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Fully Automated Method using Dispersive Liquid Liquid Micro Extraction (DLLME) for Extractable and Leachable studies

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Introduction

Dispersive Liquid Liquid Micro Extraction (DLLME) is a technique used to extract analytes from a product matrix and provide a level of enrichment for trace analysis testing.

A dispersive agent is added to the sample prior to the addition of a chlorinated solvent, either dichloromethane or chloroform. For the work with GSK chloroform was used.

The dispersive agent allows the chlorinated solvent on addition to form an emulsion thus creating a huge surface area for the chlorinated solvent to extract the analytes of interest. After the solution is mixed for a period of time, the sample is centrifuged and the bottom layer is injected onto the GC.

Manual DLLME can become time consuming and require large volumes of sample and solvents. The use of a robust fully automated method allows an analyst to perform other laboratory tasks and increases throughput with overnight analysis.

Anatune and GSK have recently developed a completely automated solution, using fast agitation and centrifugation. Figure 1 shows instrumentation which can carry out this solution.

Figure 1 - Dual Head MPS xt with Centrifuge (CF-200) and Vortex (mVorx) on a GC/Q-TOF

A video is enclosed below which shows the addition (in slow motion) of dichloromethane to a solution containing Propan-2-ol and water. It can be seen how quickly the dichloromethane is broken up into an emulsion.

Enclosed Video

Figure 2 shows the solution after it has been centrifuged at 4500 rpm. The vial is a high recovery vial enabling withdrawal of a low volume (typically less than 50 µL) of the chlorinated solvent from the bottom of the vial.

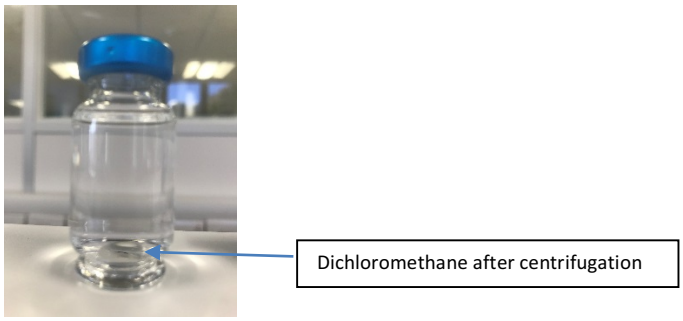

Figure 2 – High recovery vial with low volume of the chlorinated solvent present at the bottom of the vial (GSK use Chloroform).

Instrumentation

Dual Head GERSTEL MPS
GERSTEL mVORX
Agilent GC/Q-TOF 7200
Anatune CF-200 Centrifuge

Method

2 mL of a biopharmaceutical drug product sample was transferred manually to a 10 mL vial. Three different stock solutions containing the analytes listed in Table 4 were prepared in Propan-2-ol at the levels stated in Table 1. Additionally, an internal standard of Fluorene d10 was added to each test mix level at a concentration of 13.5 µg/mL, so equivalent to 0.33 µg/mL per sample. 2,4-ditertbutylphenol was in the test mix at five times the concentration to allow for intrinsic quantities found in the drug product.

Level	Spike Conc [µg/mL]	Final Concentraion in sample [µg/mL]
1	13.5	0.33
2	68.0	1.66
3	136.5	33.3

Table 1 Test mixture spiking levels

After addition of the test mixture to the biopharmaceutical drug product, 4.5 mL of Propan-2-ol was added to a 10 mL vial to crash out the biopharmaceutical protein. The vial was then transported to the mVORX and vortexed at 3000 rpm for 30 seconds. The vial was then centrifuged at 4000 rpm for 10 minutes. Up to 6 vials can be centrifuged at one time.

After centrifugation, 1.5 mL of the extract was added to a 10 mL High recovery vial. 75 µL of Chloroform was then added to the high recovery vial and vortexed for 30 seconds at 3000 rpm. 4.0 mL of water was added as a disperser and vortexed again for 30 seconds at 3000 rpm. The solution was then centrifuged at 4500 rpm for 5 minutes. From the Chloroform solvent layer 35µL was added to a high recovery GC vial ready for injection onto the GC/Q- TOF

Results

Precision was carried out on six samples spiked at 0.33 µg/mL which resulted in 10% RSD or less for all components of the test mix.

