Validation of Automated Extraction (Partition Chromatography) of β-Carotene using VERSA Mini Liquid-Liquid Extraction Workstation

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I. Abstract

To provide an automated solution to the liquid-liquid extraction (partition chromatography) process for downstream applications, Aurora Biomed Inc has validated the VERSA Mini Liquid-Liquid Extraction Workstation. For this validation, the water-alcohol (1:1) sample was spiked with β-carotene at 850 µg/ml. The sample (liquid phase) and the hexane solvent (organic phase) were mixed either by auto-shaker provided on the deck of the workstation, or by auto pipette-action of the workstation. The extraction profile showed that 90.1, and 9.3% of the active compound was partitioned in the first, and second extraction, respectively. The third, fourth, and fifth extraction had 0.3, 0.1, and 0.02% efficiency, respectively. The mixing of the sample and the solvent was effectively carried by the auto-shaker at 1100 rpm. The performance of the shaker at 700 rpm was also compared with 1100 rpm. The latter speed was observed to be more effective than the former. The extraction profile of the automated operation was found to be better than manual performance.

II. Introduction

Liquid-liquid extraction (partition chromatography) is among the most widely used sample preparation methods. This technique normally uses the immiscible solvent pair for the extraction of active component. Over the years, this old technique has seen few changes but still is among the most popular in routine sample preparation although it is time consuming to perform[1]. Therefore, the ability to automate this process allows for much faster sample preparation.

Aurora Biomed's VERSA Mini Liquid-Liquid Extraction workstation was developed to provide more efficient sample enrichment, faster sample preparation, and easier performance, compared with manual extraction, in additions to increase increasing throughput. This workstation is ideal for researchers using liquid-liquid extraction techniques in research areas such as combinatorial chemistry, medicinal chemistry, biotechnology and diagnostic science.

III. Objectives

- To validate the workstation
- To optimize the automated protocol
- To compare automated versus manual performance
- To study the reproducibility of the automated performance
- To observe efficiency of the automated performance

IV. Materials & Methods

The validation of the automated process of liquid-liquid extraction on VERSA Mini workstation was conducted as follows:

1. Sample preparation: The samples were prepared by spiking β-carotene (850ug /ml) in water : alcohol (1:1) solution as an aqueous phase. Organic solvent hexane was used as an extraction solvent to provide an organic phase in the partition process.

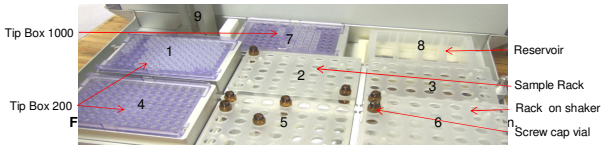
2. Deck equipment (Figure 1):

a. **Sample rack:** The samples added to 2ml screw-capped vials were placed in a rack on deck position 2.
b. **Target rack:** The target rack containing appropriate number of vials was placed on the shaker position 8.
c. **Reservoir:** Alcohol and hexane were contained in the reservoir at deck position 8.
d. **Tip boxes:** The tip boxes (200 µL and 1000 µL) were placed on the deck position 1 and 4, respectively.

3. Automation protocol: The automated protocol for liquid-liquid extraction is depicted in Figure 2.

4. Manual Performance: The process was also carried manually using a standard manual pipettor

5. Analysis: After the extractions,the samples were evaporated and were reconstituted in hexane just before taking the absorbance (OD_{450}) of B-carotene using Spectrumlab 22C spectrophotometer [2].



