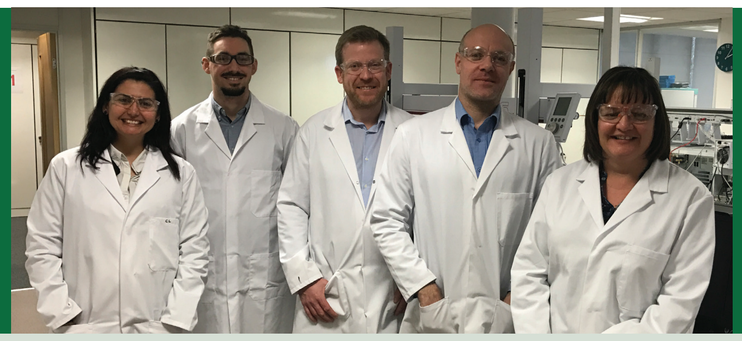
Automated Solutions using Smart SPE to solve challenging applications



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Introduction

Over the last 8 years, Anatune have developed several solutions using ITSP (Instrument Top Sample Preparation). This technique is now also known as Smart SPE. This poster provides a summary of solutions using Smart SPE to allow the reader to gain an understanding of how established and robust Smart SPE is. One of the major advantages of Smart SPE is that it uses positive pressure to accurately control the flow rate through the cartridge making the solutions developed extremely robust. Applications within this poster 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 at Guy's and St Thomas' Hospital. 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 and more recently Affinity 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 (Smart SPE (ITSP)) and ITSP tray

Amino acid analysis

Amino acids are both structural building blocks of proteins key and metabolites in primary metabolism. Amino acids measurements are routinely done in several different fields. In clinical medicine, amino acids are analysed for the diagnosis of metabolic disorders, in the food industry amino acids are used for quality control, process control and nutritional labelling while in the biotech and pharmaceutical industries, amino acid analysis is an integral tool in peptide identification and characterization.

We've recently been building on some proof-of-concept work done by Dr Katja Dettmer using alkyl-chloroformate derivatives automated with the GERSTEL MultiPurpose Sampler (MPS). Figure 2 shows the derivatisation carried out.

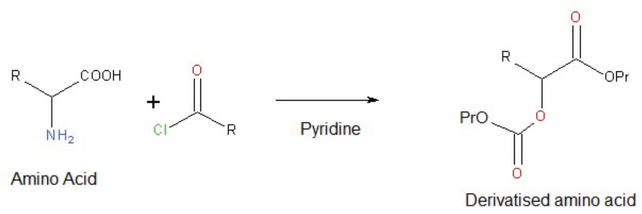

Figure 2. Derivatisation of amino acids

These derivatives benefit from being fast and robust and can be applied to a range of aqueous samples without the need for complex sample clean-up. In addition to Katja's original work, we have added a cation exchange clean up (prior to derivatisation) using Smart SPE, GERSTEL Multi Position Vortexer (mVORX) and Anatune CF200 Robotic Centrifuge to deliver a fully automated solution for amino acid determination. Table 1 shows some of the initial results obtained from an 8 point calibration.

Linearity & Precision 2.5-400 ng/mL	Linearity	R^2	%RSD
Alanine	y = 0.0050x - 0.003	0.998	3.25
Sarcosine	y = 0.0008x - 0.002	0.998	4.13
Glycine	y = 0.0061x - 0.014	0.991	3.62
α-Aminobutyric Acid	y = 0.0098x - 0.034	0.987	2.79
Valine	y = 0.0077x - 0.015	0.994	4.11
β-amino-*iso*-butyric acid	y = 0.0030x - 0.001	1.000	2.51
Leucine	y = 0.0083x – 0.035	0.995	3.61
Isoleucine	y = 0.0061x – 0.020	0.995	4.40
Threonine	quadratic	1.000	18.53
Serine	y = 0.0009x – 0.001	1.000	31.36
Proline	y = 0.0177x – 0.022	0.994	4.44
Aspargine	y = 0.0030x – 0.002	0.999	3.60
*Aspartic Acid	y = 0.0031x – 0.005	0.999	4.91
Methionine	quadratic	0.999	4.09
Glutamic Acid	y = 0.0006x – 0.004	0.997	7.34
*4-hydroxyproline	y = 0.0048x – 0.020	0.996	n.d.
*Phenylalanine	y = 0.0021x – 0.003	0.998	6.05
α-amino-adipic Acid	y = 0.0006x – 0.006	0.993	8.37
Ornithine	quadratic	0.998	9.55
Lysine	y = 0.0007x – 0.006	0.995	6.91
Histidine	quadratic	0.997	24.84
Tyrosine	y = 0.0087x – 0.003	0.999	9.00
Tryptophan	y = 0.0045x – 0.004	0.999	6.08
*Linear calibration range 2.5-100 ng/mL			

Table 1 Linearity of derivatised amino acids

Automated Vitamin D2/D3 analysis 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

Figure 3 shows Anatune's most recent configuration for Vitamin D analysis.

