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Stir Bar Sorptive Extraction Based on Restricted Access Material for the Direct Extraction of Drugs and Metabolites in Biological Fluids

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Abstract

An experimental biocompatible stir bar sorptive extraction (SBSE) device was prepared using a restricted access material (RAM) as the SBSE coating. The biocompatible SBSE approach was advantageous in terms of simplifying the sample preparation procedure.

The RAM-SBSE bar was able to simultaneously fractionate the protein component from a biological sample, while directly extracting caffeine and its metabolites; overcoming the present disadvantages of direct sampling in biological matrices by SBSE, such as fouling of the extraction coating by proteins.

Analytical Figures of Merit for Caffeine Determination in Plasma:

- Limit of detection (s/n = 3) for caffeine = 25 ng/mL
- Linear over the range of 0.5-100 µg/mL (R^2 = 0.9981)
- Injection repeatability and intra-assay precision % R.S.D. < 8%

The RAM-SBSE device was simple to use, robust and re-useable with over 50 direct extractions of caffeine and its metabolites in biological fluids without significant signal loss.

INTRODUCTION

Stir bar sorptive extraction (SBSE) is a novel extraction technique that utilizes glass stir bars coated with polydimethylsiloxane (PDMS) for extraction of organic compounds, such as environmental contaminates, in aqueous samples. The device operates similar to a conventional magnetic stirring rod, with the PDMS coating enabling direct sample clean-up and analyte preconcentration while stirring. The higher extraction phase ratio of SBSE also provides better recovery and sample capacity over microextraction approaches such as solid phase microextraction (SPME).

Although SBSE has been used for biological sample analysis, limitations with the available PDMS coating exist and include lack of selectivity for more polar compounds and potential fouling of the coating from protein adsorption during extraction in biofluids. This leads to poor reproducibility and recovery of analytes from biological fluids.

The applicability of the SBSE approach to biological fluid analysis was extended via a biocompatible absorbent coating of particles, known as restricted access materials (RAM). The RAM coating was able to fractionate a sample into the protein matrix and the analytes, while simultaneously extracting low molecular weight compounds into the phase's interior (see Figure 1). Various extraction phases, such as C_4, C_8, C_{18} and ion exchange are available to provide a wide range of selectivity.

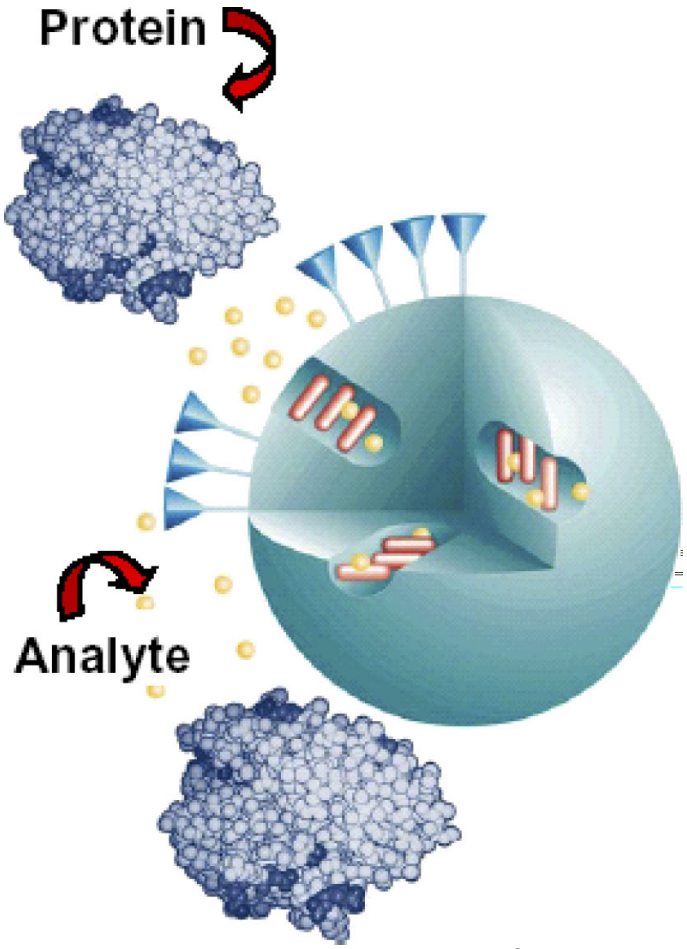

Figure 1. Schematic representation of a restricted access material (RAM).

