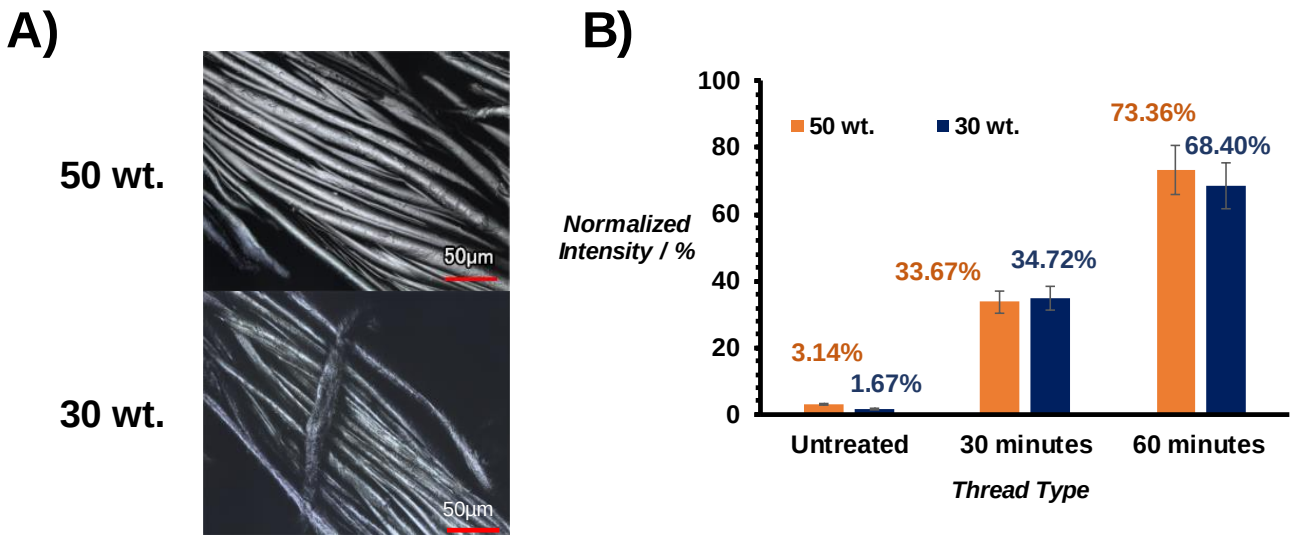
Supporting Information

Microsampling with Cotton Thread: Storage and Ultra-Sensitive Analysis by Thread Spray Mass Spectrometry

Devin J. Swiner, Sierra Jackson, George R. Durisek, Bridget K. Walsh, Yaman Kouatli, and Abraham K. Badu-Tawiah*

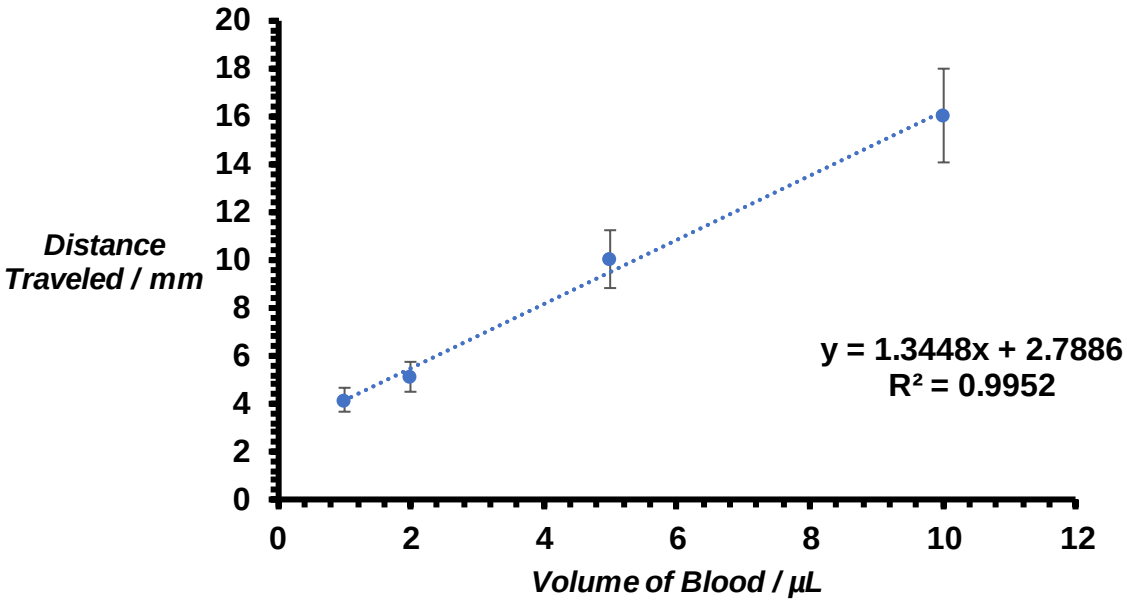
Supporting Information is summarized below:

