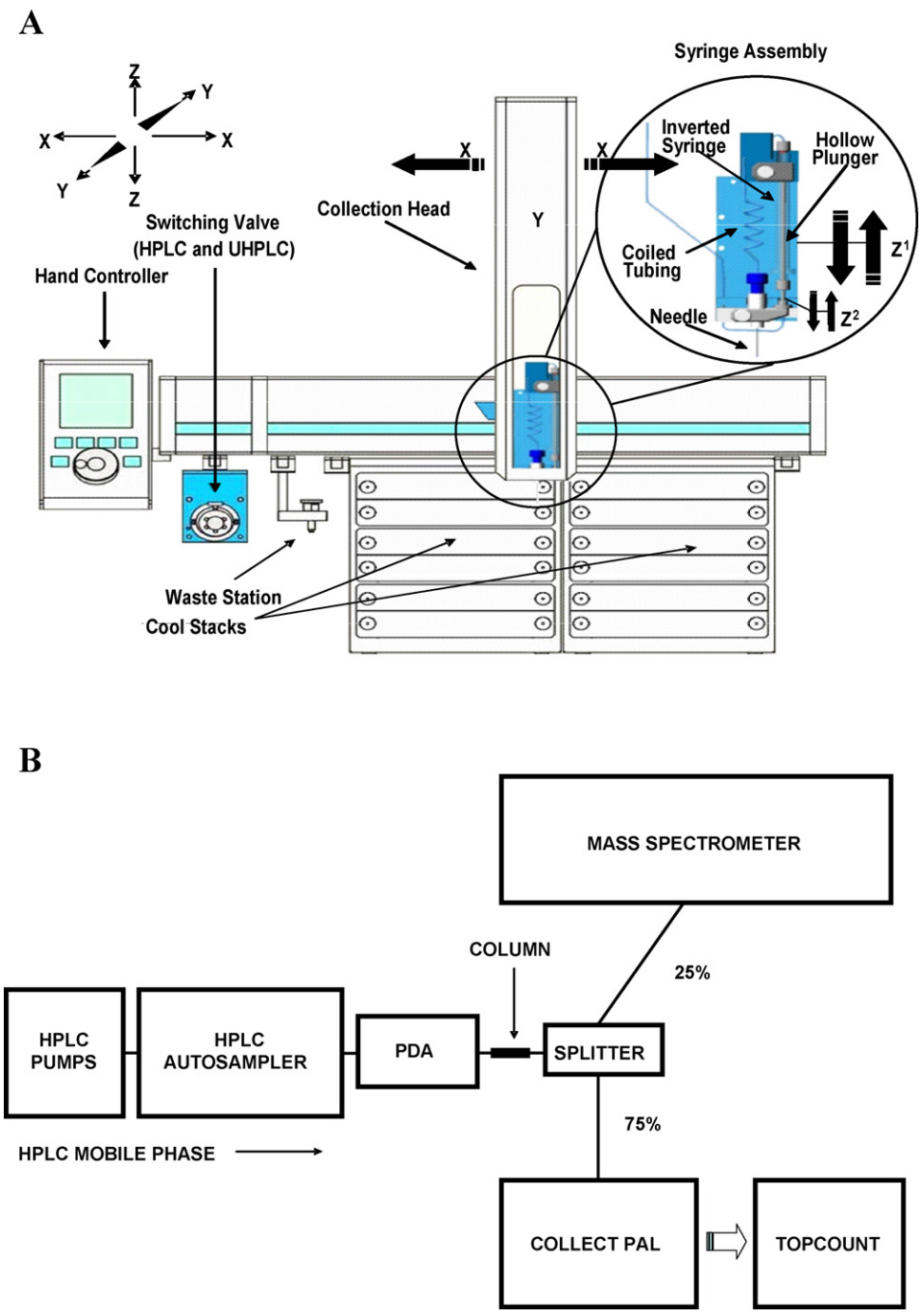

Fig. 1. (A) Schematic diagram of the 24 plate Collect PAL with illustrated *X*, *Y* and *Z* movements of the syringe plunger, syringe assembly and collection head where Z^1 represents the vertical movement of the syringe assembly and Z^2 represents the vertical movement of the syringe plunger. (B) Typical system configuration for HPLC radioprofiling with simultaneous LC/MS/MS analysis. For the analyses conducted in this study, the HPLC eluate was split post-column with 75% of the eluate directed to a fraction collector for radioprofile generation and 25% of the eluate directed to an API 4000 Qtrap mass spectrometer.

Most conventional fraction collectors have a maximum capacity of four 96-well or 384-well plates per HPLC run. They may also be capable of collecting samples into various-sized test tubes or vials. Collection is triggered manually or by contact closure. Typically, the HPLC eluate is dispensed into the plates by dripping or spraying as the fraction collector head moves from well to well. 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 this process generally produces acceptable radioprofile data, it is labor-intensive. In addition, due to the mechanism for dispensing the HPLC eluate, the potential exists for contamination and the loss of a small amount of sample as the fraction collector head moves between wells and between plates.

