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Note

A SepPak unit for batch processing serial blood plasma samples for PET

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Abstract: A prototype blood plasma processor unit was designed and tested for generic PET-tracer metabolite analyses, using solid-phase-extraction cartridges (SepPaks). An assay method for FLT (3′-deoxy-3′-[F-18]fluorothymidine) and its blood metabolite: FLT-glucuronide, in serial, patient-derived samples was developed to evaluate the device. The unit is simple to construct from readily available components and can process up to six samples in parallel for high throughput. The precision of sample results was evaluated in a test–retest trial and was 98%. A syringe pump method for sample application and SepPak elution proved to be a reliable technique. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: PET; plasma metabolite; SepPak; FLT; sample processor

Introduction

To perform a kinetic analysis of dynamic PET imaging data it is essential to follow the declining fraction of blood plasma activity that is specific to the parent radiopharmaceutical, and sometimes a specific metabolite.[1] Typically, eight to 12 serial blood plasma samples need to be analyzed to construct the appropriate plasma input function(s). The number of samples required is dictated by the curvature of the blood time–activity curve, the rapidity of drug metabolite production, the time required for sample processing and the half-life of the radionuclide. In the setting of a busy PET imaging suite, limited access to a shared counting instrument may also be a factor.

Rapid solid-phase-extraction (SPE) methods for drug metabolite analysis[2] offer a practical, cost-effective alternative to time-consuming HPLC methods. However, processing of multiple samples can also be challenging and requires considerable focus to avoid handling errors. Herein, we report on the design and testing of a versatile multi-sample SPE processor unit. This portable unit was easily assembled from standard parts. It has been used for routine metabolite analysis of [F-18]FLT (3′-deoxy-3′-[F-18]fluorothymidine) in PET studies. The unit separates FLT from its glucuronide conjugate in diluted plasma samples.

Results and discussion

SPE methods are used to process samples, such as blood plasma, by extracting or voiding a desired analyte. A variety of SPE stationary phases are available for extracting ionic or lipophilic species. For convenience, multiple samples are typically processed in parallel using a panel of SPE cartridges connected to a vacuum manifold. However, flow control through these cartridges poses problems. For example, sample breakthrough is an issue when flow is too fast or crudely controlled. Cartridge packing densities may also differ, which leads to a range of flow and performance. This can cause incomplete sample extraction and result in poor quantitation.

To standardize our use of SepPak cartridges for SPE, we designed a unit that relies on syringe pumps to better control fluid flow through the SepPaks. The device schematic and the physical layout are shown in Figures 1–3. Up to six samples can be processed at once. The specific configuration uses a tandem pair of SepPaks (anion exchange-QMA and C18) to separate FLT–glucuronide (FLT-G, a blood metabolite) from FLT, respectively. In our experience, the syringe pump

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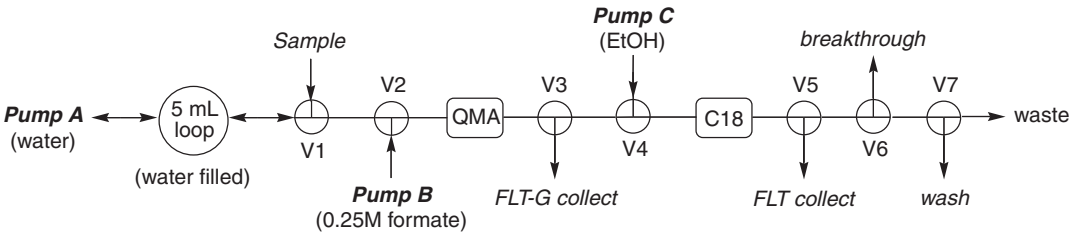

Figure 1 Schematic for the dual-SepPak (QMA/C-18) FLT/metabolite processor.

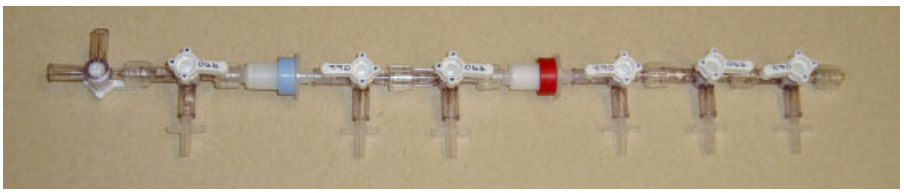

Figure 2 Quick-fit processing units (*left to right*, V1-2, QMA, V3-4, C18, V5-7; male luer slips on V2 and V4 are press-fitted to the structural support, while all others drain into collection tubes).