Figure S1. Thread ionization efficiency as a function of thread weight



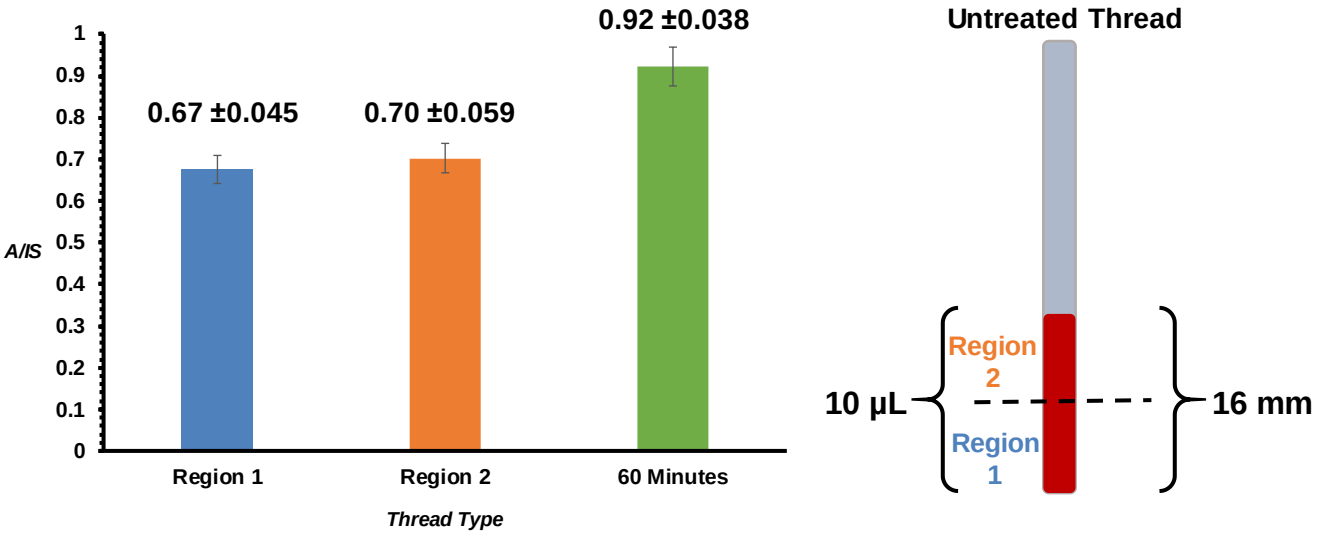
A) 3D optical images of commercially available 30 and 50 wt. untreated thread. Thread sizes are determined by their weights with larger weights having smaller diameters (~360 µm vs. ~350 µm, respectively). As seen in the 3D images, there is no significant difference in thread appearance. All threads have similar, unidirectional subfiber configurations regardless of thread weight and treatment. B) Normalized ion intensity derived thread spray MS analysis of cocaine as a function of both thread size and treatment type. With increasing treatment time, there is an increase in ionization efficiency due to decreased analyte absorption into the threads' subfibers, leaving analyte molecules on the surface readily available for ionization. This phenomenon is independent of thread weight. There was no significant difference between thread weight for each treatment time.

