USING LIQUID LEVEL DETECTION TO MEASURE PARTITION COEFFICIENTS OF VANILLIN IN BIPHASIC SOLUTIONS

APPLICATION NOTE AN1049

BENEFITS

- Liquid level detection to fully automate sample preparation of biphasic mixtures
- Allows for precise automated control over probe position in liquid sample regardless of sample volume or container

ADDRESSED ISSUES

- Reduction of consumable use via automation
- Elimination of additional manual steps needed without liquid detection capabilities
- Complete automation of sample preparation process involving complex samples

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INTRODUCTION

Sample preparation for any form of analysis can be tedious and time-consuming, and efforts to automate this process have been long and fruitful. Today, many different types of liquid handlers exist that can perform a variety of functions to increase throughput tremendously. However, sometimes samples can be complex and harder to work with than normal, leaving manual preparation the only feasible solution. For example, in the branch of liquid-liquid chromatography, biphasic solutions are extremely common. If a liquid handler's probe position cannot be set when aspirating, a complex sample like a biphasic solution would need to be split into two different tubes to sample each layer for analysis. With the liquid level detection (LLD) capabilities that Gilson liquid handlers offer, samples from both layers of a biphasic solution can be analyzed without needing to have each layer in a separate tube. This can greatly shorten workflows, increase efficiency, and increase the scope of what automation can take over in terms of sample preparation.

In liquid-liquid chromatography, there is a well-developed, commonly used system of solvents known as the Arizona solvent system[1]. It is a set of biphasic systems comprised of 23 different starting ratios of heptane, ethyl acetate, methanol, and water categorized from A, being the most polar, to Z, being the least polar. This well-developed system offers a great starting point in method development for creating separation workflows but can require the preparation of many samples that naturally separate into two layers. If using a liquid handler to dispense the appropriate amounts of solvent, it would normally then need to be manually separated into two vials before allowing automation to continue sample preparation (Figure 1). To screen through the entire Arizona series without liquid level detection, one would need to prepare 23 biphasic solutions and then split each one to make 46 separate solutions before letting automation take care of the rest of the preparation, which can be extremely time-consuming and still require a technician to handle separating the phases in the middle of the workflow.

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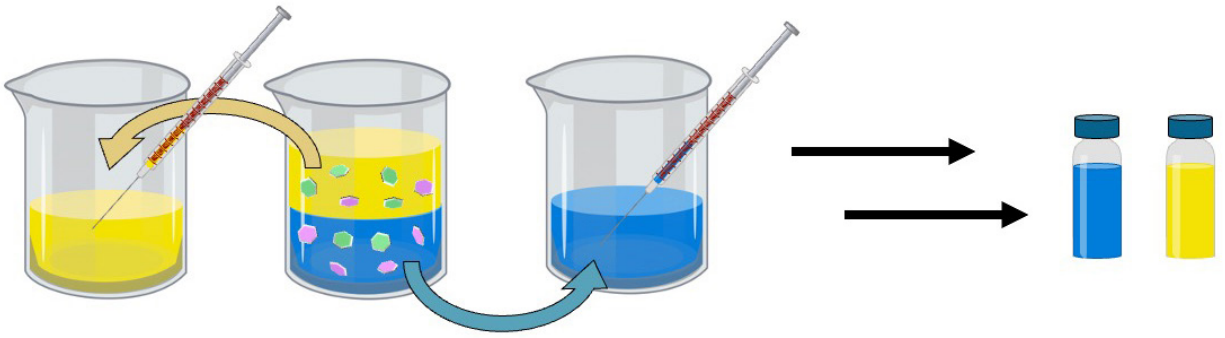

Figure 1

Without liquid level detection, a biphasic sample needs to have the different layers manually separated to have automation make a sample of each layer.

