Chromatography Technical Note No AS 163

Automated Solutions using Small Scale SPE to Solve Challenging Applications

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Introduction

Over the last 8 years, Anatune have developed several solutions using ITSP (Instrument Top Sample Preparation). Most of these solutions have been covered in previous application notes in more detail than in this note. However, this provides a summary so the reader can gain how established and robust ITSP is.

Applications within this note cover, environmental, food and flavour and clinical sectors.

Anatune started using ITSP as part of the automated clean-up step for the analysis of vitamin D2 and vitamin D3 in Human serum. The solution uses LC/MS/MS. Sample throughput is over 200 samples per week. Further solutions, have included enrichment of N-Nitrosodimethylamine (NDMA) in water samples and analysis of Metaldehyde in water for an environmental application with Severn Trent Water. Both of these methods are using GC/MS/MS. Recently, ITSP has been used for enriching taste and odour compounds (TOCs) in water. ITSP has also be used for filtration.

Figure 1 shows a standard ITSP cartridge and also the 96 position ITSP tray which is attached to the MultiPurpose Sampler (MPS) rail.

Figure 1. Photo of ITSP and ITSP tray

Vitamin D2/D3 solution-LC/MS/MS method

Vitamin D, along with calcium, promotes proper bone growth in children and aids in the prevention of osteoporosis in older adults. Vitamin D is present in two forms, Vitamin D3 and Vitamin D2. Both D2 and D3 vitamins are metabolised in the liver to form 25-Hydroxyvitamin D2 (25OH-D2) and 25-
Hydroxyvitamin D3 (25OH-D3), respectively.

A fully automated solution for Vitamin D, has been running successfully for a number of years for Viapath at Guy's and St Thomas' Hospital whereby they run hundreds of patient samples every week. This fully automated method uses a protein precipitation followed by centrifugation and Solid Phase Extraction through ITSP. At Guy's and St Thomas' Hospital, this is run on an Agilent 6460 MS/MS.

Instrumentation

Gerstel MultiPurpose Sampler (MPS) 2 XL
Anatune CF100 Centrifuge and agitator
ITSP solutions, Instrument Top Sample Preparation (ITSP)
Agilent 1200 LC
Agilent 6410 Triple Quadrupole (Multimode source- APCI (+)) in Multiple reaction monitoring mode

Method

The MPS adds 40 µL of internal standard solution (25-OH Vitamin D3-d6 50 ng/ml) to the serum, followed by 200 µL of a 0.2 M zinc sulphate solution to enhance the sensitivity of the assay. Following this, 500 µL of methanol is added to the vial for protein precipitation. The vial is then moved using magnetic transportation to the CF100 centrifuge whereby the contents are thoroughly vortexed for 1min and then centrifuged at 3000 rpm for 1 minute to obtain a clear extract. A 10 mg C18 ITSP cartridge is solvated with and then water. 500 µL of the supernatant is then loaded onto the cartridge and then washed with a small amount of methanol/water. The cartridge is then dried with 250 µL of air. Analytes are eluted with one 100 µL aliquot of methanol into a 300 µL high recovery vial. 40 µL of HPLC grade water was then added. The solution is then injected directly into a switching valve with a 20uL loop attached. A 2.5 minute gradient LC method was used.

Results

Calibration curves were constructed for 25-OH D2 and 25-OH D3. Linear calibrations were achieved from the Chromsystems four point serum calibration standards. Correlation coefficients of 0.999 and 0.998 were obtained for 25-OH D2 and 25-OH D3 respectively. See Figure 2.

Cal Level	Analyte µg/L	
	25-OH D2	25-OH D3
Std_01	3.8	4.7
Std_02	7.6	9.4
Std_03	15.2	18.8
Std_04	30.4	37.5

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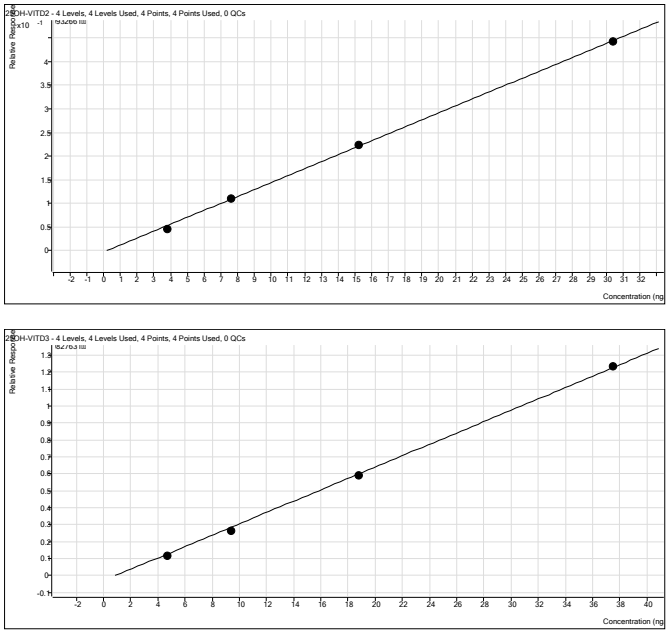

