Automation of Sample Preparation and Introduction into NMR Tubes for Spectroscopy using VERSA™ Workstation

I. Abstract

Automation of sample preparation and introduction into nuclear magnetic resonance (NMR) spectroscopy tubes is required for high throughput NMR analysis, as high-throughput applications are hindered by manually loading and handling individual NMR tubes. However, automating NMR sample preparation is a challenging project as it may involve serial dilutions, mixing of different reagents and samples and liquid transfers. To solve these problems, Aurora developed a protocol enabling automation of all NMR-associated tasks, such as sample preparation and sample introduction using Aurora's 8-channel VERSA™ 1000 Workstation equipped with a plate shaker and ReagentDrop liquid handling module. In the validation protocol, bovine serum was added to serially diluted reagents in 96-well format, mixed with on-the-deck plate shaker, followed by addition of another reagent, mixed again, and followed by dispensing into the bottom of 5 mm NMR tubes arranged in 96-well format. Using this configuration, the VERSA™ 1000 can prepare 4 sets of 96 NMR tubes (396 NMR tubes) in one load, in one hour.

II. Introduction

An NMR tube is a thin glass walled tube (1 – 5 mm diameter, with fill volume from 5 – 550 µL) used to contain samples in NMR spectroscopy (Figure 1). NMR spectroscopy is a versatile technique with applications including: qualitative and quantitative purity analysis for synthetic samples, quality control of compound libraries, screening small protein-binding molecules for pharmacological applications, analyzing metabolites in biological fluids like blood and serum, and searching for biomarkers or drug degradation products[1]. However, sample preparation provides a productivity bottleneck challenge for automation[2]. Dispensing highly volatile or viscous solutions into typical 5 mm ID NMR glass tube presents unique problems.

Automatic sample preparation systems for NMR spectrometers, which permit running multiple samples unattended, have been commercially available for some years[3]. In this era of information technology, NMR automation utilizing sample preparation systems has become a valued tool for both industry and academia. Depending on the laboratory's needs or goals, automation may involve high-throughput screening, overnight automation or multi-user open access, for example. Here we report preliminary studies into the feasibility of automating the preparation of such samples where prepared samples may be removed by temporarily interrupting the run.

III. Objectives

1. To automate sample preparation for NMR tubes
2. To carry auto dilutions in series in 96-well format.
3. To automate a method for handling sensitive biomaterials like blood for analyte analysis.
4. To check for cross contamination

IV. Materials & Methods

The validation of this process was carried on VERSA™ 1000 Workstation and the set up was conducted by mimicking a procedure using food color solutions

A. Deck setup configuration: The configuration of the workstation deck set up is shown in figure 1a.

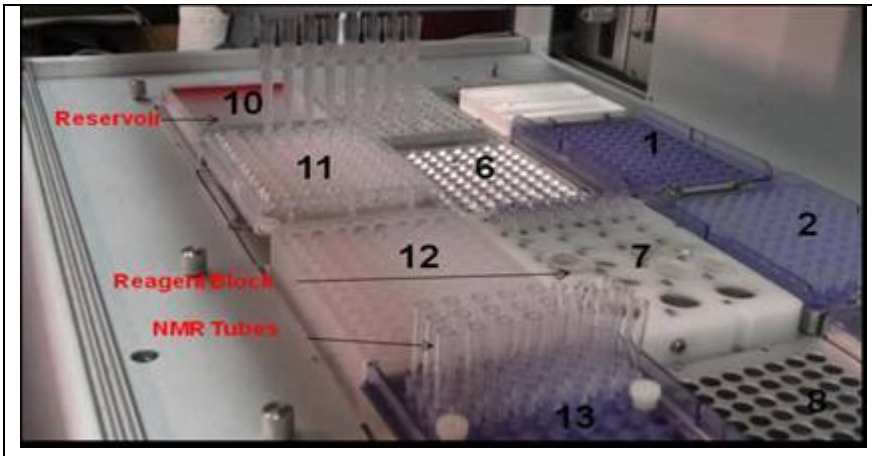

Figure 1. a: Deck configuration of the workstation showing NMR tubes, and other accessories. **b.** Showing relative size of NMR tube

B. Procedure: The following procedure was followed for validation of the workstation for NMR sample prep:

1. Addition of diluent to the preparation plate: 100 µL of the diluent was added to the preparation plate using the VERSA™ 1000's ReagentDrop feature.

2. To the preparation plate containing 100 µL of diluent added at the step # 1, 100 µL of the another reagent (spiked with rubidium) from reservoir placed on the deck was added to the first column of the plate using 8-channel robotic pipettor.

3. A two fold serial dilution of the reagent added at step # 2 into the diluent, was carried using 8-channel robotic arm. This step was followed by addition of 50 µL of sample to the reaction plate. The preparation plate was shaken on the automatic shaker for 30 second at 900 rpm to mix the reagents. The sample from the plate was analyzed on ICR8000 for comparison with manually processed samples.

4. To a deep well reaction plate set up on the deck next to the preparation plate, 300 µL of another reagent was added using ReagentDrop device.

5. 50 µL of the diluted reagent was transferred from the preparation plate to the corresponding deep well reaction plate. The reaction plate thus prepared was ready for the transfer of its contents to the corresponding NMR tube arranged in 96 well-SBS format.

6. Using 8-channel pipettor, 350 µL of the reaction volumes were transferred from the deep well plate to the NMR tubes.

V. Results

The results from the robotic automation of the process are presented below supported by the data.

