Automated sample preparation methods for mixing and centrifugation

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Introduction

This poster details two different applications whereby both methods required automated mixing and automated centrifugation.

The first application is a methanolic extraction of soil for determination of BTEX (GC method). The second application is an assay for a pharmaceutical tablet which involves tablet dissolution then automated vortexing and centrifugation (LC method).

Methanolic Extraction

Significant levels of benzene, toluene and the xylenes (BTEX) can be a found in soil due to environmental pollution. Petrol spills and leaking underground storage tanks can be some of the common causes. Different extraction approaches have been tried in the past such as vapour partitioning. However, methanolic extractions have been shown to be far more robust to extract and recover VOCs from soil [1].

Figure 1 GERSTEL Dual Head, mVorx (automated vortexer) and centrifuge with Agilent GC-MS (5977) at Anatune

Figure 2 GERSTEL Dual Head, mVorx and centrifuge with Agilent LC 1260 with Diode Array detection at Anatune

Pharmaceutical tablets contain excipients which are not soluble in standard aqueous organic solvents typically used to dissolve the active ingredient for HPLC analysis. It is usually necessary to centrifuge or filter the sample prior to analysis.

Instrumentation

GERSTEL MultiPurpose Sampler MPS 2 XL
Maestro Version 1.4.8.14/3.5
Anatune CF200 Centrifuge
GERSTEL mVorx (Vortexer)

Methanolic Extraction

Agilent 5977 MSD
Agilent GC 7890A

Assay on Pharmaceutical Tablet

LC Agilent 1260 with diode array detector

Methanolic Extraction Method

A set of garden soil samples were prepared in methanol and spiked with benzene, toluene, ethyl benzene, ortho, meta and para xylene standards at six different concentrations between 66 ppb to 1.7ppm relative to the soil weight taken. A further low level spike was prepared at 3ppb. A deuterated toluene and benzene mixture was used as an internal standard. A blank soil sample was also prepared.

Using the Dual-Head MPS, mVorx and CF200, each sample was vigourously mixed at 3000 rpm for a short period of time, then centrifuged at 4500 rpm. An aliquot of the clear supernatant was taken and added to a saturated salt solution in a 20 ml vial. The second MPS head with a heated 2.5 ml syringe was then used to carry out conventional headspace analysis.

Pharmaceutical Tablet Method

One whole tablet was transferred to a 10 ml glass screw vial. 2 ml of water were added and the sample was then vortexed for a period of time in order to fully disperse the tablet. Once dispersed, 2 ml of methanol was added to the same vial and the sample was further vortexed for 20 minutes. Once agitation was completed, the sample was centrifuged at 4500rpm for 5 minutes. Using the MPS, an aliquot of supernatant solution was diluted to 10 ml with sample solvent and vortexed for 1 min at 3000 rpm to homogenize. This was then automatically injected into a 10 ul loop for HPLC analysis. A standard isocratic LC method was used with a C18 phase HPLC column.

Results – Methanolic Extraction

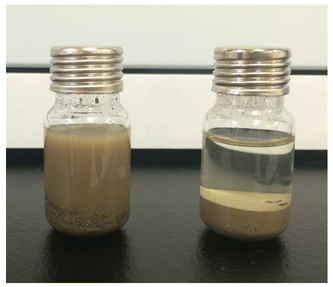

Figure 3 shows typical soil samples before and after centrifugation.

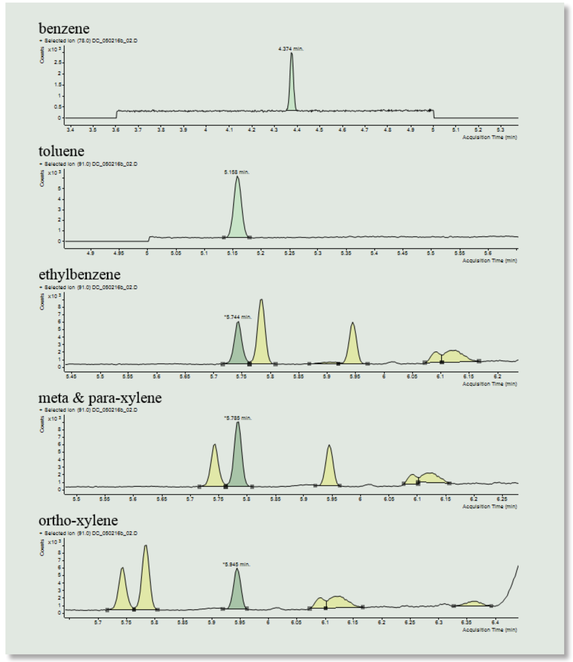

Figure 4 Figure 4 SIM chromatograms for each analyte at 3ppb in garden soil.

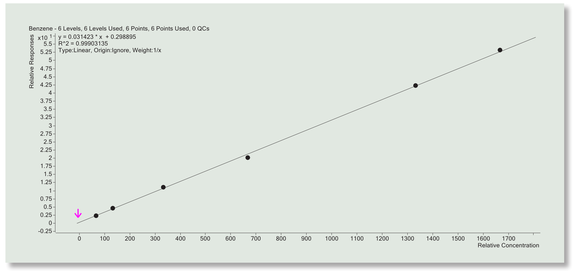

Figure 5 six point calibration of benzene from 66 ppb to 1.7ppm

Good linearity was also observed for ethyl benzene, toluene, and the xylenes.

Results – Pharmaceutical Tablet

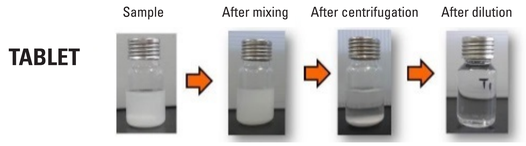

Figure 6 Stepwise sample preparation of the pharmaceutical tablet analysis

	Active	
Sample ID	**RT**	**Area**
1	13.8	7560
2	13.8	7611
3	13.8	7552
4	13.8	7531
5	13.8	7591
6	13.8	7418
7	13.8	7569
8	13.8	7562
9	13.8	7540
Average	**7548**	
SD	**55**	
RSD%	**0.7**	

Table 1 Showing precision obtained for 9 separate tablets prepared by the automated method detailed in this note.

Figure 7 shows the ultrasonic bath option now available.

Conclusions and Future work

By the use of the CF200 Centrifuge and the mVorx vortexer instrumentation, it is possible to fully automate difficult sample preparation for LC and GC applications.

We are currently evaluating an automated ultrasonic bath which will definitely add to similar applications using pharmaceutical tablets.

If you would like to discuss this further, please do not hesitate to contact us, either by emailing enquiries@anatune.co.uk, or call us now on +44 (0)1223 279210.