Figure S2. Blood volume as a function of distance traveled on the thread



Calibration curve of sample volume as a function of distance travelled along the thread surface. Untreated threads were dipped in blood samples of varying volumes and the distances they travelled were measured with a caliper. Error bars are represented with replicates of 3. This curve suggests that capillary action in the unidirectional thread fibers can permit the estimation of collected blood volume via length travelled. This has potential in eliminating coffee-ring or volcanic effects that often cause uneven distribution of blood in paper.

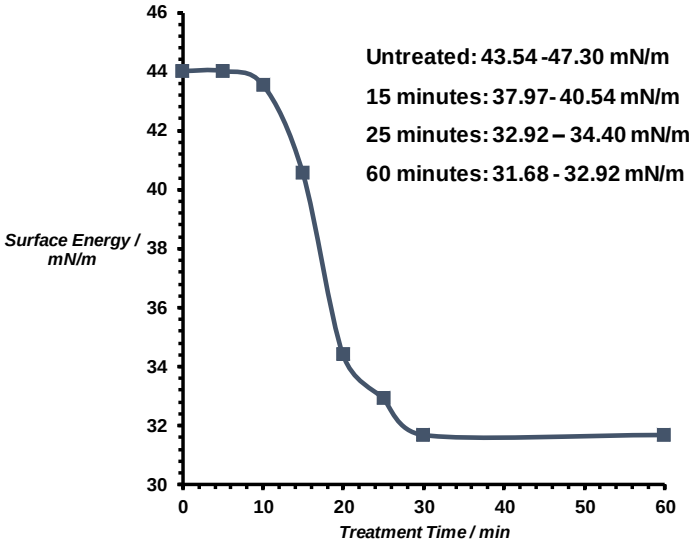
Figure S3. Thread diffusion study in blood samples



Blood diffusion in thread was studied using the distribution of diazepam (A) and its internal standard (IS) to measure analyte-to-internal standard (A/IS) ratios. Untreated thread was dipped in 10 µL of blood samples spiked with diazepam (100 ppb). The region with soaked blood (16 ± 2 mm distances) was then cut into two sections: regions 1 and 2. Each region was placed inside a glass capillary containing a piece of 60 minutes treated thread to facilitate thread spray MS analysis of diazepam. Spray solvent (ethyl acetate with 50 ppb internal standard) was added and allowed to extract for 60 seconds before MS analysis. As shown, there is no significant difference between Regions 1 and 2 for untreated thread. For reference, the 60 minutes treated thread was sampled (dipping into a 10 µL blood sample) to show that the ionization efficiency is greater for this sampling platform.

Figure S4. Surface energy estimation via bracketing

Solvent	σ (mN/m)	χ_{ACN}	χ_{H2O}
1	62.36	0.0149	0.9851
2	55.92	0.0298	0.9702
3	49.39	0.0576	0.9484
Ethylene Glycol	47.30	-	-
DMSO	43.54	-	-
Quinoline	43.12	-	-
4	40.54	0.0950	0.9050
5	37.97	0.1227	0.8773
Cyclohexanol	34.40	-	-
6	32.92	0.2541	0.7459
7	31.68	0.3959	0.6041
8	31.45	0.4851	0.5149
9	30.95	0.5913	0.4087
10	29.30	1	0