A Gilson GX-271 Liquid Handler[2] was programmed with custom tasks to dispense the four solvents used in the Arizona solvent system series into 23 different tubes containing a small amount of vanillin, in amounts that lead to a biphasic solution comprised of 1 mL of each phase. Immediately after, the liquid handler then transferred an aliquot of both the resulting top and bottom layer of each tube using liquid level detection capabilities into separate HPLC vials for further analysis. In approximately 90 minutes, the GX-271 was able to use liquid level detection to prepare all 46 unique vanillin samples from 23 biphasic solutions. Four pairs of these samples were randomly selected and underwent further HPLC analysis to calculate the partition coefficient of vanillin made by the liquid handler. These values were then compared to similar samples prepared manually using standard pipettes to confirm the accuracy of the GX-271.

MATERIALS AND METHODS

All reagents and solvents were obtained from Sigma-Aldrich and used as is. Instrumentation included a GX-271 and a Gilson VERITY® 4120 Dual with Tee Syringe Pump. A code 338S and 345 rack were used on the GX-271 to hold HPLC vials and 16 x 150mm test tubes, respectively. Heptane, ethyl acetate, methanol, and water were contained in 700 mL containers on the GX-271 bed. Analytical HPLC analysis of samples was performed on a Shimadzu LC2030C 3D Plus HPLC configured with a Restek Raptor ARC-18 column.

A small quantity of vanillin was distributed across the 23 different test tubes. Using Gilson TRILUTION® LH Software, the first method was constructed using 23 custom dispense tasks — one corresponding to the solvent ratios of each letter of the Arizona series. A subsequent transfer method was composed of two back-to-back transfer tasks utilizing liquid level detection to create the 46 HPLC samples, one using the tube bottom as the target for the bottom layer aliquot and one targeting the top layer using liquid level detection for the other aliquot. These transfer steps were performed immediately after dispensing solvent across all 23 tubes. Four Arizona systems of vials were then selected for further HPLC anaylsis, and two more sets of samples were remade with the liquid handler to test the consistency of the liquid handler. 0.1 µL from each selected vial was injected onto the Restek analytical HPLC column and relative concentrations of vanillin between the top and bottom layer for each pair of samples were compared to calculate vanillin's partition coefficient in each solvent system.

The same analysis was performed on a set of samples prepared in triplicate by hand and compared with the automatically prepared samples. Manual samples were prepared by adding the four solvents in ratios corresponding to each letter of the Arizona system into a test tube. The tube was then shaken by hand, allowed to settle, and 1 mL of each layer was then added to a separate vial containing a small amount of vanillin. This vial was shaken, and a small aliquot of

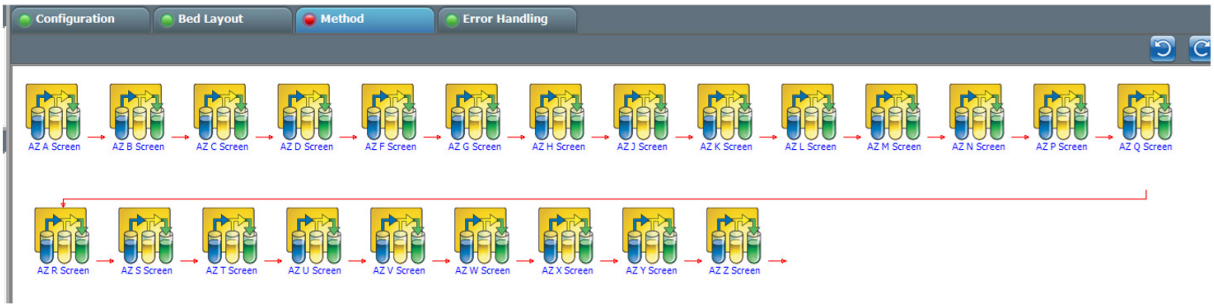

Figure 2

Custom tasks available to dispense 2 mL of mixed solvent to create a 1:1 mixture of organic/aqueous layers corresponding to each Arizona letter.

Table 1

Solvent ratios for selected Arizona system letters and vanillin partition coefficients (K_p) calculated from the analysis of manually and automatically prepared samples. Measurements were performed in triplicate.

