On-line Microfluidic Extraction Enables Highly Efficient and Sensitive Direct Elution from Dried Blood Spots

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Introduction

Dried blood spots (DBS) have rapidly emerged in the pharmaceutical industry as a highly cost effective method for low-volume sampling, storage, and retrieval of specimens prior to assay by liquid chromatography/tandem mass spectrometry (LC-MS/MS). Prior to analysis, a punch (typically 3 mm) from the spot (typically 15 µL) is subjected to solvent extraction and subsequent analysis by conventional (mm bore) LC-MS/MS. Inefficiencies in extraction and volume handling limit assay sensitivity. Insufficient limits of quantification often preclude the use of DBS for high potency and/or inhaled compounds. Here we present a novel workflow utilizing (1) micro DBS punches (< 0.4 mm) and (2) a microfluidic flow-through extraction cell combined with segmented flow electrospray.[1]

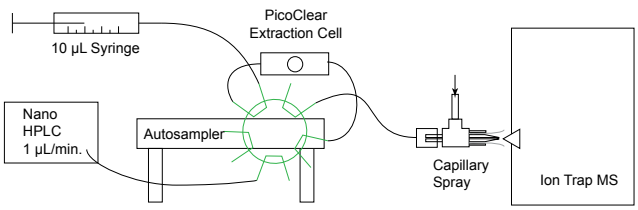
Methods

DBS Card preparation:

- Whatman FTA® or FTA® DMPK
- 20 µL Spots of rat blood containing sitamaquine (varying concentrations up to 10,000 ng/mL)
- D_{10} Internal standard of sitamaquine (spotted at 1,000 ng/mL)
- Air dried, then packaged in poly bags w/dessicant

Micro-punch with segmented elution:

- 0.4 mm ID custom-made stainless steel punch from DBS
- Punch transferred into internal bore of PicoClear union
- Union connected into loop of autosampler injection valve with 50 µm ID tubing
- An air-gapped 0.20 µL slug of 75% ACN transferred to union
- 120 Sec. wait period; valve switched to pumped flow (Eksigent)
- Data collection on Thermo LCQ Deca Ion trap with custom built capillary flow ESI source (CSP, New Objective, Inc.)
- SRM mode: 344.5 > 271 transition



Elution from Microfluidic Punches:

Rat blood aliquots (20 µL) containing sitamaquine were spotted on FTA or FTA Elute cards (Whatman) Micro-punches (0.35 mm ID) from the spot were obtained using a custom fabricated biopsy punch and transferred directly into an elastomer core extraction cell (PicoClear Union, New Objective Inc.; 0.4mm ID). Fused-silica tubing (50 µm ID) was connected to the inlet and outlet of the cell. Compression of the elastomer core effectively sealed the inlet and outlet tubes in the cell to the micro-punch at zero dead volume. The extraction cell was placed in the injection loop of an autosampler (Leap CTC) connected to a nanoflow LC pump (Eksigent). The outlet of the autosampler was connected to a nanospray equipped ion trap mass spectrometer (Thermo Finnigan LCQ Deca) operated in (pseudo) SRM mode. The 344 to 271 m/z transition was monitored.

Once mounted in the loop of the autosampler injection valve, approx. 0.25 µL of extraction solvent (75% acetonitrile) was transferred into the cell from a 10 uL gas-tight micro-syringe. The injection loop was switched from load to inject after a 120 sec extraction period. Once the valve was switched, the nanoflow pump (1 µL/min.) pushed the extraction punch into the nanospray ionization source equipped with a junction style high voltage contact. Stable spray ionization was observed using a 20 µm ID fused-silica emitter (sheath gas assisted) at 2.5 kV.

Figure 1 *Preparation of punch for microfluidic extraction*

A 0.4 mm punch from the DBS card is transferred to the center of an elastomer core union. Tubing is connected to the inlet and outlet of the union. The elastomer is compressed making a conformal seal around the DBS punch.

