AUTOMATION OF POST-PURIFICATION ANALYSIS SAMPLE PREP

TECHNICAL NOTE TN224

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INTRODUCTION

Following successful purification of small molecule drug candidates, it is necessary to pool the fractions, dry down those pooled fractions, and then prepare samples for analysis from this dried solid. Usually, the analysis of choice is a combination of analytical LCMS and NMR. To do this, it is necessary to re-dissolve the dried solid in a known quantity of a suitable solvent and then transfer small aliquots from this sample into the appropriate analysis tubes and vials.

This technical note shows how the use of a suitable Gilson liquid handler and TRILUTION® LH Software makes it is possible to automate this process. Automating this process reduces manual handling errors and provides traceability where previously there was none. Additionally, it also frees up laboratory staff time.

INSTRUMENT REQUIREMENTS

Liquid Handler

This application is best applied to the GX-281 Liquid Handler (Figure 1). The GX-281 Liquid Handler has a large capacity bed and sufficient space for the dried down samples, racks to hold NMR tubes, and LCMS vials. The application also requires the use of an Orbital Shaker for which the GX-281 Liquid Handler has appropriate mounting hardware available to allow the use of the Orbital Shaker on its bed.

FIGURE 1
GX-281 Liquid Handler

Orbital Shaker

The Orbital Shaker (Figure 2) is an accessory available for the GX-281 Liquid Handler that allows a Code 200-series rack to be shaken in an orbital or vortexing motion. It is fully automated and moves only to one side allowing racks to be used alongside it. Additionally, it has a defined home position that it will return to every time it is stopped. This ensures that the probe can locate the vessels held in the rack correctly time after time. It can also be controlled by the TRILUTION LH, enabling automated start, stop, and speed control. The connection is by GSIOC, therefore, a suitable GSIOC controller is required as part of the system, such as a 508 Interface Module.

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The standard orbital shaker is designed for mounting on the right-hand side of the GX-281 Liquid Handler due to its right-sided orbital movement. For high throughput laboratories, the capacity of this application can be increased by use of an additional orbital shaker. For this purpose there is a special version of the Orbital Shaker that shakes to the opposite side and can therefore be mounted on the left of the GX-281 Liquid Handler. Having two shakers helps maximize capacity in this application as the dry-down tubes or vials are likely to be the largest vessels in use.

FIGURE 2
Orbital Shaker

There are also specials for two rack wide orbital shakers: SPL-2541-HDW (left hand) and SPL-2093C-HDW (right hand).

To avoid uncontrolled movement of the orbital shaker(s) during use, there are mounting kits available for use with the GX-281 Liquid Handler. These secure the shaker(s) to the bed through the use of screws, so that even at high speeds the shaker(s) will not move.

Racks

In order to maximize the benefit of the system to the laboratory, it is important to make sure that the racks chosen fit the laboratory workflow. The orbital shakers need to be fitted with racks suitable for the dry-down vessels. In the case of a Biotage V10 evaporator this will be a Gilson code 204 rack for use with 20 mL glass vials. If a Genevac system is used for dry-down then the racks will be different and will depend on the particular laboratory workflow. It is important to remember that the orbital shaker will add height to the dry down rack that sits on top of it; the GX-281 Liquid Handler has up to 210 mm of clearance.

The racks chosen for the LCMS analysis must fit the format required by the LCMS instrument in use in the laboratory. Commonly these will be glass 2 mL vials, but it may also be that 96 deep well plates are used. There are suitable racks in the Gilson range to accommodate both of these and one LCMS rack should be sufficient for the output from two orbital shakers.

FIGURE 3
Rack SPL-1293-HDW

The racks chosen for the NMR analysis must fit the format required by the NMR instrument in use. These may be individual NMR tubes in either 3 mm or 5 mm outer diameter (Figure 3) or 96 tube format packs such as those for use with the Bruker SampleJet. There are suitable racks in the Gilson Specials range to accommodate both of these and one NMR tube rack should be sufficient for the output from two orbital shakers.

A rack heightener (RH1) is available for use with racks that accompany the orbital shaker. This heightener will raise any standard code 200-series rack to the same height as racks placed onto the shaker. One of these heighteners will be required for each of the LCMS and NMR racks.

Bottle racks can also be added to this configuration to provide additional solvents if the solvent inlets on the GX Solvent System are insufficient or if minimizing solvent usage is important.

METHOD

TRILUTION LH provides all the control necessary to fully complete this post purification process (Figure 4). The sample tubes or vials need to be placed into the racks on the orbital shakers and sufficient receiving tubes and vials placed into the LCMS and NMR racks.