AZ system	Heptane	Ethyl Acetate	Methanol	Water	K_p (manual)	K_p (auto)
B	1	19	1	19	19.02±2.23	16.08±1.68
J	2	5	2	5	4.91±0.87	5.26±1.07
N	1	1	1	1	0.33±0.15	0.43±0.11
S	5	2	5	2	0.030±0.005	0.026±0.001

each layer was then subjected to the same analysis performed on the samples prepared by the GX-271.

RESULTS AND DISCUSSION

Using TRILUTION LH, custom tasks were made for the preparation of each Arizona letter (Figure 2). Each task was designed to aspirate and dispense the four solvents, heptane, ethyl acetate, methanol, and water, for any given Arizona letter in one concerted action. The volumes needed for each Arizona letter were able to be preprogrammed into the task to dispense a total of 2 mL of the four solvents that would separate into equal 1 mL phases to increase automation efficiency (Figure 2). Each Arizona letter was made to be its own task, so that in future runs there would be flexibility in deciding which specific letters are to be tested if a full A-Z screen is not needed.

Next, for transferring the solution into HPLC vials for further testing liquid level detection allowed for a two-step transfer task to make two different samples from the same tube. The first was a normal dispense step that used the “tube bottom” setting as its target to transfer the bottom layer into a vial. The second step was also a dispense task but employed the liquid level detection functionality to stop probe movement as soon as it detected solution contact. This allowed for precise aspiration of the top layer of the biphasic sample into a separate vial and showcased the GX-271’s ability to prepare two unique samples from the same tube. This also allowed us to use half the number of test tubes compared to if liquid level detection was unavailable.

The time taken to prepare a full A-Z screen of 46 samples was approximately 91 minutes; approximately 50 minutes to dispense all 23 combinations of the four solvents and approximately 41 minutes to then transfer an aliquot from all 46 layers into individual HPLC vials.

Extrapolating the time it took to prepare the manual samples to a full AZ screen was only a little bit longer than the time it took for the liquid handler to prepare the full set. However with automation, samples can be prepared overnight without a technician present and the rinse step between dispense steps allows for the liquid handler to use a single probe to carry out all the tasks. Preparing a full set of 46 samples manually would present countless opportunities for human error to be introduced and most likely go through over 100 tips if using pipettes.

From the total pool of vials, samples corresponding to four randomly selected Arizona letters (B, J, N, S) were then analyzed and compared against samples made manually using pipettes. Samples for these four Arizona systems were made in triplicate both manually as well as automatically. Each prepared sample was then analyzed via HPLC in triplicate to get consistent values. Data shown in Table 1 shows the comparison of the calculated partition coefficient of the four tested Arizona systems using both automated and manual methods. The data shows that the partition coefficients of vanillin in the tested Arizona letters are comparable between the automated preparation and manual preparation, and in many cases the variance using the automated liquid handler was smaller even without the need for an additional dedicated automated mixing step.

CONCLUSION AND BENEFITS

The Gilson GX-271 Liquid Handler can help automate sample preparation to streamline processes and workflows. In this application note, we have demonstrated just one example of this by taking a common but complicated screening method for optimizing liquid-liquid chromatography run parameters and automating the process. We have also showcased the use of liquid level detection in the precise liquid handling of biphasic solutions. Performing a full Arizona screen manually could take a technician several hours to accomplish, with the added risk of possible human errors.

The benefits highlighted in this article include:

- Reduced labor hours
- Streamlined sample preparation of complex solutions
- Decreased opportunity for human error
- Reduced consumable use

While not discussed here, the GX-271 is configured with a bed that can accommodate many different racks with a multitude of formatting options for additional applications. This lends itself to being capable of automating other complex preparative processes as well.

REFERENCES

1 https://doi.org/10.1016/j.chroma.2020.461426

2 https://www.gilson.com/default/gx-271-liquid-handler.html