Recently, researchers have been investigating the use of Ultra High Performance Liquid Chromatography (UHPLC) in metabolite profiling experiments [6–8]. The use of 384-well plates for collection, combined with the relatively lower flow rates utilized with UHPLC will greatly enhance resolution and will shorten run times over current 96-well HPLC methodology, but will require a fraction collector that is able to precisely dispense the UHPLC eluate into the smaller wells of the 384-well plates.

The Collect PAL (LEAP Technologies, Carrboro, NC) was developed to improve sample throughput and to address some of the deficiencies of the conventional fraction collector. The present studies were conducted to evaluate the performance of the Collect PAL with regard to the precision of dispensing selected fraction volumes of HPLC mobile phase. In addition, the reproducibility of the system for generating metabolic profiles of [^{14}C]buspirone, a compound that produces a complex radioprofile and has a well-characterized metabolism [9,10], was determined with multiple test samples and was compared to data generated using a conventional fraction collector.

2. Experimental

2.1. Fraction collectors

2.1.1. Collect PAL

The Collect PAL (Fig. 1A) was developed to be capable of collecting fractions in plates or tubes. It features a custom-designed collection head that is fully controlled by LEAP Shell software. During operation, the eluate exiting the HPLC column flows into an inverted syringe inside the collection head, where it is dispensed through a detached needle into each well. The syringe has a hollow-core plunger which moves down with each aspiration step and moves up with each dispensing step. A segment of coiled HPLC tubing in the collection head enables movement of the plunger. The syringe barrel acts as a temporary reservoir for the mobile phase during these well-to-well movements. This design feature permits the eluate to continuously flow while the collection head is in movement, without any dripping in between wells or plates. When the Collect PAL is configured with two, 6-drawer, Peltier Cool Stacks, up to 24 plates can be included per run; a maximum of 4 plates are accessible for each individual injection, corresponding to the number of plates available in the same horizontal plane at one time. The current Collect PAL firmware limits the maximum number of 96- or 384-well plates that can be loaded per analytical run to 24.

Major parameter settings include the penetration depth of the delivery needle (mm), the plunger retract distance (mm) and the plunger aspiration and dispense speeds (mm/s). There is also a sample collection delay setting (s) which can allow for a period of time at the beginning of an injection when sample collection will not occur. These settings should be optimized by the user and are dictated by the type of plates that will be used (96- or 384-well, shallow or deep-well), the mobile phase flow rate, and collection rate. By adjusting *x*, *y* and *z* position settings the Collect PAL can be optimized to accommodate specific racks, which would permit collection into larger-sized test tubes and vials (e.g. for the isolation of metabolites for further structural characterization and testing).

During development, the Collect PAL fraction collection procedure was optimized using plates that were sealed with TopSeal S film (PerkinElmer Life and Analytical Sciences). In the process of delivering the HPLC eluate to each well, the sample delivery needle punctured the film covering and then dispensed the sample.

2.1.2. Gilson FC 204

The Gilson Model FC 204 is a conventional fraction collector that is routinely used in our laboratory [11–13] and is widely used among other drug metabolism groups across the industry [14–16] in the generation of radioprofiles for drug metabolism samples. This fraction collector is designed with a tray that can hold a maximum of four plates per HPLC run. The HPLC eluate is dispensed into open plates as the collection head moves over each well. The system is capable of dispensing into 96-well plates, however, it is not designed to dispense into 384-well plates. The FC 204 has several built-in programs which utilize specific racks and *x* and *y* position settings that can be optimized to accommodate larger-sized test tubes and vials for collection.

2.2. Materials

[^{14}C]Buspirone with a specific activity of 27 μCi/mg and a radiochemical purity > 97.5% was synthesized at Bristol-Myers Squibb Co. (Princeton, NJ). β-Nicotinamide adenine dinucleotide phosphate, reduced form (NADPH) was purchased from Sigma–Aldrich Co., (St. Louis, MO). Potassium phosphate (monobasic and dibasic) was purchased from EM Science, (Gibbstown, NJ). Deepwell LumaPlate™-96 and -384 well plates and TopSeal S film were purchased from PerkinElmer Life and Analytical Sciences Inc., (Waltham, MA). Glacial acetic acid was purchased from J.T. Baker, (Phillipsburg, NJ). Type I reagent grade water was prepared using a Milli-Q water system (Model No. ZMQS6000Y, Millipore Corp, Billerica, MA). All organic solvents were of HPLC grade. A pooled, mixed gender preparation of human liver microsomes (HLM) was purchased from BD Gentest, (Woburn, MA). Blank EDTA human plasma was purchased from Bioreclamation, Inc. (Hicksville, NY). Control dog bile and human urine were obtained in-house from a male bile duct-cannulated beagle dog and a normal, healthy volunteer.