Analyte	% RSD
4-Methyl Benzaldehyde	1.7
Dodecane	3.8
1,6-Dibromohexane	2.8
Tetradecane	5.0
4-Pentylphenol	1.9
2,4-Ditertbutylphenol	5.3
Butylated Hydroxytoluene	1.6
1-Bromododecane	10.2
3,5-di-Tert-Butyl-4-hydroxybenzaldehyde	2.2
Octadecane	1.8
1-Bromopentadecane	2.3

Table 2 – % RSD for 6 different extractions at 0.33 µg/mL

Recovery experiments were carried out at the three concentration levels and three preparations of each. These were carried out to ensure there were no matrix effects from the biopharmaceutical product.

Analyte	% Recovery		
	0.33 µg/mL	1.66 µg/mL	33.3 µg/mL
4-Methyl Benzaldehyde	106.7	104.3	99.2
Dodecane	108.0	108.9	102.0
1,6-dibromohexane	101.2	100.6	101.4
Tetradecane	111.6	107.6	102.9
4-pentylphenol	98.0	104.1	101.7
2,4-ditertbutylphenol	132.1	102.5	92.6
Butylated Hydroxytoluene	102.8	108.2	101.0
1-bromododecane	114.1	102.9	101.9
3,5-ditertbutyl-4-hydroxybenzaldehyde	100.5	100.5	101.2
Octadecane	101.1	102.4	102.1
1-bromopentadecane	99.7	97.8	101.1

Table 3. Recovery experiments performed at three different concentrations

Analyte	r^2
4-Methyl Benzaldehyde	0.995
Dodecane	0.998
1,6-dibromohexane	0.984
Tetradecane	0.997
4-pentylphenol	0.991
2,4-ditertbutylphenol	0.911
Butylated Hydroxytoluene	0.979
1-bromododecane	0.995
3,5-ditertbutyl-4-hydroxybenzaldehyde	0.998
Octadecane	0.997
1-bromopentadecane	0.998

Table 4. r^2 values for the linearity experiments

Figure 3 shows a linearity plot for 3,5-Di-Tert-Butyl-4-Hydroxy benzaldehyde

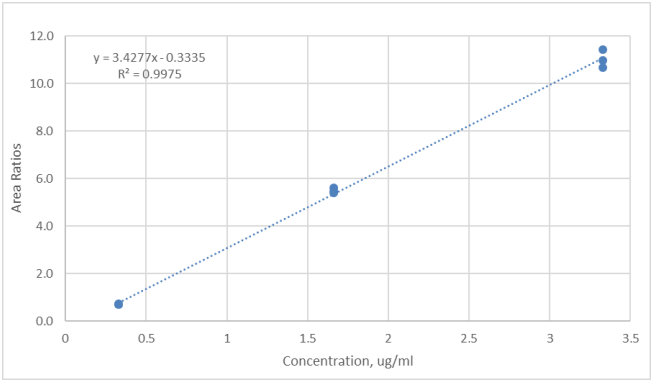

Figure 3. linearity plot for 3,5-Di-Tert-Butyl-4-Hydroxy benzaldehyde

Figure 4 shows a typical chromatogram of a sample spiked with test mix at the 0.33µg/mL level.

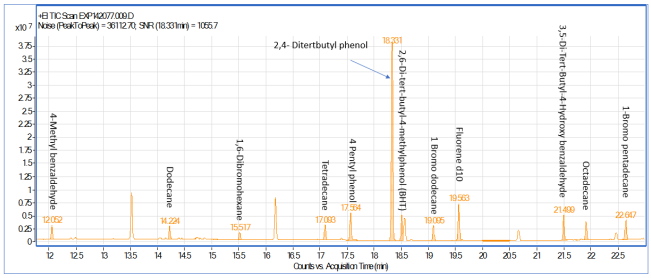

Figure 4. Chromatogram of Sample at 0.33 µg/mL level

Discussion

It is now possible to fully automate Dispersive Liquid Liquid Micro Extraction using the high recovery vials with automated mixing and centrifugation. This will increase throughput within GSK over the manual method. Please let Anatune know if you need any further information.