The novel RAM SBSE device could be directly stirred in biological fluids for analyte extraction without fouling of the coating from proteins and therefore minimizing sample preparation and extraction solvents requirements.

Its ability to directly extract a model compound (caffeine) and various metabolites from plasma, followed by liquid desorption and HPLC-UV analysis was studied.

EXPERIMENTAL

RAM coated stir bars (17 mm x 2 mm o.d.) were prepared with various coating procedures using a 2 part epoxy as a binding agent for the RAM particles.

Extraction:

- An extraction time profile was obtained by stirring the RAM SBSE bar at 1000 RPM in spiked rat plasma samples for various periods of time and measuring the levels of caffeine extracted.
- After the extraction is completed, the bar is removed with a steel rod and placed in the desorption vial.

Desorption:

- A desorption time profile was obtained by stirring the RAM SBSE bar at 1000 RPM in 75:25 (v/v) H_20:ACN.
- After desorption was complete, the stir bar is removed and the sample is injected for HPLC-UV analysis.

Results and Discussion

The coating immobilization procedure is critical since it must withstand the frictional forces associated with high stirring rates during SBSE, providing multiple extractions (> 50) in complex sample matrices.
A fairly uniform coating of the RAM particles on the SBSE device was observed in contrast to the initial smooth surface topography of the glass stir bar support (Figure 2).

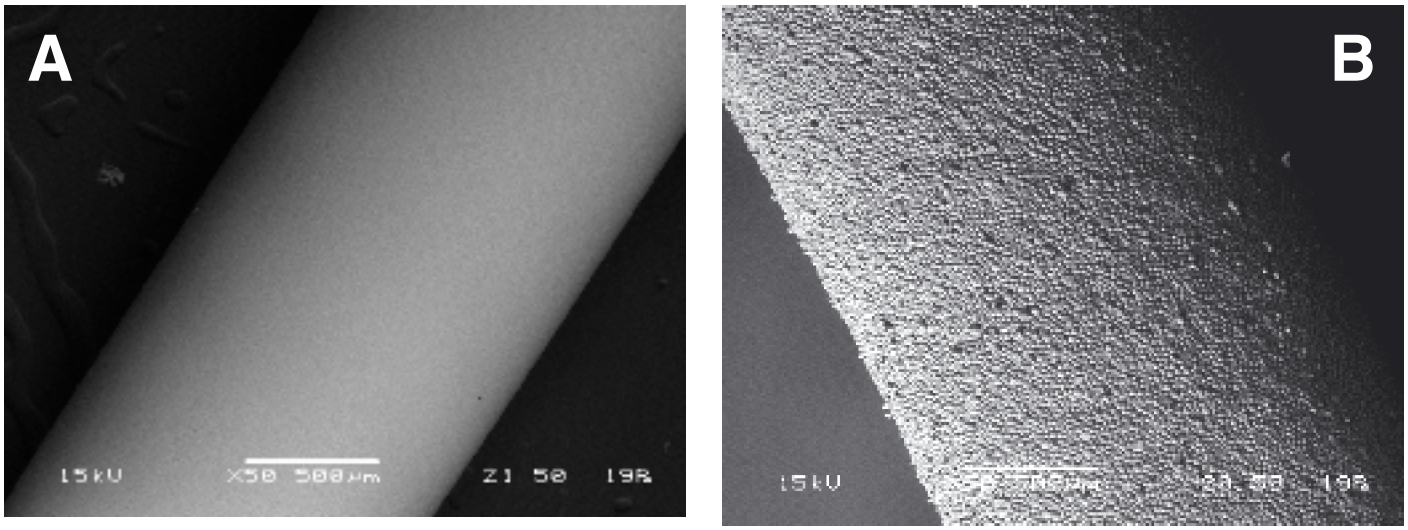

Figure 2. Scanning electron micrographs of glass stir bar (A) and RAM-SBSE bar coating (B).