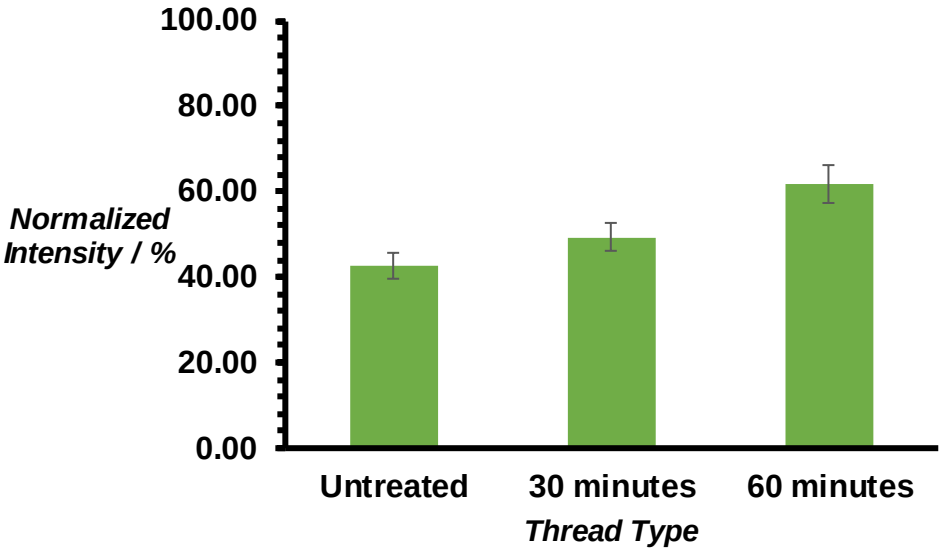


Bracketing experimental curve for threads with increasing treatment times as a function of relative surface energies. Corresponding solvents and surface tensions are also included in the table[1].

A quantitative measure of hydrophobicity is a substrate's surface energy; lower surface energies correlate to more hydrophobic surfaces. Typically, hydrophobicity is determined with contact angle measurements using water droplets and measuring the angle it makes with the substrate. The issue with adopting this methodology for the analysis of threads it two-fold: the threads are nonplanar and porous which would yield incomplete results due to the existence of variable wetting states at different time points. To quantify the surface energies of the untreated and treated cotton threads, analysis via bracketing was conducted. Total wetting (zero contact angle) occurs when the surface tension of the wetting liquid is less than the critical energy of the surface. Therefore, the basic idea in this bracketing experiment is that a liquid drop will wet a surface only when the wetted surface has a lower energy than the initial dry surface. Only such an exothermic reaction will proceed. Thus, casting a drop of selected "inert" liquid onto a surface will lead to only two outcomes: 1) liquid droplet wets the surface and so its surface tension is lower than the critical energy of the surface, and 2) liquid droplet does not wet the surface meaning the surface tension of the liquid is higher than the critical energy of the surface.

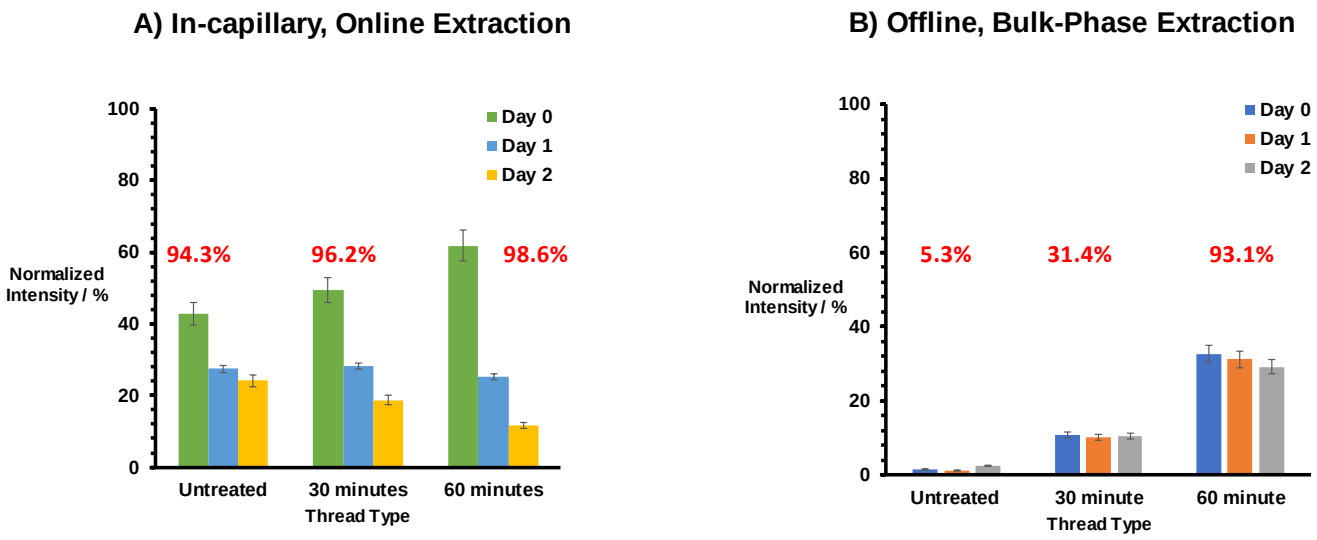
The implementation of this bracketing method utilized solvents of known surface tensions. Solvents 1-7 (Table above, Figure S4), with different mole fractions of acetonitrile (ACN) and water (H_2O), were used in 10 µL droplets along the length of the thread to obtain a range of potential surface tension values. Once visual wetting occurred for multiple droplet spots on the thread for a specific solvent, ethylene glycol, dimethyl sulfoxide (DMSO), quinoline, cyclohexanol, and solvents 8-10 were used to narrow (bracket) the range to approximately +/- 1 mN/m. Figure S4 shows the results of the wettability study. Untreated cotton thread has a surface energy value between 43.12- 43.54 mN/m, which is similar to that of various microcrystalline cellulose (e.g., 44 mN/m for Ceolus KG-802) determined using capillary intrusion[2]. We expect the silanization treatment to decrease surface energies. We investigated the effect of treatment time and found that some of the treated cotton threads could be resolved with the range of solvents that were used. For example, a 15-minute treated thread has a surface energy between 37.97- 40.54 mN/m, while the 60-minute treated thread had a surface energy between 31.68-32.92 mN/m. It can be concluded from this data that as the treatment time increases, the surface energy of the thread decreases confirming that hydrophobicity increases as well.