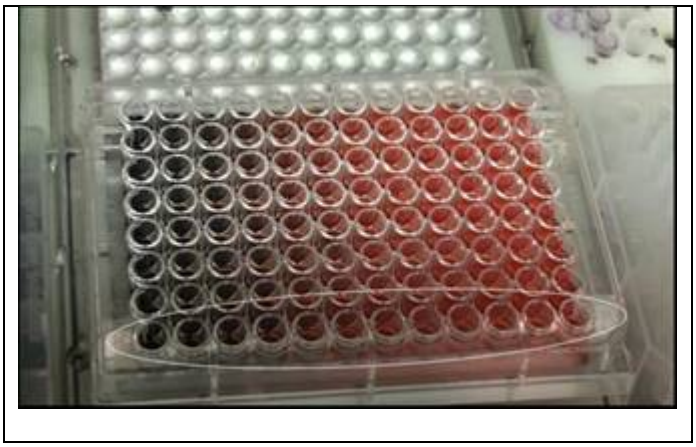

Figure 2.The preparation plate showing two fold serial dilution of the reagent followed by addition of the artificial blood sample. The reaction plate is lying on the plate shaker equipped on the deck of the workstation.

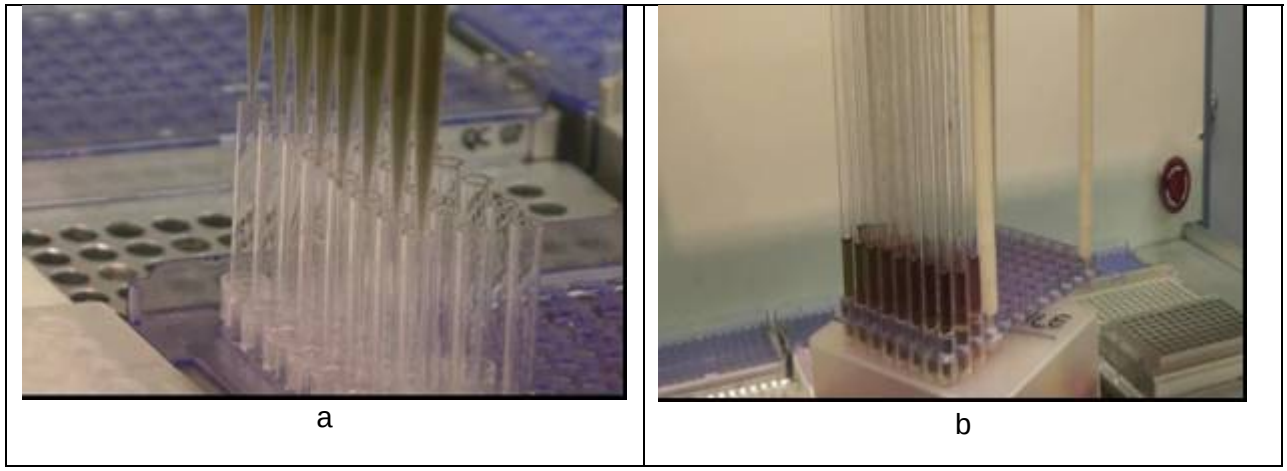

Figure 3. **a.** Robotic arm transferring samples to the NMR tubes (upper part shown) using 8-channel syringe pipettor. **b.** View of the filled NMR tubes (bottom part shown) and a uniform distribution of the samples into the tubes.

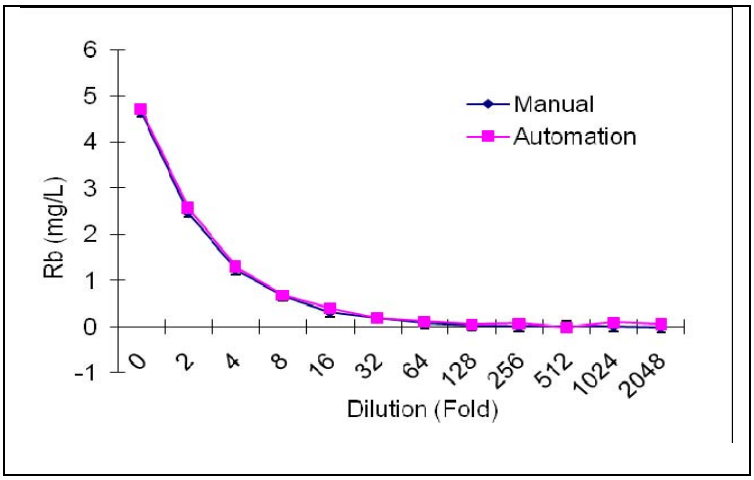

Figure 4: Comparison of data from manual and automated dilution of samples showing standard deviation error bars (n=8).

VI. Conclusion

- VERSA™ 1000 workstation can be used to prepare samples for NMR spectroscopy.

- VERSA™ 1000 Workstation is capable of automating processes involved with reagent additions and dilutions (pipette mixing or shaker mixing).

VII. References

1. Soares DP and Law M. NMR: review of metabolites and clinical applications. Clin Radiol. 2009 Jan;64(1):12-21

2. Gary A. McClusky et al. Automation Of Structure Analysis In Pharmaceuticals R&D http://www.zymark.com/

3. Robert Meinecke. http://www.esrf.eu/events/conferences/past-conferences-and-workshops/psdiXV/Talks/RobertMEINECKE.pdf