RESTEK Pure Chromatography

Benefits of Centrifugation on Oral Fluid Samples

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Introduction

Oral fluid is an increasingly popular matrix for drugs of abuse analysis. The matrix itself is simple to collect and work with, however most collection kits add a few extra variables to consider. Among these variables is the removal of liquid from the sponge into the buffer solution to ensure full recovery of matrix and analytes. There are two primary techniques to empty the sponge: manual compression or centrifugation. These procedures can be paired with devices that aid in the technique, such as devices to help with manual compression or those to help hold the sponge in place while centrifuging. The primary objective is to maximize volume recovery, to ensure complete analyte recovery. In this study, manual compression and centrifugation will be compared to known controls to assess volume and analyte recovery.

Sample Preparation

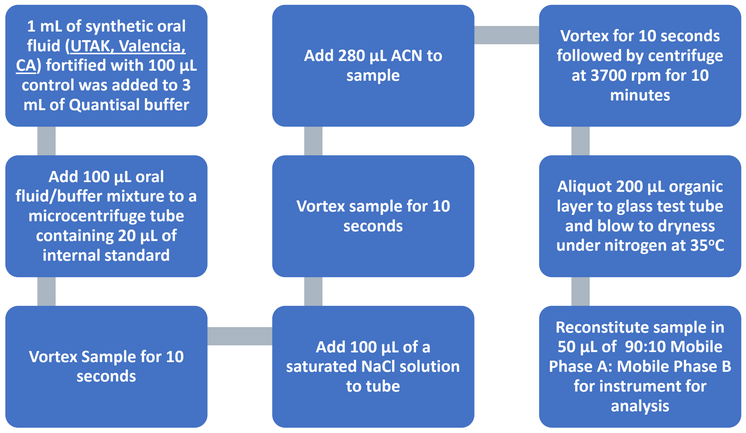
Techniques for Emptying the Sponge

Manual Compression
- Pressing the sponge against the side of the tube to attempt to drip as much liquid out of the sponge as possible.

Centrifugation
- Folding the sponge above the buffer solution and centrifuging the sample to remove liquid from the sponge.

Sample Extraction

Figure 1. SALLE Workflow for Oral Fluids.



Sample Preparation Time Comparison

It can be argued that the addition of a centrifugation step can add time to the method; a comparison was done to calculate the time per sample using manual compression versus centrifugation. Results can be found in Table 1, below. While the time is longer for centrifugation at lower sample volumes, when preparing 96 samples, the sample preparation takes about 20 minutes less time when compared to the manual compression technique.

Table 1. Volume Recovery Comparison Based on Volume.

Technique	12 Samples	48 Samples	96 Samples
Centrifugation	13.27 min	28.80 min	36.13 min
Manual Compression	7.20 min	23.07 min	57.60 min

**There may be an additional time increase for centrifugation time depending on size of centrifuge.*

Volume Recovery

To determine volume recovery for emptying the sponge two different experiments were tested. One using weight and the density of oral fluid, while the other used gravimetric volume of the oral fluid to determine how much was recovered from the sponge.

Determination of Volume Recovery by Density

In this experiment, 1 mL of oral fluid was collected on the sponge. Because some liquid is always retained in the sponge, the buffer was not included in this experiment since the ratio of oral fluid to buffer was unclear. The sponge then underwent each of the techniques and the oral fluid in the tube was weighed. This was completed in triplicate to obtain an average weight for each technique. The calculation was completed using the density of the oral fluid, which was found to be 0.9932 g/mL. To calculate total volume recovered; the weight was converted using the density, using equation $d = M/V$. To highlight the potential loss of sample; the volume of oral fluid being retained by the sponge was also calculated.

Table 2. Recovery Comparison Based on Density.

Technique	Avg Weight (mg)	Volume (µL)	Volume Retained in Sponge (µL)
Manual Compression	142.7	143.7	856.3
Centrifugation	718.9	723.8	276.2

**This experiment used oral fluid only, no buffer was used.*

Determination of Volume Recovery by Gravimetric Analysis

The next experiment for volume recovery was to measure the actual volume using a graduated cylinder. In this experiment, 1 mL of oral fluid was added to a sponge, and the sponge was added to the tube with the buffer (3 mL). The samples then underwent the different techniques in triplicate. The liquid in the tube was poured into a graduated cylinder and the volume was recorded. Table 2 below outlines the data for this experiment.

Figure 2. Volume Recovery for Manual Compression

Figure 3. Volume Recovery for Centrifugation

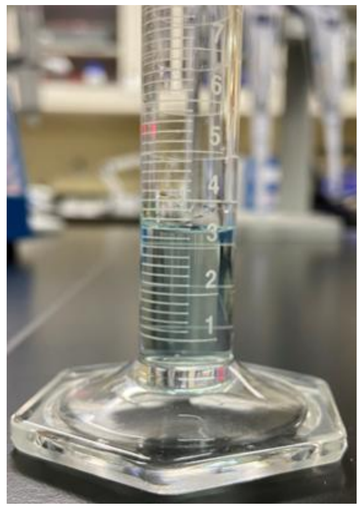


Table 3. Recovery Comparison Based on Volume.