Figure S5. Ion yield diazepam when using different thread types



Normalized ion intensity of neat solutions of diazepam dried onto various thread types. Samples of diazepam (log *P* 2.82; 100 ppb) were dried onto various thread types and analyzed via thread spray MS. The diagnostic MS/MS product ion intensity from the dried sample was normalized against the corresponding intensity obtained during a wet spray analysis. Overall, >40% of the targeted analyte was able to be detected from each substrate using the 60 s in-capillary online extraction, compared with direct analysis of the same analyte solution without drying.

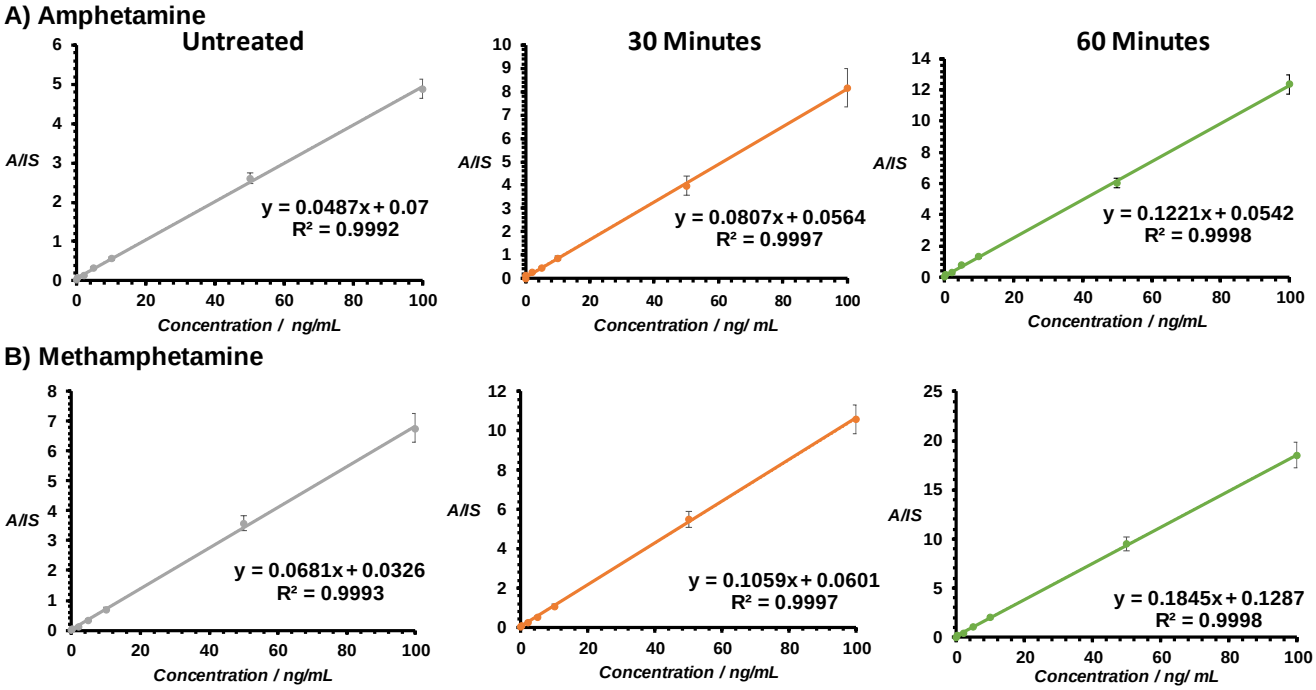
Figure S6. Online vs. offline extraction and thread reusability study