2.3. Methods

2.3.1. System precision assessments

2.3.1.1. Delivery to 12 mm × 32 mm vials. Gravimetric experiments were conducted to evaluate the precision of the Collect PAL to deliver a constant volume of HPLC mobile phase to each fraction. Two commonly used HPLC mobile phase solvents and one mobile phase solution were selected for this assessment: acetonitrile, methanol and acetonitrile–water (50:50, v/v). Each mobile phase was dispensed into fifty-four 12 mm × 32 mm glass vials fitted with magnetic caps and were run in duplicate or triplicate experiments using an HPXL pump with 5SC pump heads and pressure module system (Rainin Instrument, Oakland, CA) with the Collect PAL as the fraction collector. Each mobile phase was tested at 0.5 and 1 mL/min flow rates and with collection times of 10 and 15 s, respectively. The vials were placed in a MT-54 tray and tared using a LEAP Balance PAL (LEAP Technologies, Carrboro, NC) and then placed into the Collect PAL. After collection using the specified run conditions, the tray was returned to the Balance PAL and the net weight of each vial was calculated and reported using a SAG285 (Mettler-Toledo, Columbus, OH) with a 4 decimal place integrated system onto the Balance PAL. The weights of individual fractions were plotted and the average, standard deviation and CV were determined using Excel 2003 SP3 (Microsoft Corp., Redmond, WA).

2.3.1.2. Delivery to 96-well plates. [^{14}C]Buspirone was added to a solvent solution containing acetonitrile and 10 mM ammonium acetate (50:50, v/v), resulting in a mobile phase with an activity concentration of approximately 3000 dpm/mL. This mobile phase was delivered at a flow rate of 1.0 mL/min with an HPLC system comprised of a LC-10AD VP pump and a DGU-14A degasser (Shimadzu, Columbia, MD) and was fractionated into 96-well Deepwell Luma plates at 10 s/well using the Collect PAL or Gilson fraction collectors. After collection, the plates were dried overnight using a SpeedVac AES-2010 (Thermo Fisher Scientific, Waltham, MA) set at 45 °C. The holes punctured in the film covering each well of the plates by the dispensing needle of the Collect PAL were sufficient to facilitate complete drying of these plates while under vacuum. After the plates were dried, they were counted in a TopCount Model C991200 microplate scintillation and luminescence counter (PE Life

and Analytical Sciences, Waltham, MA) for 10 min per well. The mean radioactivity in counts per minute (CPM) per well and the % CV were reported for each plate.

2.3.2. Plate storage

Additional 96-well plates were prepared using the Collect PAL to dispense mobile phase containing [^{14}C]buspirone as described in Section 2.3.1.2. After collection, the plates were stored in the cool stacks of the Collect PAL at 4 °C for 24 or 60 h to simulate the effect of an overnight or over-the-weekend run before drying and counting as described in Section 2.3.1.2.

2.3.3. Radioprofile assessment

2.3.3.1. Sample preparation. [^{14}C]Buspirone was incubated with human liver microsomes (HLM) in order to generate a mixture of metabolites that could be used in radioprofiling experiments. Each incubation contained [^{14}C]buspirone (250 μM), HLM (4 mg/mL protein conc.), and NADPH (2.4 mM) in 0.1 M phosphate buffer (pH 7.4) at a total volume of 1 mL. The reactions were carried out at 37 °C in a shaking water bath. After 1 h, the reactions were stopped by adding an equal volume of ice-cold acetonitrile. The suspensions were vortexed, centrifuged for 5 min at 3000 rpm and the resulting supernatants were then transferred to new tubes and dried under nitrogen in a TurboVap (Zymark, Hopkinton, MA) at ambient temperature. To create test samples containing buspirone and its metabolites, the dried residues were reconstituted in 1 mL volumes of human plasma, human urine or dog bile. Prior to analysis, the plasma sample was also treated with an equal volume of acetonitrile to precipitate any proteins. The sample was centrifuged at 3000 rpm for 5 min and the resulting supernatant was utilized for analysis. Urine and bile samples were analyzed directly without further processing. The metabolism test samples, fortified with [^{14}C]buspirone and its metabolites, were transferred to glass conical injection vials for use in subsequent experiments.

2.3.3.2. Radioprofile generation. To evaluate the reproducibility for generating radioprofiles in 96-well plates, duplicate sets of plasma extracts, and urine and bile samples (10 μL each) were injected in triplicate into an HPLC system comprised of a CTC PAL autosampler (Leap Technologies, Carrboro, NC), a pair of Shimadzu LC-10AD VP pumps and a DGU-14A degasser which utilized either the Collect PAL or Gilson fraction collector to fractionate the HPLC eluate into 96-well plates. A Zorbax Eclipse XDB-C18, 3.5 μ, 4.6 mm × 150 mm column (Agilent Technologies, Santa Clara, CA) maintained at ambient temperature was used for the chromatographic separations. The HPLC solvent system consisted of mobile phases A (10 mM ammonium acetate) and B (acetonitrile) with the following HPLC gradient: Mobile phase B was increased from 10 to 90% over a 0–40 min interval, held at 90% for 2 min, ramped back to 10% in 1 min and then re-equilibrated at 10% for 5 min. The total run time was 48 min. The mobile phase flow rate was 1 mL/min and the post column eluate was split as indicated in Fig. 1B, with 75% of the HPLC eluate directed to the fraction collector. Both fraction collectors were programmed to collect the HPLC eluate into 96-well plates at 10 s/well. The plates were dried and processed as described in Section 2.3.1.2. Nine representative peaks, present in the radioprofiles of each matrix, were selected to assess the precision of the fraction collectors. The radioactivity attributed to each of the 9 peaks was determined and reported as a percentage of the total sample radioactivity recovered over the entire chromatographic run, as is typically done in a metabolism study. The inter-run precision (% CV) for the 9 peaks was reported.