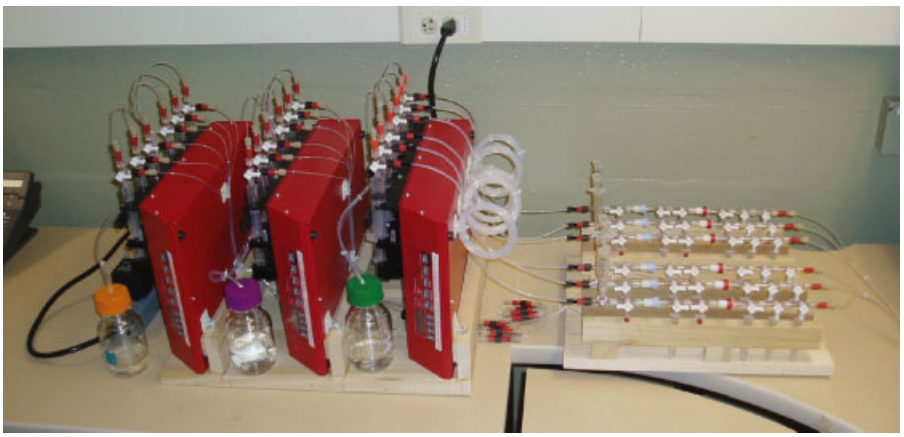

Figure 3 Full layout of the multi-sample tandem SepPak (QMA/C-18) processor. Pumps A–C (right to left) are shown. The sample loops (six) on Pump A are directly connected to the processing lanes (six) through V1 in each lane. Each processor lane receives inputs from all three pumps.

offered the best way to accurately meter flow/volume and clear voids to minimize sample channeling. For example, SepPaks were properly eluted only if the eluting solutions were delivered by positive displacement in a coordinated fashion. This was done with individual syringes, held in parallel on a multiple syringe pump. If the eluting solution was delivered from a common distribution manifold, the resulting passive flow through individual SepPaks led to >30% variation in delivered volumes ($n = 6$). With that method it was impractical to ensure that every SepPak was eluted with at least 4 ml, which was the collection tube limit.

A header + loop technique was useful for sample injection (2 ml), since the loop is back flushed by Pump-A. The loop (5 ml) is sufficiently large to protect the water supply on Pump-A from contamination when the sample is loaded. Manual three-way stopcocks are used to direct flow and their operation logic is listed in Table 1. The system void volume (1.6 ml, including SepPak contributions) was minimized by directly connecting stopcocks (see Figure 2). This was important since a counting tube holds 4 ml. The processing manifolds are rapidly installed, removed or replaced using luer fittings. The luer-slip-adapters act as both anchor posts and fluid delivery nipples. For convenience, SepPaks are conditioned while in place.

Processor operation provided repeatable results with duplicate sets of [F-18]FLT diluted plasma samples (six/set), where the %FLT content ranged from 74 to

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Table 1 Dry unit to start (position indicates which 3-way valve-port is *blocked*: 0 = central; + = toward loop; − = toward waste)

	V1	V2	V3	V4	V5	V6	V7
Conditioning							
Line C prime	0	0	0	+	0	0	0
Wash	0	0	0	0	0	0	0
Line B prime	0	+	0	0	0	0	0
Wash	0	0	0	0	0	0	0
Processing							
Loop load	−	0	0	0	0	0	0
Loop inject	0	0	0	0	0	0	0
BT collect	0	0	0	0	0	−	−
Wash collect	0	0	0	0	0	0	−
QMA/C18 elute	0	+	−	+	−	0	−
Recycling							
Reset/wash	0	0	0	0	0	0	0

Table 2 Comparison of methods (hand vs processor) for the assay of the %FLT in diluted serial blood plasma samples ($n = 6$)

Sample	Hand process	Processor unit		
	Series A	Series B and C		Means (A,B,C)
1	96.9	97.6	98.4	97.6
2	95.6	95.4	96.3	95.8
3	91.7	92.7	93.2	92.6
4	87.2	89.8	91.1	89.4
5	84.1	84.6	85.5	84.7
6	72.5	74.7	74.7	74.0

Mean percent deviation (A−C) = 0.8% ($n = 18$, stdev. 0.7%). Mean percent deviation (A−B and A−C) = 1.8% ($n = 12$, stdev. 1.3%). Mean percent deviation (B and C vs Mean B,C) = 0.53% ($n = 6$, stdev. 0.32%).

96% (Table 2). SepPaks were used twice and, while still in-place, they were reconditioned with water between cycles. The processing and collection tube replacement cycle took less than 20 min. On average, the precision was >98% and the sample breakthrough was <2%. In addition, QMA cartridges (formate form) had no affinity for FLT and C18 cartridges had no affinity for FLT-G and the recovery of captured counts from SepPaks was quantitative.

A simpler, alternative manual method has been reported for [F-18]FLT metabolite analysis. It uses a whole blood sample and a QMA cartridge.[3] Nearly identical results were obtained by comparison with our method provided that whole blood samples were loaded onto QMA cartridges as slowly as possible. However, we did notice the restriction could be lifted if blood samples were diluted with water (1:7). While the simplicity of a whole blood/QMA method is attractive, there is no way to detect if sample breakthrough has occurred. The processor device described here offers a convenient, standardized way to process samples and offers a means to detect sample breakthrough.