In-capillary, online extraction (A) is compared to an offline, bulk-phase extraction (B). Each thread of each type was extracted multiple times (Days 0-3). The results indicate the used thread can be reanalysed after storage.

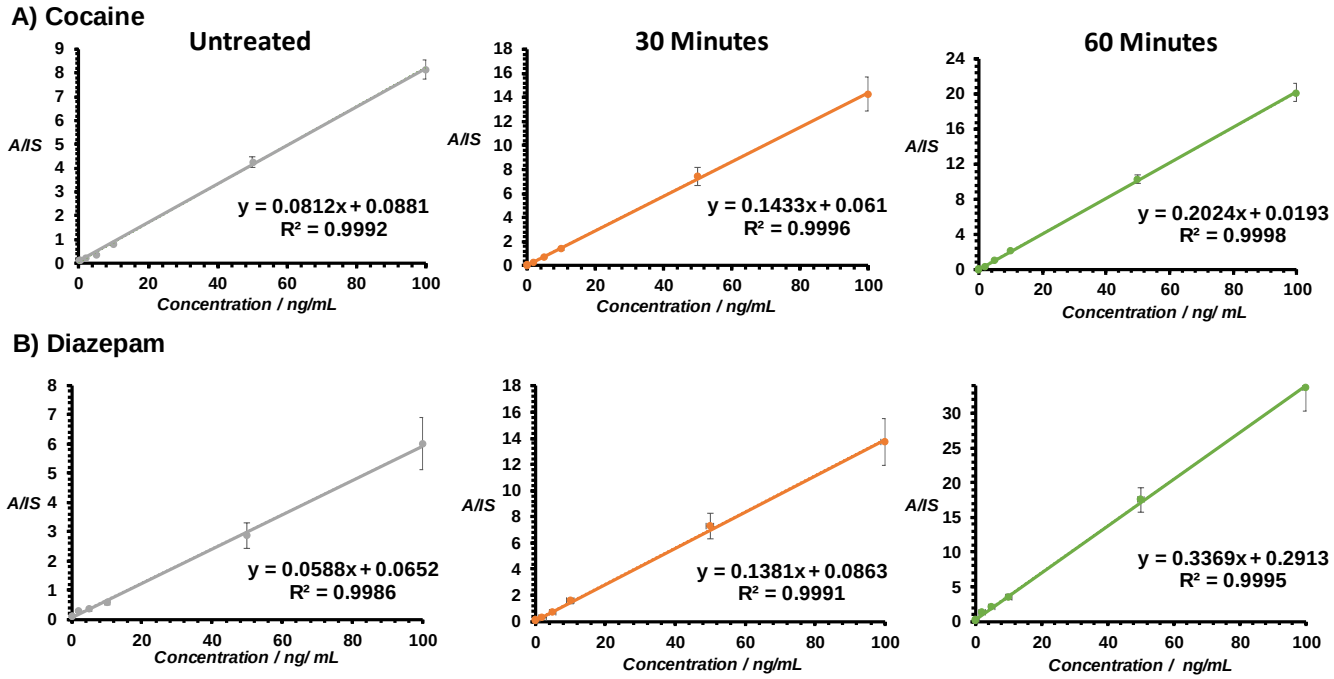
We investigated the use of multiple online and bulk phase extraction analyses separately, Figure S6, for a single thread for dried diazepam samples to find if one method yielded a higher extraction efficiency than the other. To conduct this study, we dried 10 µL of 100 ppb diazepam solutions on a single thread, of each treatment time, and conducted a 60 s online extraction analysis (Day 0) – allowing the extraction/spray solvent to sit inside the capillary for 60 s before MS analysis. We then took that thread, stored it in ambient conditions, and repeated this for Days 1 and 2 and tracked the normalized ion intensity. We conducted the same workflow for another set of threads and instead of performing an online in-capillary extraction, we set them in excess solvent (50 µL) for 60 s and used the collected sample for analysis. This excess solvent volume was optimized to yield the same final volume (20 µL) for thread spray analysis. The extracted solution was then analyzed with a fresh, dry thread. The set of threads that had dried diazepam were stored and re-analyzed for two subsequent days. Overall, for the online extraction set of threads, we were able to extract ~94% - 98% analyte from the substrate. This is because even after the first 60 s extraction period, the spray solvent continues to extract more analyte during MS analysis time, since the thread that the analyte was deposited on is the same thread that is used to spray. The bulk phase extraction set of samples had a wider range of extraction efficiencies, with the untreated thread at ~5% and the 60-minute treated thread at ~93%. This is analyte dependent and diazepam, given its hydrophobicity, can dry on the surface of the hydrophobic threads, making it more available to be washed off in excess solvent. It will absorb into the subfibers of the hydrophilic thread, making it less available to solvent for extraction. Hydrophilic analytes, however, will be more readily extracted in a unit time compared with hydrophobic compounds due to less favourable interactions on the hydrophobic thread. This explains why 80% extraction efficiency was obtained for benzoylecgonine (logP –0.59, Figure 2A) in 60 min treated thread compared with 60% for diazepam (log *P* 2.82; Figure S6A).