Figure 2- Calibration curves for 25-OH D2 and 25-OH D3

The method was validated by running tri level Vitamin D serum controls obtained from UTAK Laboratories. Dried control materials were reconstituted with water to provide solutions with target concentrations of 10 ng/ml for 25-OH D2 and 25-OH D3 (See Table 1)

Sample	Analyte	
	25-OH D2	25-OH D3
QC_Low_01	9.944	10.480
QC_Low_02	9.727	9.512
QC_Low_03	10.672	10.339
QC_Low_04	9.987	10.309
QC_Low_05	9.789	9.951
QC_Low_06	9.277	8.915
QC_Low_07	10.560	9.453
QC_Low_08	10.120	9.840
Average	9.899	9.918
SD	0.455	0.603
% RSD	4.597	6.080

Table 1 - Showing results of the low serum control samples.

NDMA solution-GC/MS/MS method

Formation of N-Nitrosodimethylamine (NDMA), in treated sewage and environmental waters has been known for around 40 years. NDMA is formed as disinfection by product during chloramination of wastewaters and drinking waters [2-4]. It has not been recognized as a potential drinking water contaminant until quite recently.

NDMA is a very polar molecule and is difficult to extract from water on most SPE phases. It has been found that coconut charcoal gives the best retention of NDMA and this stationary phase was chosen for this study.

Instrumentation

Agilent 7000 GC-Triple Quadrupole EI source (Agilent GC 7890A).
GERSTEL MPS 2 XL-*xt* Dual Head HS-enabled.
ITSP solutions, Instrument Top Sample Preparation (ITSP)
CIS 4 PTV fitted with septum-less head.

Figure 3: – Analytical solution for automated NDMA analysis.

Method

Using the right MPS fitted with a 2.5 ml headspace syringe (SPE needle), the ITSP (coconut charcoal) cartridge was conditioned with 750 µl dichloromethane. 1000 µl of methanol was then loaded, followed by 2000 µl of HPLC grade water to equilibrate the cartridge. 10 ml of solution A containing NDMA was loaded and the cartridge was dried for 15 minutes with nitrogen using the headspace syringe. Drying is a critical step, to get the best recovery of NDMA and NDMA-d6 from the cartridge. After drying, 400 µl of dichloromethane is used to elute the NDMA and NDMA-d6 into a 2ml GC vial. The left MPS head fitted with a 10 µl syringe is then used to inject 10 µl of the extract into the Cooled Injection System (CIS 4).

Results

Figure 4 shows a 7 point calibration in extracted water from 0.25ng/L to 15.25 ng/L (NDMA in water)

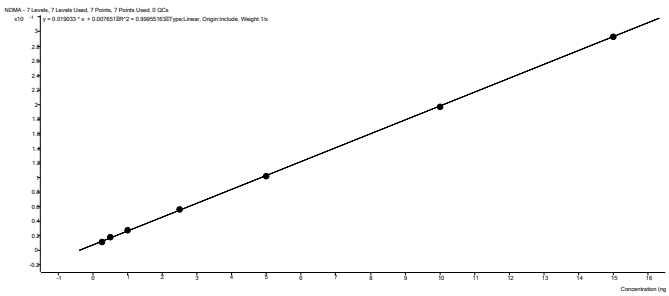

Figure 4 calibration curve for NDMA in water.

Metaldehyde solution-GC/MS/MS

Damage to crops from slugs and snails is a growing problem in the UK. Metaldehyde, a white, crystalline solid compound, is principally used as a contact molluscicide, commonly applied in the form of slug pellets. It is estimated that over 8 % of the area covered by arable crops is treated with Metaldehyde.

An ITSP method similar to NDMA has been developed.

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Extraction procedure:
Using the right MPS fitted with a 2.5 mL headspace syringe (SPE needle), the ITSP cartridge (Biotage ENV) was conditioned with 2 mL dichloromethane. 2 mL of methanol was then loaded, followed by 2.5 mL of HPLC grade water to equilibrate the cartridge. 10 mL of sample containing Metaldehyde was loaded and the cartridge was dried for 15 minutes with nitrogen using the headspace syringe. Drying is a critical step to get the best recovery of Metaldehyde and Metaldehyde-d16 from the cartridge. After drying, 400 µL of dichloromethane is used to elute the Metaldehyde and Metaldehyde-d16 into a 2ml GC vial. The left MPS head fitted with a 10 µL syringe is then used to inject 10 µL of the extract into the Cooled Injection System (CIS 4).

A seven-point calibration for Metaldehyde was prepared in water at concentrations ranging from 0.04 µg/L to 0.8 µg/L keeping the Metaldehyde –d16 consistent at 0.32 µg/L.