Under the same chromatographic conditions, representative radioprofiles were generated by collecting the HPLC eluate in 384-well plates using the Collect PAL. In order to accommodate the smaller capacity of each well, the Collect PAL was programmed to collect the HPLC eluate at 4.6 s/well. The plunger movement (12 mm) and penetration depth (4 mm) were also optimized for the increased rate of movement of the collection head. After collection, the plates were dried and counted as described in Section 2.3.1.2. Radioprofiles were prepared by plotting the resulting net CPM values against time-after-injection. Radioactive peaks in the chromatographic profiles were reported as a percentage of the total radioactivity recovered during the entire HPLC run.

2.3.3.3. Identification of metabolites by LC/MS/MS. To facilitate the identification of drug-related components in the buspirone metabolism samples, the HPLC eluate was split post-column and 25% of the flow was directed into a QTrap 4000 mass spectrometer (Applied Biosystems MDS Sciex, Ontario, Canada), as depicted in Fig. 1B. The mass spectrometer was equipped with an API probe and was operated in the positive mode with the following parameters: 300 °C turbo ion temperature, 50 psi GS1 and GS2 turbo ion gas setting, 30 curtain gas setting, 5500 V ion spray current and 50 eV collision energy. Peaks corresponding to buspirone and its metabolites were identified via their protonated molecules and by their mass spectral fragmentation patterns as compared to previously reported data [9]. The following metabolites/protonated molecules were monitored by multiple reaction monitoring (MIM): buspirone, *m/z* 386; 1-pyrimidinylpiperazine (1-PP), *m/z* 165; monohydroxylated metabolites, *m/z* 402: 3′-hydroxybuspirone (3′-OH-Bu), 6′-hydroxybuspirone (6′-OH-Bu) and other unidentified monohydroxylated metabolites (mono-OH-Bu); buspirone *N*-Oxide (Bu *N*-oxide), *m/z* 402; and dihydroxylated metabolites, *m/z* 418: 5,6′-dihydroxybuspirone (5,6′-di-OH-Bu), and other unidentified dihydroxylated metabolites (di-OH-Bu). As per the method of Yao et al. [17], identification of any of these protonated molecules in the MIM analysis was set to trigger the acquisition of an enhanced product ion scan.

3. Results and discussion

3.1. Gravimetric assessment

At flow rates of 0.5 and 1.0 mL/min, the Collect PAL was capable of precisely delivering 0.083-mL (10 s) and 0.25-mL (15 s) fractions,

Table 1
Gravimetric assessment of the precision of the Collect PAL to fractionate 3 different mobile phases into vials. Flow rates of 0.5 or 1.0 mL/min and collection times of 10 or 15 s, respectively, were utilized.

Mobile phase	Flow rate (mL/min)	Collection interval (s)	Average sample weight (g) (n = 54)	S.D.	% CV
Acetonitrile	1.0	15	0.2532	0.004	1.4
	0.5	10	0.0628	0.002	3.2
Methanol	1.0	15	0.2552	0.002	0.6
	0.5	10	0.0750	0.003	4.0
Acetonitrile/water 50:50 (v/v)	1.0	15	0.3045	0.004	1.2
	0.5	10	0.0972	0.004	4.1