Experimental

Sample processor components

The commercially available components were: (1) three programmable 6-place syringe-pumps (NE-1600, New Era Pump Systems, Farmingdale, NY); (2) 3-way stopcocks with swivel male Luer-lock (MX5311L, Medex, Dublin, OH); (3) male luer slip adapters (57-02, Intl Medical Industries, Pompano Beach, FL); (4) Luer-Lok plastic syringes (5, 10, 20 ml, BD, Franklin Lake, NY); (5) from ChromTech/Upchurch Scientific (Apple Valley, MN): Tefzel tubing ($\frac{1}{8}$ in OD, 2.4 mm ID); Teflon tubing ($\frac{1}{16}$ in OD, 0.75 mm ID); Quick connect $\frac{1}{4}$–28 luer adapters (male and female); $\frac{1}{4}$–28 flangeless fittings with ferrules for $\frac{1}{16}$ in and $\frac{1}{8}$ in OD tubing; (6) Accell Plus QMA SepPak cartridges and SepPaks Plus C18 cartridges (Waters, Milford, MA); (7) 75 × 13 mm (OD) plastic counting tubes.

Structural supports for sample processing units and collection tubes

Six supports, in parallel, were attached to a 16 × 12 in (L × W) baseboard, using 1 in elevating blocks (2/lane). Supports were spaced to match with the syringe pump (Figure 3). Supports were made from 13.75 × 0.75 × 1.5 in (L × W × H) stock. Holes were made for either fluid lines (V2, V4) or collection tubes (V3, V5, V6, V7). The hole for V2 (ref. point) was made 2 in from the leading edge and subsequent holes were made at 70, 108, 179, 215 and 253 mm. The fluid line fittings for (V2 and V4) were recessed into the stock so the top of the exposed female luer was flush with top of the collection tubes. Processing units (Figure 2) were connected to their supports with male luer slips (Figure 2). In operation, the flanges of luer slips on V3, V5, V6 and V7 rested on collection tube rims. Luer slips on V2 and V4 were the only ones secured by female luer fittings for fluid delivery.

Support for syringe pumps

On each of the three required syringe pumps (A–C), syringes (six/pump) were held vertically by supporting the syringe pumps on-edge (Figure 3). The pumps were mounted on a 19 × 16 in (L × W) baseboard and spaced

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7.5 in apart (base–base). A vertical attaching support for the pumps was made from $15 \times 0.75 \times 2.5$ in stock ($L \times W \times H$). An additional $15 \times 0.25 \times 1.125$ in piece was glued to the inside, upper edge of the vertical support to compensate for the pump's rubber feet.

Sample preparation

[F-18]FLT was prepared as previously described.[4,5] Radiochemical purity was $\geqslant 99\%$, with specific activity >1 Ci/μmol (EOB). Blood samples (3 ml each) were collected at the University of Washington Medical Center under IRB approval. Whole blood was collected into pre-heparinized tubes, typically at 5, 10, 15, 20, 30, 45, 60 and 90 min, post-infusion. Whole blood aliquots were centrifuged for 3 min at 8000 rpm (Eppendorf, Model 5415). One portion of the separated plasma (0.5 ml) was counted and another (0.5 ml) was diluted with 3.5 ml of water. Samples were counted using a Packard (Downers Grove, IL) Cobra II Auto-Gamma instrument.

Processor operation

The units were assembled as shown in Figures 2 and 3. Pump syringes were primed by flushing to and from their reservoirs. Pumps were run at 4 ml/min and valve positions were selected according to the specific tasks listed in Table 1. QMA and C18 SepPaks were activated with 5 ml of 0.25 M formate and ethanol, respectively, and then washed with 10 ml of water. In each case, a diluted blood plasma sample was first loaded into a 5 ml syringe, which was then attached to V1. The sample was injected into the loop when the 3-way stockcock on Pump-A (stopcock-A) was directed to the water reservoir. Stopcock-A was then redirected to the pump. The sample solution was pumped through the SepPaks at 2 ml/min over 2 min and breakthrough activity was collected at V7. The line was then washed with 4 ml of water, at 4 ml/min, and a wash sample was collected from V6. The QMA and C18 SepPaks were eluted with 4 ml of formate and ethanol, and eluates were collected at V3 and V5, respectively. To process less than six samples, unused lanes have their pump flows directed back to their respective reservoirs. If an odd number of lanes are needed, a dummy sample lane is included to balance loads.

Recycling SepPak units

The processing units are recycled by: (1) loading new collection tubes; (2) discarding the injection syringe; (3) back flushing V1 (0.5 ml) and (4) flushing the attached unit with 10 ml of water.

Conclusion

A prototype blood plasma metabolite analysis unit was tested for sample processing with SepPaks. The unit processes up to six samples in parallel and can be rapidly reconditioned for a second batch of samples. This suggests that it may be useful for other fluorine-18 tracers and, possibly, carbon-11 labeled compounds.

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