Technique	Avg Volume (mL)	Volume Retained in Sponge (mL)
Manual Compression	3.2	0.800
Centrifugation	3.4	0.600

Analyte Recovery

Analyte recovery was assessed by spiking a known concentration into synthetic oral fluid followed by saturation of the sponge with the oral fluid before submerging it in the buffer solution. This concentration was analyzed against a calibration curve that was prepared without using the kits and only using oral fluid and buffer solution, eliminating any recovery issues from the kit. The two recovery techniques were compared and assessed for accuracy, precision, and peak area. These results can be found in Figure 4, 5, and 6.

Analyte Recovery Continued

Figure 4. Accuracy of Analyte Recovery for Manual Compression vs Centrifugation.

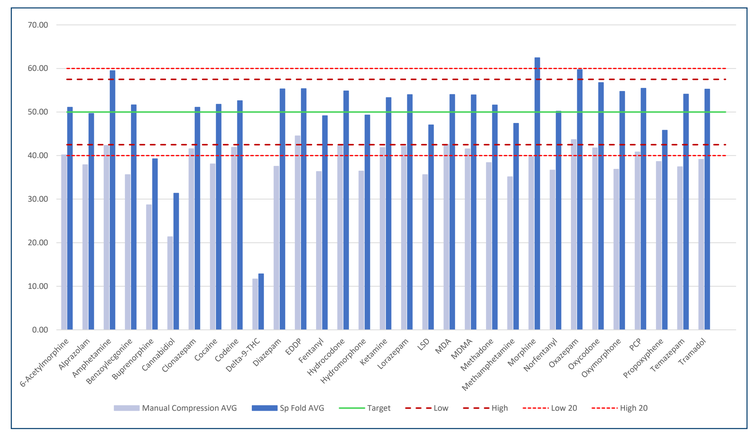


Figure 5. Precision of Analyte Recovery for Manual Compression vs Centrifugation.

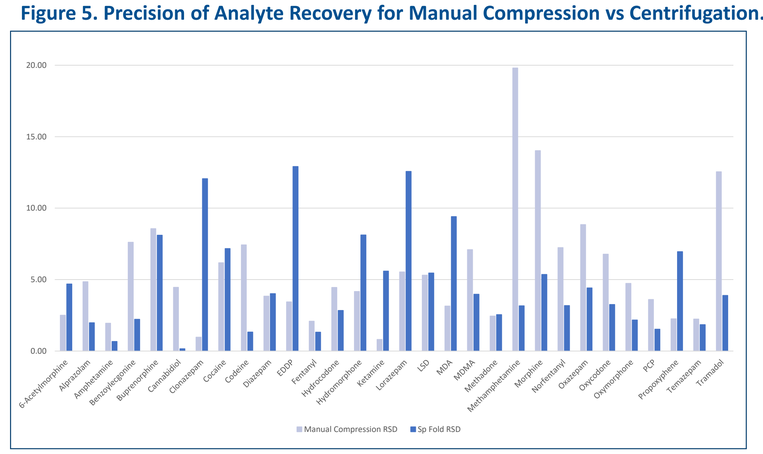
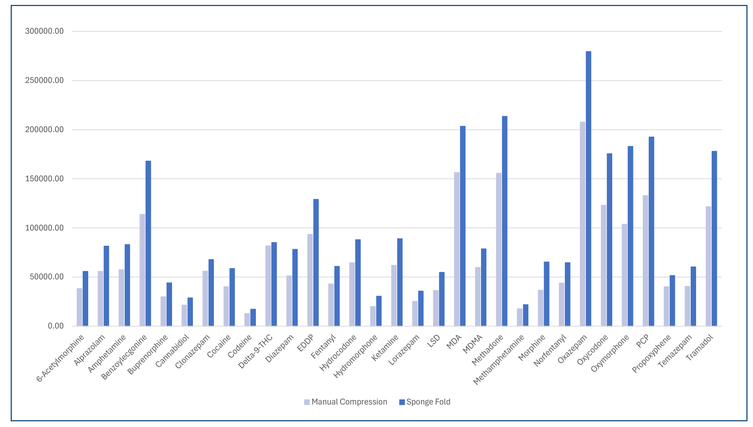


Figure 6. Peak Area for Manual Compression vs Centrifugation.



When using centrifugation, peak area increased for all analytes compared to the manual compression technique. Additionally, 28 of the 31 analytes were greater than +/- 15% of the target value when using manual compression. This improved to just 6 being outside of the acceptable window when using the centrifugation technique. The centrifugation technique also aided in precision, showing 18 analytes having lower %RSD when using this technique compared to manual compression.

Conclusions

Overall, there are many benefits to oral fluid samples when using centrifugation as an extraction technique. When preparing large batches of samples, it is faster to use centrifugation versus manual compression. Volume recovery increased when using this technique as compared to manual compression. Results shows increase in volume, peak area and recovery for all analytes. When performing quantitation analysis, the centrifugation technique benefits both accuracy and precision as well.

Disclosure: I have (or a member of my immediate family has) a financial relationship with a company as defined in the AACC policy on potential bias or conflict of interest.