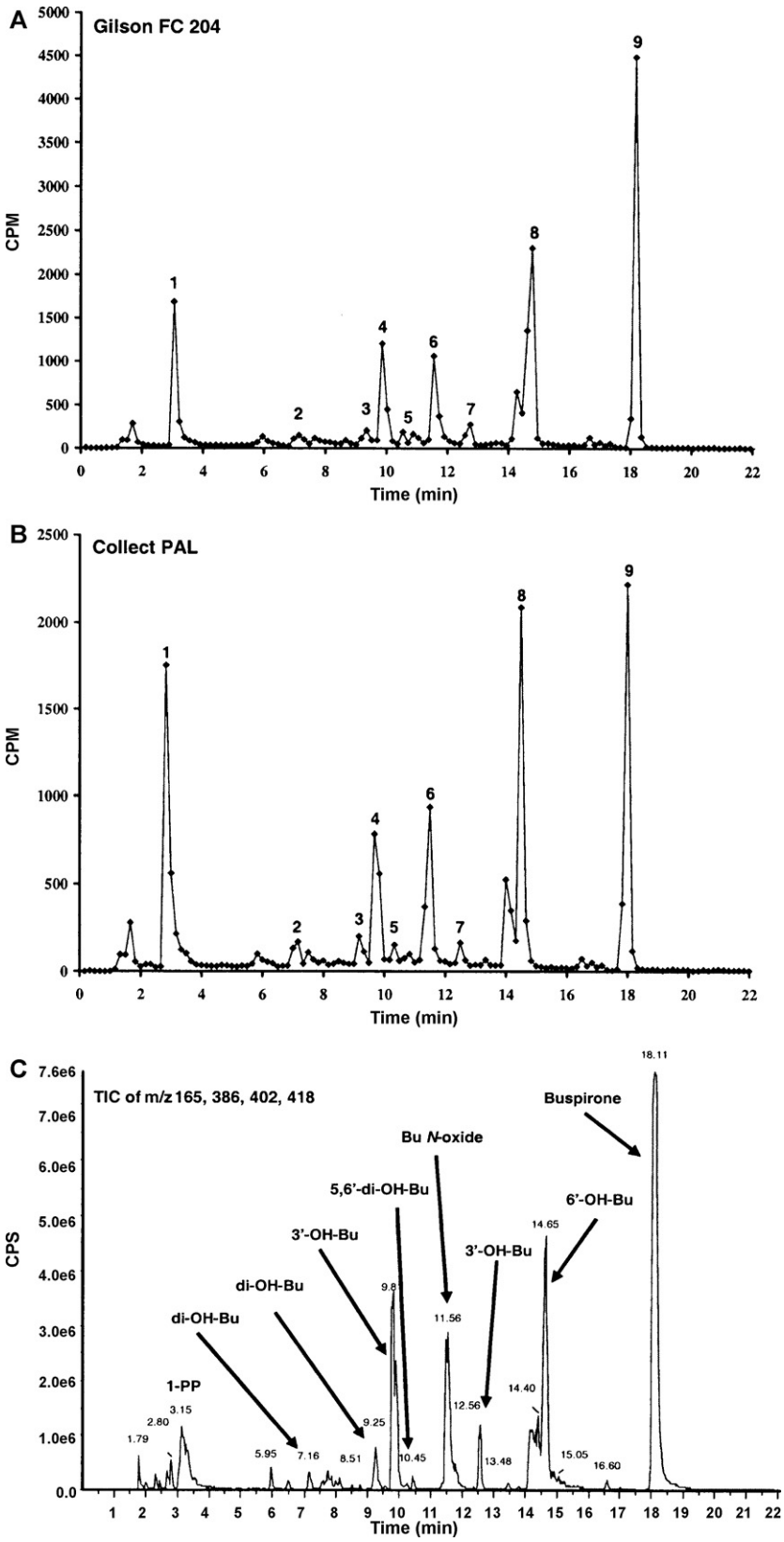

Fig. 2. Comparison of representative [^{14}C]buspirone metabolite profiles in human plasma collected at a flow rate of 1.0 mL/min and a fractionation rate of 10 s/well using the (A) the Gilson FC 204 and (B) the Collect PAL. (C) Corresponding multiple-ion monitoring (MIM) chromatogram of selected masses for the identification of buspirone and its metabolites.