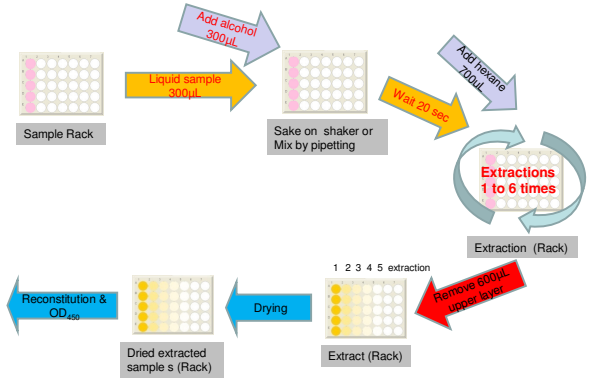


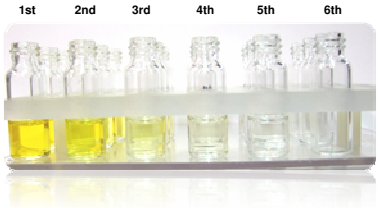
Figure 2 Automation protocol for liquid-liquid extraction using VERSA Mini workstation

V. Results

Q 1: Was it possible to auto-aspirate the organic phase following phase partition?

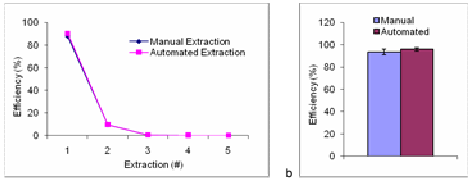
Answer: As shown in the picture, the height of the tip was auto adjusted to aspirate the organic phase only leaving a desired amount of the upper layer.

Q 2: Did the automated extractions appear visually effective?



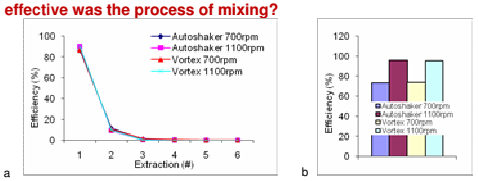
Answer: The comparative visual appearance of the extractions shows that the first extraction was quite effective and the fifth one was significantly clear of the active compound.

Q 3: How many extraction steps were effective in comparison to the manual performance?



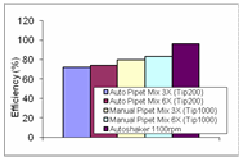
Answer: The extraction of β-carotene was found to be effectively carried out in the first two extractions where about 90.1 and 9.3% of the active compound was procured, respectively. The automated extraction was slightly better than the manual performance as shown in figure b.

Q 4: How was mixing of the aqueous phase (sample) and the organic phase (hexane) carried out on the deck, and how effective was the process of mixing?



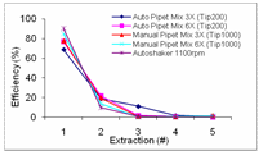
Answer: The mixing of the sample was carried using shaker. Two different speeds (700 rpm and 1100 rpm) of the shaker were tested. The 1100 rpm speed was found to be significantly effective as it partitioned 90.1%, while at 700 rpm, the extraction was 86.1%. In the manual performance, the mixing was carried by vortexing at (700, and 1100 rpm). The results were quite comparable.

Q 4: Can the workstation mix the aqueous phase (sample) and the organic phase (hexane) by pipet-action? How effective was the mixing process?



Answer: The mixing of the solvent and the sample was also studied by pipette-action using a 200µL tip. Three times pipette-mixing was observed to be less effective than six times pipette-mixing where the liquid was aspirated from the bottom of the vial and dispensed at the top of the liquid layer. In addition, in the manual performance, when mixing was carried with a 1000µL tip, the mixing was found to be slightly better than automated. However, the automated shaking appeared more effective than manual or automated pipette-mixing.

Q 5: What was the extraction profile of the pipette-mixing?



Answer: The profile of the first to fifth extraction clearly show the efficiency of pipette-mixing and shaker mixing at different steps.

VI. Conclusion

The VERSA Mini Liquid-Liquid Extraction workstation can effectively carry extraction protocols, increasing throughput and matching or increasing accuracy and precision over manual performance.

VII. References

1. Majors RE: LCGC, 2002; 20(11): 1098–1113 .
2. Saushkina AS and Karpenko VA: Pharm Chem J, 2006; 39: 618-620

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