Figure S7. Calibration curves for amphetamine and methamphetamine



Calibration curves for A) amphetamine and B) methamphetamine in 10 µL whole blood samples for concentrations 0.2 – 100 ng/mL range. Each data point has 5 replicates for each thread type, untreated, 30 minutes, and 60 minutes treated threads.

Figure S8. Calibration curves for cocaine and diazepam



Calibration curves for A) cocaine and B) diazepam in 10 µL whole blood samples for concentrations 0.2 – 100 ng/mL range. Each data point has 5 replicates for each thread type, untreated, 30 minutes, and 60 minutes treated threads.

Scheme S1. Surface Area Comparisons

Surface area of a cylinder:

$$A = 2\pi rh + 2\pi r^2$$

r = radius of the thread (determined by microscopy)

h= distance blood travels along thread

Untreated Thread:

$$r = 0.175\ mm$$

$$h = 16\ mm$$

$$A = 2\pi(0.175\ mm)(16\ mm) + 2\pi(0.175\ mm)^2 = 17.79\ mm^2$$

60- minute Treated Thread:

$$r = 0.175\ mm$$

$$h = 0.2\ mm$$

$$A = 2\pi(0.175\ mm)(0.2\ mm) + 2\pi(0.175\ mm)^2 = 0.41\ mm^2$$

References

[1] A. A. Rafati, A. Bagheri, M. Najafi, *J. Chem. Eng. Data* **2010**, *55*, 4039–4043.

[2] D. F. Steele, R. C. Moreton, J. N. Staniforth, P. M. Young, M. J. Tobyn, S. Edge, *AAPS J.* **2008**, *10*, 494–503.