Figure 5 shows a 7 point calibration in extracted water from 0.04 µg/L to 0.8 µg/L

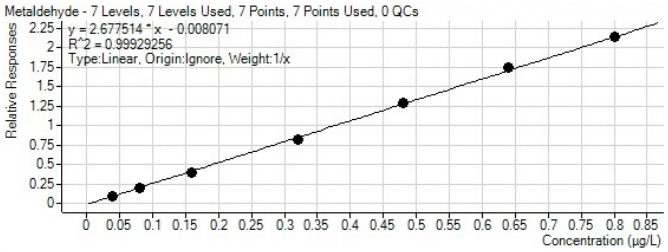

Figure 5: Linearity plot of Metaldehyde in water

Taste and Odour compounds in water by GC/MS/MS

Taste and Odour compounds (TOCs) are regularly monitored by water companies due to their very low odour detection threshold (ng/L). These affect the public perception of the drinking water quality as public may consider water is not of high quality if it has an odour. TOCs have a range of polarities and they can be extracted from water using similar methodology to both NDMA and Metaldehyde methods already developed by Anatune.

Method

35 mL of water sample was transferred to 40 mL amber vials and added with 1 g of NaCl and 5 mL MeOH, respectively.

Calibration standards and samples were prepared by spiking with a methanolic mixed standard solution of the target analytes and a methanolic standard solution of the two internal standards (2,4-dichlorophenol D3 for Phenols and 2,4,6-trichloroanisole D5 for Taste and Odour compounds).

The ITSP procedure was carried out using the right MPS fitted with 2.5 mL headspace syringe (SPE needle).

The left MPS head fitted with a 10 µL syringe was used to inject 10 µL of the extract into the Cooled Injection System (CIS 4) for GC-MS analysis

After solvating and equilibrating the ITSP cartridge 10 mLs of sample was passed through the ITSP cartridge. The cartridge was then dried for 15 minutes with clean Nitrogen and eluted with acetone.

Results

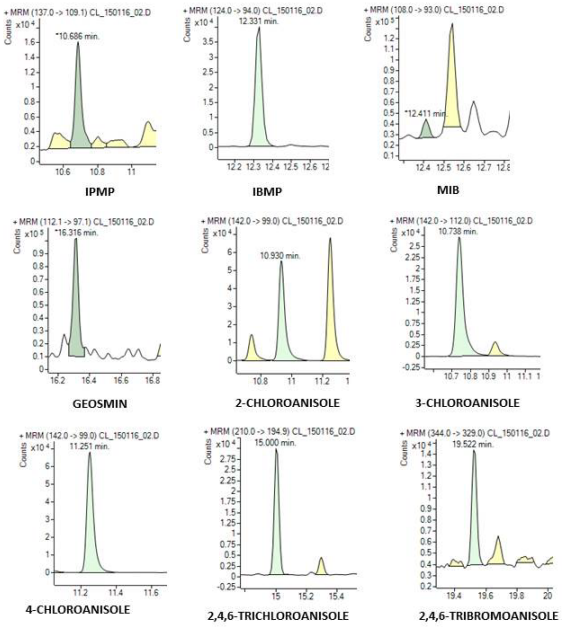

Figure 6: SRM extracted chromatograms at 1 ng/L for all target TOCs
Good linearity was obtained for all TOCs. LODs were calculated as 3 SD of the blank. Table 2 summarizes R^2 and LODs for all the target compounds and Figure 3a and b show an example of calibration curve plots obtained for MIB and Geosmin.

Compound ID	*LOD (ng/L)*	*R^2*
2-Isopropyl-3-methoxypyrazine (IPMP)	0.7	0.9898
2-Isobutyl-3-methoxypyrazine (IBMP)	0.6	0.9949
MIB	1.0	0.9947
Geosmin	0.6	0.9975
2-chloroanisole	0.6	0.9952
3-chloroanisole	0.8	0.9952
4-chloroanisole	0.7	0.9966
2,4,6-trichloroanisole	0.9	0.9964
2,4,6-tribromoanisole	1.1	0.9958

Table 2: LOD and R^2 linearity coefficients for the target TOCs

Filtration for UPLC by ITSP

As many laboratories look to increase their sample throughput, ultrahigh performance liquid chromatography (UHPLC) has become a very popular technique utilizing very high pressure systems to reduce run times to a few minutes with improved resolution.

However to gain these advantages very narrow tubing with very small internal diameters, tiny particle sizes and frit porosity means that any particulates in the samples you are analyzing can cause the system to become blocked very easily. These blockages can cause more down time than you save. Filtration is strongly recommended.

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Discussion

This combined application note shows how versatile ITSP and robust is. Severn Trent Water routinely use ITSP for the analysis of metaldehyde running up to 100 water samples per week.

Vitamin D assay using ITSP for over 200 patent serum samples per week is being performed at Guy's and St Thomas' Hospital.

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