An extraction time profile was prepared by stirring the RAM-SBSE device in caffeine solutions over a period of time, followed by desorption and analysis (Figure 3).

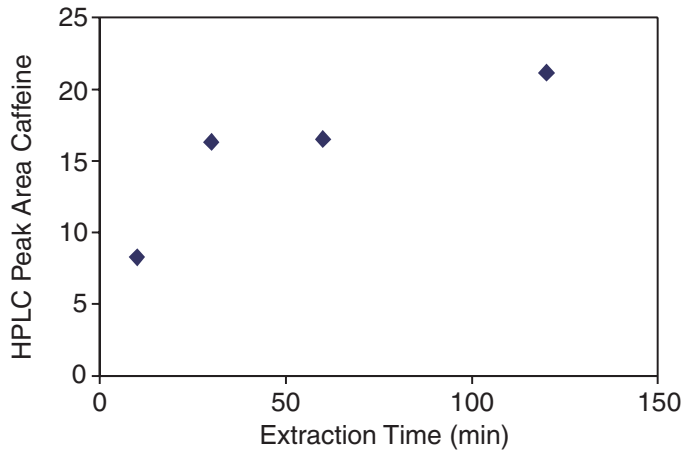

Figure 3. RAM-SBSE extraction time profile of caffeine spiked in rat plasma.

Approximately one hour of extraction was required for the RAM-SBSE bar to reach equilibrium. Since equilibrium is not required for analysis, the developed method utilized an extraction time of 30 minutes to limit the overall analysis time.

Desorption of the extracted analytes was accomplished by stirring the RAM SBSE device in an appropriate solvent. The composition of the desorption solvent was investigated to ensure complete removal of the analytes from the C_{18} extraction coating of the stir bar. A desorption time profile was recorded to determine the time required to ensure quantitative removal of the extracted analytes (Figure 4).

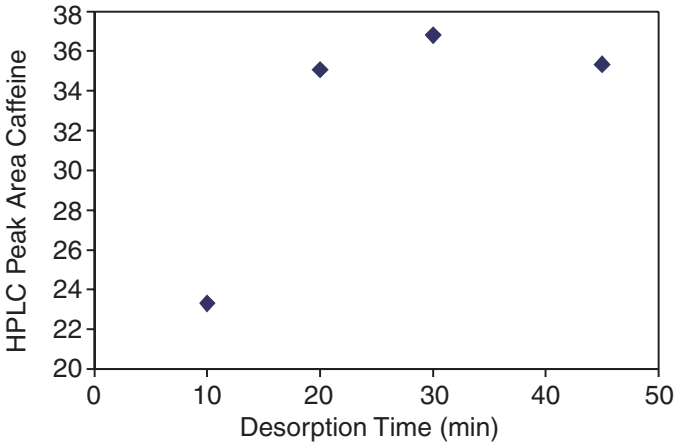

Figure 4. RAM-SBSE desorption time profile of caffeine spiked in rat plasma.

Sample carry-over was evaluated by completing successive desorption steps in fresh solvent; with no detectable amount of caffeine observed in subsequent desorptions.

The robustness of the stir bar was illustrated with over 50 extractions with minimal loss of extraction efficiency (Figure 5). The RAM-SBSE device could withstand repeated and direct exposure to plasma, while providing a consistent HPLC peak area for caffeine extraction.