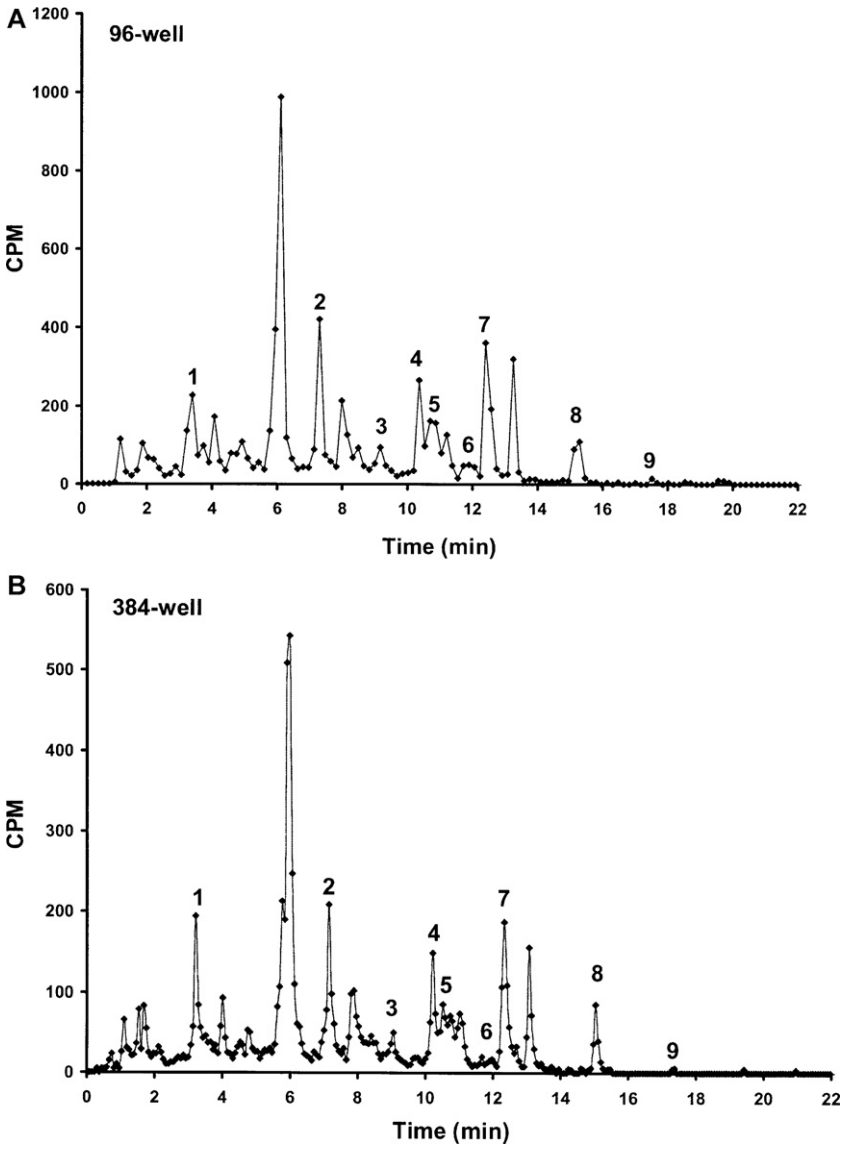

Fig. 3. Comparison of HPLC-radioprofiles of dog bile samples collected (A) at a fractionation rate of 10.0 s/Well into 96-well plates and (B) at a fractionation rate of 4.6 s/Well into 384-Well plates at a flow rate of 1.0 mL/min.

respectively, of 3 different mobile phases (acetonitrile, methanol and acetonitrile-water (50:50, v/v) into 12 mm × 32 mm glass vials (Table 1). For each mobile phase, the gravimetric measurements of the 54 fractions were ≤4.1 and ≤1.4% CV at 0.5 and 1.0 mL/min, respectively.

3.2. Radioactivity assessment

At a flow rate of 1.0 mL/min, the Collect PAL was capable of fractionating a mobile phase of 10 mM ammonium acetate in water/acetonitrile 50:50 (v/v) containing ^{14}C radioactivity into 96-well plates (Table 2). The measured radioactivity dispensed by the Collect PAL demonstrated well-to-well consistency, with a precision within 5.3% CV at 0-time. In comparison, the precision of the measured radioactivity dispensed by the current technology, the Gilson FC 204, was within 7.5% CV. In addition, there was minimal impact on the radioactivity determinations when plates were stored in the Peltier stack at 4 °C for up to 60 h prior to processing, for example due to the adsorption of the compound to the sides of the wells as a result of evaporation.

3.3. Chromatographic assessment

HPLC radioprofiles of the plasma, urine and bile test samples fortified with [^{14}C]buspirone and its metabolites, obtained utiliz-

Table 2
Reproducibility of fractionating samples into 96-well plates using the Collect PAL, Gilson FC 204, and manual pipettor. HPLC mobile phase containing 3000 dpm/mL of radioactivity was dispensed in 167-μL fractions into a 96-well plate at a rate of 10 s/well using the Collect PAL or Gilson 204 FC fraction collectors. For comparison, one plate was spiked by manually pipetting an equivalent amount of mobile phase into each well. Additional plates collected with the Collect PAL were stored in the cool stacks at 4 °C for 24 or 60 h to simulate overnight or over weekend collections.

Fraction collector	Storage time (h)	CPM/well (n = 96)	
		Average	% CV
Collect PAL	0	443	5.3
	24	453	4.4
	60	396	7.1
	Average	431	5.6
Gilson FC 204	0	404	7.5
Manual pipettor	0	496	4.0

Table 3
[^{14}C]Buspirone metabolite profile data for representative plasma, urine and bile samples collected in triplicate at a flow rate of 1.0 mL/min and a fractionation rate of 10 s/well using the Collect PAL and the Gilson FC 204. The % of sample radioactivity for the 9 selected peaks in each matrix was used as an indicator of reproducibility.