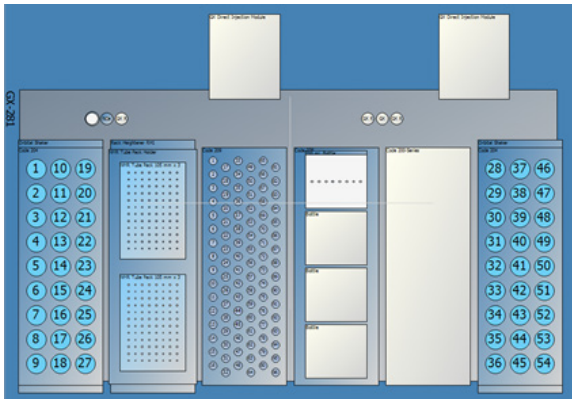

FIGURE 4
Example Bed Layout

On starting TRILUTION LH, the GX-281 Liquid Handler will then deliver the required quantity of solvent to each of the vials in the sample racks and the shakers will be turned on. The shaking speed and duration is configurable in the software and is something that should be optimized to the specific laboratory's needs. The speed required will need to be adjusted based on the tubes or vials used, vortexing in a wide vial is achieved at a slower speed than in a narrow vial. The duration required will be dependent on the types of samples used and the preceding dry-down method. The actual speed required should be determined manually and then pre-set on the front of the unit for the auto start/stop method. This can be established through GSIOC as well.

Once re-dissolving is complete, the GX-281 Liquid Handler will then proceed to transfer aliquots of the samples to each of the analysis vials and tubes. If capped vials are used for the LCMS analysis, then a septum piercing probe is required and the probe position in the vial should be set to just off the bottom with no probe movement during the dispense. This will allow for excess air to escape from the vial as it is displaced by the sample. A slow dispense speed should also be used.

When dispensing into the NMR tubes, it is important to set the probe position to the bottom of the tube but with liquid level following (LLF) activated. In this way the probe will move up the tube as the liquid is dispensed. If this is not set, the tube may overflow due to the probe taking up significant volume within the NMR tube. This kind of dispensing of sample will ensure that the NMR tube is properly filled and will avoid the introduction of airlocks or bubbles to the tube.

TRILUTION LH has the ability to number racks in almost any order to match upstream and downstream processes. This is a vital part of sample traceability within the laboratory workflow.

CONCLUSION

This kind of liquid handling application can be set up on a GX-281 Liquid Handler with minimal customization using the orbital shaker and an additional special rack for NMR tubes. Racks are available to suit almost any laboratory workflow in terms of input vials and tubes from a dry-down device and output vials and tubes for use on common brands of HPLC, UPLC, and NMR. For higher throughput laboratories, deuterated solvents can be used on the solvent system pump and for lower throughput laboratories the solvents can be housed in a bottle rack to minimize loss of expensive solvent.

The application is easy to run using TRILUTION LH and allows for traceability where the equivalent manual process has none. Additionally TRILUTION LH offers support for the import of sample lists from spreadsheets if required.

EXAMPLE CONFIGURATION

GX-281 LIQUID HANDLER WITH GX SOLVENT SYSTEM	
PART NUMBER	**DESCRIPTION**
261030	GX-281 LIQUID HANDLER NO BARCODE,110-220
26034555	RINSE STATION,GX 175MM FC
26034551	RINSE STATION,GX 175 MM
26036198	GDE FT ASSY, GX-281, 3-WAY INSERT 1.5MM
2603614112	LOWER PROBE HOLDER 1.5MM
2507252	PROBE,220X1.5X.4MM BEV MICRO .7 TIP
499424013	TUBING,1.1ML FEP STRAIGHT .8MM ID

ORBITAL SHAKERS AND RACK OPTIONS	
PART NUMBER	**DESCRIPTION**
251711	ORBITAL SHAKER
SPL-1707-HDW	ORBITAL SHAKER,LEFT HAND
26037101	LOCATOR, 281 ORBITAL SHAKER, RIGHT
26037102	LOCATOR, 281 ORBITAL SHAKER, LEFT
2504604	RACK,CODE 204 27 28 X 57MM 20ML SCINT
2504625	RACK,CODE 225 30 25 X 150MM VIALS
2504608	RACK,CODE 208 70 18 X 150MM TUBES
2504609	RACK,CODE 209 96 12 X 32MM 2ML VIALS
SPL-1745A-HDW	RACK,215 BRUKER BIOSPIN NMR TUBE
SPL-1293-HDW	RACK,215 NMR TUBES 3MM
2504628	RACK,CODE 228 4 500/700ML REAGENT BOT
543701700	BOTTLE, SOLVENT, 700ML, 4/PK
25045514	RH1 RACK HEIGHTENER ASSY, 215

TRILUTION LH SOFTWARE AND PC	
PART NUMBER	**DESCRIPTION**
21100005	TRILUTION 4.0 VERIFIED CONTROL PC
21063024	TRILUTION LH 4.0 LICENSE, LIFETIME