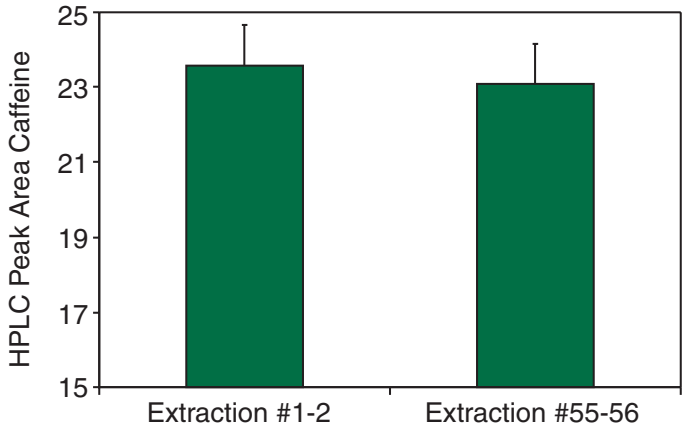

Figure 5. HPLC RAM-SBSE method for caffeine extraction from plasma.

The effect of plasma matrix on the recovery of caffeine was evaluated by comparison to extraction of caffeine in PBS. As shown in Table 1, multiple extractions in both matrices produced similar results indicating complete recovery of caffeine from plasma with good reproducibility (R.S.D. = 8 %).

Table 1. Recovery and Reproducibility of Caffeine Extraction by RAM SBSE.

Sample No.	HPLC Peak Area	
	Caffeine* (PBS)	Caffeine* (Plasma)
1	24	32
2	25	30
3	25	27
4	28	32
5	30	30
Average	27	30
% RSD	8.0	8.0

*Caffeine concentration = 5 µg/mL

Caffeine recovery in Plasma compared to phosphate buffer saline (PBS) = 110%.

A calibration curve was prepared for caffeine analysis in rat plasma using the RAM-SBSE approach. The curve showed very good linearity over a 2 order of magnitude concentration range.

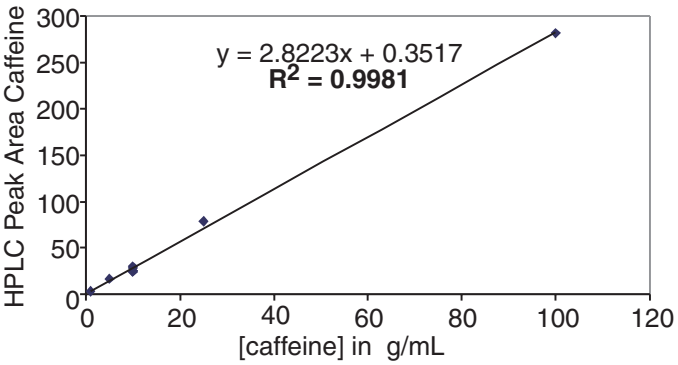

Figure 6. RAM-SBSE calibration curve for caffeine spiked rat plasma.

Blank extractions from plasma were performed to rule out the presence of interferring compounds from the biological matrix extracted by the RAM-SBSE bar that could co-elute with caffeine. An absence of chromatographic peaks at the analyte's retention time confirmed the selectivity of the method.

The results from the RAM-SBSE extraction were compared to results obtained from a standard protein precipitation method. The RAM SBSE provided a much cleaner extract than protein precipitate plasma as can be clearly seen when comparing the resulting chromatograms (Figure 7A and figure 7B). This effect was very pronounced early in the chromatogram, which is significant since many metabolites (polar compounds) will elute early on commonly used C_{18} analytical columns. The plasma spiked with metabolites was successfully extracted using the RAM SBSE device.