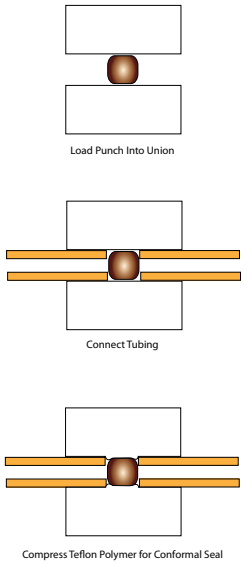


Figure 2 *Segmented flow extraction from the punch*

A 0.2 uL aliquot is created with a 10 uL syringe and transferred to the punch. After a 120 sec, delay the segment is transferred to the capillary flow ESI source by switching the valve from the syringe to the HPLC pump. The segment is air-gapped on both sides to eliminate Taylor dispersion in the transfer line.

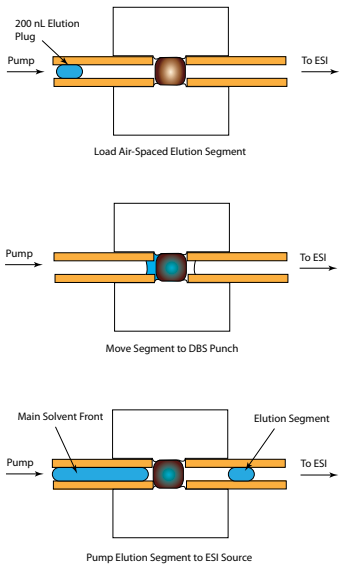


Figure 3 *Micro-punch in dried blood spot (DBS)*

A 0.4 mm punch through the dry, blood-spotted FTA

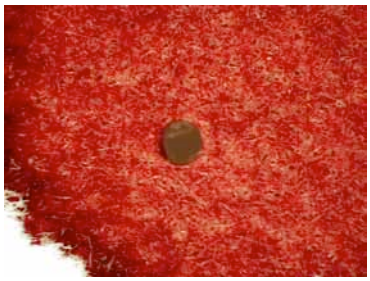


Figure 4 *Multiple punches from a single µL DBS*

Ahlstrom 226–15 punches from ≈ 1/3 spot

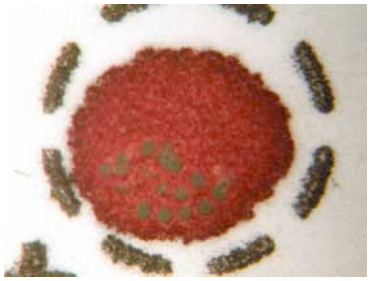


Figure 5 *Transfer punch to first tube (optional)*

The punch is transferred to an FEP tube for optional storage and subsequent transfer for analysis

Figure 6 *Multiple punches from a single µL DBS*

The punch is transferred into the center of a PicoClear union

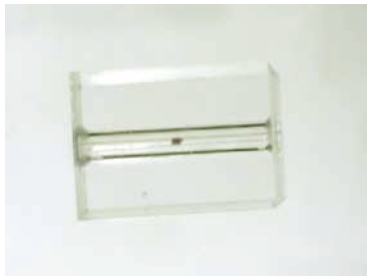


Figure 7 *Insert is installed in PicoClear union body*

The PicoClear insert, with the dried blood spot punch, is inserted into a standard PicoClear union body

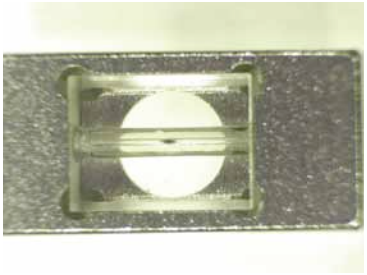


Figure 9 *Union is inserted into the autosampler injection valve*

The union with punch is loaded into the injection loop of a 6-port valve on the autosampler

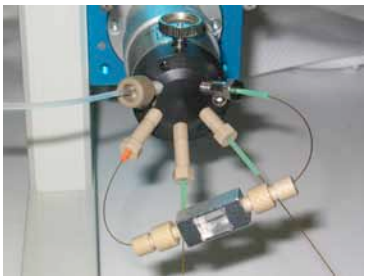


Figure 8 *PicoClear union compressed*

Under compression, the soft FEP core will seal around the punch forming a plug at high pressures (> 3,000 psi). The gaps before and after the punch are intentional and important.