Peak #	Peak ID[a]	% Sample radioactivity (n = 3)			
		Collect PAL		Gilson FC 204	
		Average	% CV	Average	% CV
Plasma					
1	1-PP	17.1	1.3	10.9	1.4
2	di-OH-Bu	2.7	1.1	1.9	9.1
3	di-OH-Bu	2.4	6.1	1.4	13.1
4	3′-OH-Bu[b]	8.4	0.5	4.4	10.0
5	5,6′-di-OH-Bu	2.9	1.0	6.6	3.1
6	Bu *N*-oxide	8.8	3.4	8.3	3.8
7	3′-OH-Bu[b]	2.2	0.8	2.4	10.4
8	6′-OH-Bu	15.5	1.6	18.3	1.9
9	Buspirone	16.6	2.5	22.2	6.2
Urine					
1	1-PP	12.6	2.7	12.5	2.1
2	di-OH-Bu	1.9	9.0	1.8	1.1
3	di-OH-Bu	1.9	1.7	1.2	17.3
4	3′-OH-Bu[b]	6.5	2.8	4.2	11.5
5	5,6′-di-OH-Bu	4.8	3.1	6.7	6.3
6	Bu *N*-oxide	7.8	2.6	7.9	0.3
7	3′-OH-Bu[b]	2.5	7.2	2.1	4.7
8	6′-OH-Bu	20.1	0.7	19.9	1.5
9	Buspirone	21.8	1.3	21.6	0.5
Bile					
1	1-PP	9.0	2.2	8.7	5.6
2	di-OH-Bu	1.6	1.0	1.6	0.5
3	di-OH-Bu[b]	1.2	9.3	0.8	8.0
4	3′-OH-Bu	2.5	2.9	2.4	1.1
5	5,6′-di-OH-Bu	6.6	2.3	6.2	1.2
6	Bu *N*-oxide	6.2	3.1	7.9	3.1
7	3′-OH-Bu[b]	3.6	2.7	2.9	5.1
8	6′-OH-Bu	19.0	1.0	16.9	1.3
9	Buspirone	30.9	2.8	29.8	3.8

[a] The following abbreviations were used for buspirone metabolites: 1-pyrimidinylpiperazine (1-PP), unspecified di-hydroxylated metabolites of buspirone (di-OH-Bu), unspecified mono-hydroxylated metabolites of buspirone (mono-OH-Bu), 5,6′-dihydroxybuspirone (5,6′-di-OH-Bu), buspirone *N*-oxide (Bu *N*-oxide), 6′-hydroxybuspirone (6′-OH-Bu).
[b] There were two 3′-OH-Bu isomers detected in the samples.

ing either the Collect PAL or FC 204 demonstrated similar peak resolution (see representative radioprofiles for the human plasma extract in Fig. 2A and B). The inter-run precision (% CV) in the triplicate assessment of 9 selected peaks in the fortified human plasma extract, human urine and dog bile sample radioprofiles with the two systems are reported in Table 3. Across the 3 matrices, the % CV was within 9.3 and 17.3% for the Collect PAL and the FC 204, respectively. Overall, the peak resolution and chromatographic reproducibility for radioprofiles generated on the Collect PAL were comparable to or better than the FC 204.

By splitting the HPLC eluate post-column and directing a percentage of the flow to a mass spectrometer, as depicted in Fig. 1B, mass spectral data for the identification of drug-related components in a representative urine buspirone metabolism sample could be collected concurrently with the radioprofile. Alignment of the radioprofiles with their corresponding MIM chromatograms supported the identification of the peaks for buspirone and its metabolites in the radioprofiles (Fig. 2C).

The Collect PAL was capable of fractionating HPLC eluate flowing at a rate of 1.0 mL/min into 384-well plates. In order to accommodate the smaller well-size of the 384-well plate, the fractionation rate had to be reduced from 10.0 s/well (used for the 96-well plates), to 4.6 s. Radioprofiles generated from a test bile sample that was fortified with [^{14}C]buspirone and its metabolites were collected into both 96-well and 384-well plates (Fig. 3). Generally, the radioprofiles were comparable, with the radioprofile collected into 384-well plates showing greater peak resolution. However, since the radioactivity under each peak was distributed among a larger number of wells, the apparent sensitivity was lower for the 384-well plates than with the 96-well plates. The use of UHPLC in conjunction with 384-well plate collection, should provide better resolution and increased sensitivity for each peak relative to the HPLC results.

3.4. Safety considerations

Special safety considerations exist when using any type of fraction collector. Most fraction collectors are designed to dispense mobile phase into open collection plates, resulting in potential exposure of scientists to solvents and potent compounds due to evaporation and aerosol formation. There is an improved safety benefit of the process described here for the Collect PAL due to the collection of the HPLC effluent into sealed plates and storage of these plates at a sub-ambient temperature.

4. Conclusion

The Collect PAL, a multiple-plate, high-throughput, high-capacity fraction collector was evaluated for precision, reproducibility and overall applicability for 96- and 384-well fraction collection. The Collect PAL utilizes Peltier cooling and is fully automated with up to 24 plate storage capability which allows for over-night and over-weekend collection, with increased sample throughput and overall productivity when compared to the existing technology used in our laboratory. An added safety benefit achieved with the use of sealed plates is the reduction in exposure of scientists to volatile solvents and potentially potent compounds.