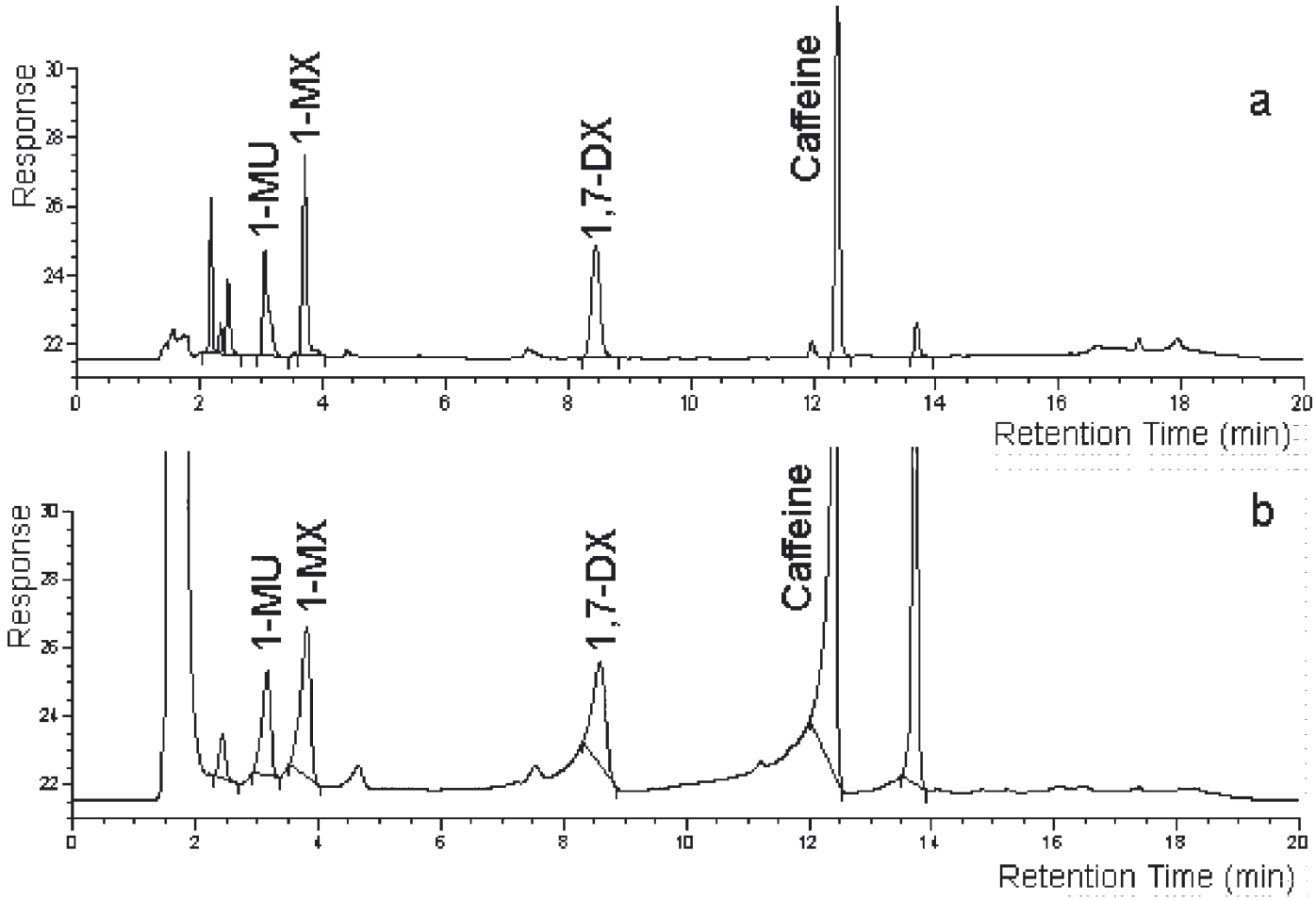

Figure 7. HPLC sample chromatograph of caffeine, 1,7-dimethylxanthine (1,7-DX), 1-methylxanthine (1-MU) and 1-methyluric acid (1-MX) in plasma (a) RAM-SBSE extraction and (b) protein precipitated plasma sample.

Conclusions

An experimental RAM-SBSE bar was developed for direct extraction and desorption of caffeine and metabolites from biological samples. The binding capacity, extraction efficiency and reproducibility of extraction were suitable for UV determination over a wide range of caffeine concentrations in plasma. The bar was reusable (> 50 times), providing cleaner extracts than existing sample prep methods and simplified sample handling.

Selectivity towards many classes of drugs is possible using with C_4, C_8, C_{18} or ion exchange extraction phases located inside the pores of the coating.

The simplicity and compatibility of SBSE with many analytical systems such as HPLC and GC extends the versatility of the RAM-SBSE device for bioanalysis. To enable a more automated procedure, extractions by RAM-SBSE bar coupled to thermal desorption GC/MS are considered for future work.

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