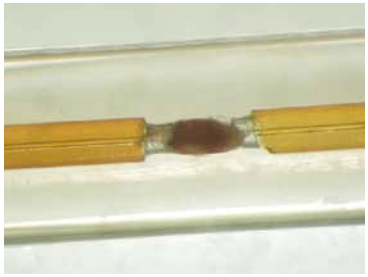


Figure 10 *Eluent solvent added*

DBS in extraction solvent from syringe. Autosampler syringe is used to "fill" the punch w/solvent (≈ 0.2 µL)

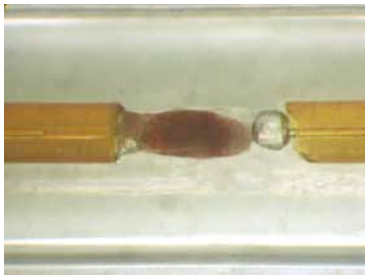
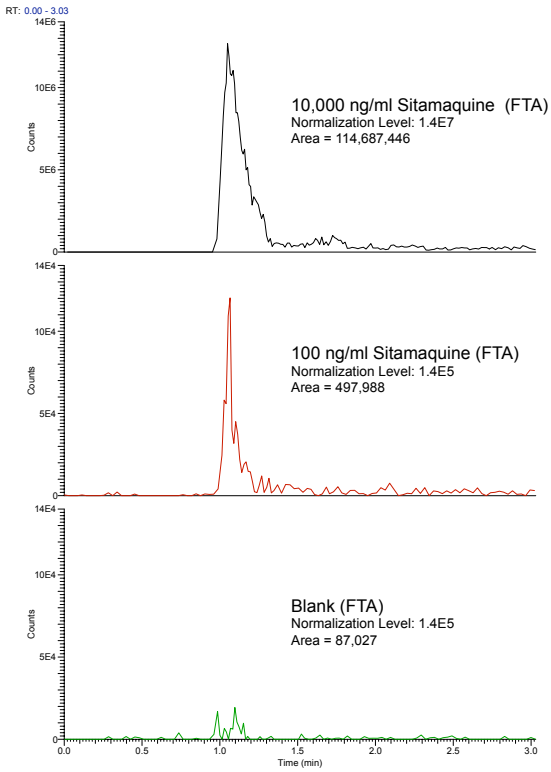


Figure 11 *SRM results on conventional 3D ion trap*



Ion trap based SRM elution profiles at two different analyte concentrations and a blank. The segmented elution enables a tight elution profile preserving high sensitivity and signal-to-background ratio. Elution is rapid, with an MS cycle time of better than 2 minutes.

15-20 µL Blood Spot		
Diameter on Card (mm)	6.50	
Thickness Card (mm)	0.53	
DBS Volume (mm)3	17.59	
	Standard	Micro
Punch Diameter (mm)	3.00	0.40
Punch Volume (mm)3	3.75	0.07
Extraction Volume (uL)	150.00	0.20
DBS Volume/Extraction Volume	0.02	0.33
Relative Extraction Efficiency		13

Extraction efficiency from DBS punches

Conclusions:

- PicoClear-based microfluidic extraction improves extraction efficiency by thirteen-fold over conventional methods
- Sample punches from DBS cards can be reduced to ≤ 0.4 mm diameter.
- Multiple (≥ 10) punches can be obtained from a single DBS spot (20 µL blood)
- The conformable core of the PicoClear union enables true flow-through elution from a DBS punch
- The punch holds at high pressure (> 3,000 psi) and is effectively compatible with nanobore LC elution
- The small elution volume (≤ 200 nL) of the air-gapped elution segment enables highly efficient extraction
- Segment flow elution yields signal-to-background ratio (S/B) ≥ 6:1 at an analyte concentration of 100 ng/mL on a conventional 3D ion trap
- Estimated LLQ of 10-20 ng/mL on an ion trap

References

1. Pei J, Li Q, Lee MS, Valaskovic GA, Kennedy RT. Analysis of samples stored as individual plugs in a capillary by electrospray ionization mass spectrometry. *Anal Chem. 2009* 81(15):6558-61

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