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References

[1] T.A. Baillie, M.N. Cayen, H. Fouda, R.J. Gerson, J.D. Green, S.J. Grossman, L.J. Klunk, B. LeBlanc, D.G. Perkins, L.A. Shipley, Drug metabolites in safety testing, Toxicol. Appl. Pharm. 182 (2002) 188–196.
[2] D.A. Smith, R.S. Obach, Metabolites and safety: what are the concerns and how should we address them? Chem. Res. Toxicol. 19 (2006) 1570–1579.
[3] Guidance for industry: safety testing of drug metabolites, US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), 2008.
[4] M. Zhu, W. Zhao, N. Vazquez, J.G. Mitroka, 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 (2005) 233–245.
[5] A.N.R. Nedderman, M.E. Savage, K.L. White, D.K. Walker, The use of 96-well scintiplates to facilitate definitive metabolism studies for drug candidates, J. Pharm. Biomed. Anal. 34 (2004) 607–617.
[6] Y. Gu, W.R. Wilson, Rapid and sensitive ultra-high-pressure liquid chromatography–tandem mass spectrometry analysis of the novel anti-cancer agent PR-104 and its major metabolites in human plasma: application to a pharmacokinetic study, J. Chromatogr. B 877 (2009) 3181–3186.
[7] B. Prasad, S. Singh, LC–MS/TOF and UHPLC–MS/MS study of *in vivo* fate of rifamycin isonicotinyl hydrazone formed on oral co-administration of rifampicin and isoniazid, J. Pharm. Biomed. Anal. 52 (2009) 377–383.
[8] G.J. Dear, N. Patel, P.J. Kelly, L. Webber, M. Yung, TopCount coupled to ultra-performance liquid chromatography for the profiling of radiolabeled drug metabolites in complex biological samples, J. Chromatogr. B 844 (2006) 96–103.
[9] M. Zhu, W. Zhao, H. Jimenez, D. Zhang, S. Yeola, R. Dai, N. Vachharajani, J. Mitroka, Cytochrome P450 3A-mediated metabolism of buspirone in human liver microsomes, Drug Metab. Dispos. 33 (2005) 500–507.
[10] H.K. Jajoo, R.F. Mayol, J.A. LaBudde, I.A. Blair, Metabolism of the antianxiety drug buspirone in human subjects, Drug Metab. Dispos. 17 (1989) 634–640.

[11] L.J. Christopher, D. Cui, C. Wu, R. Luo, J.A. Manning, S.J. Bonacorsi, M. Lago, A. Allentoff, F.Y. Lee, B. McCann, S. Galbraith, D.P. Reitberg, K. He, A. Barros Jr., A. Blackwood-Chirchir, W.G. Humphreys, R.A. Iyer, Metabolism and disposition of dasatinib after oral administration to humans, Drug Metab. Dispos. 36 (2008) 1357–1364.
[12] D. Zhang, K. He, N. Raghavan, L. Wang, J. Mitroka, B.D. Maxwell, R.M. Knabb, C. Frost, A. Schuster, F. Hao, Z. Gu, W.G. Humphreys, S.J. Grossman, Comparative metabolism of ^{14}C-labeled apixiban in mice, rats, rabbits, dogs and humans, Drug Metab. Dispos. 37 (2009) 1738–1748.
[13] V.T. Ly, J. Caceres-Cortes, D. Zhang, W.G. Humphreys, I.V. Ekhato, D. Everett, S.N. Comezoglu, Metabolism and excretion of an oral taxane analog [14C]3(-tere-Butyl-3(-N-tert-butyloxycarbonyl-4-deacetyl-3(-dephenyl-3(-N-debenzoyl-4-O-methoxy-paclitaxel (BMS-275183), in rats and dogs, Drug Metab. Dispos. 37 (2009) 1115–1128.
[14] H.P. Gschwind, U. Pfaar, F. Waldmeier, M. Zollinger, C. Sayer, P. Zbinden, M. Hayes, R. Pokorny, M. Seiberling, M. Ben-Am, B. Peng, G. Gross, Metabolism and disposition of imatinib mesylate in healthy volunteers, Drug Metab. Dispos. 33 (2005) 1503–1512.
[15] C.P. Yu, C.L. Chen, F.L. Gorycki, T.G. Neiss, A rapid method for quantitatively estimating metabolites in human plasma in the absence of synthetic standards using a combination of liquid chromatography/mass spectrometry and radiometric detection, Rapid Commun. Mass. Spectrom. 21 (2007) 497–502.
[16] C. Shaffer, C. Langer, Metabolism of a ^{14}C/^{3}H-labeled $GABA_A$ receptor partial agonist in rat, dog and human liver microsomes: evaluation of a dual-radiolabel strategy, J. Pharm. Biomed. Anal. 43 (2006) 1195–1205.
[17] M. Yao, L. Ma, W.G. Humphreys, M. Zhu, Rapid screening and characterization of drug metabolites using a multiple ion monitoring-dependent MS/MS acquisition method on a hybrid triple quadrupole-linear ion trap mass spectrometer, J. Mass Spectrom. 43 (2008) 1364–1375.