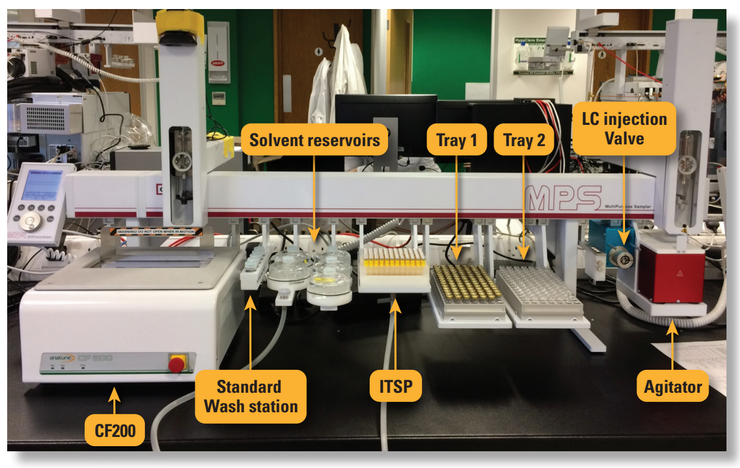

Figure 3 Photo of most recent Vitamin D set up at Anatune

At Viapath, 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 CF-100 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. HPLC Grade water, 40 µL, 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.

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. This method has been validated at Viapath.

Filtration for UPLC – Smart SPE

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 analysing can cause the system to become blocked very easily. These blockages can cause more down time than you save. Filtration is strongly recommended.

Anatune carried out some filtration work and solutions were run successfully by UHPLC with good results. Unfortunately, it is not possible to publicise this work

Metaldehyde solution using Smart SPE

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.

Using the left 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 2 mL GC vial. The right 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).

Figure 4 shows the set up at Severn Trent Water where they are routinely carrying out metaldehyde analysis.

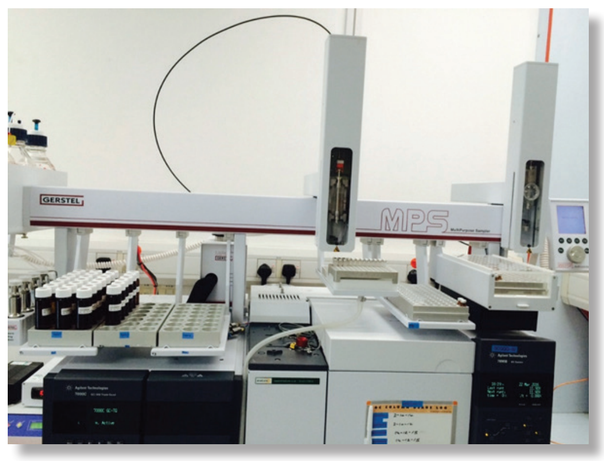

Figure 4 Metaldehyde solution at Severn Trent Water (GC/MS)

Figure 5 shows a comparison of calibration level 1 (Metaldehyde at 0.04 µg/L) and a blank.

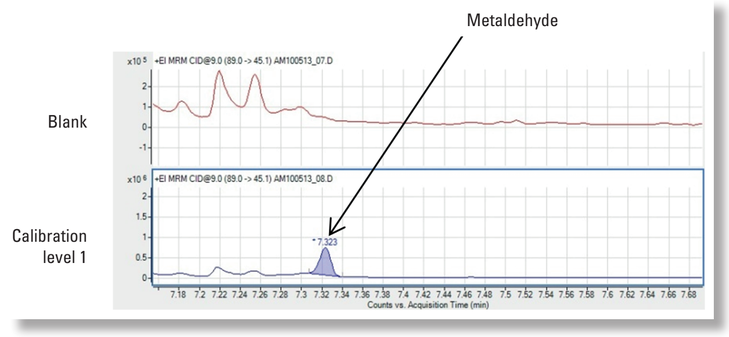

Figure 5 MRM (transition 89 to 45 m/z) chromatogram of a Blank and the calibration level 1 (Metaldehyde 0.04 µg/L)

A six point calibration in extracted water has been performed with a correlation coefficient of 0.999 achieved. The detection limit for Metaldehyde was calculated to be 0.002 µg/L.

Recovery experiments have been carried out at two different concentrations: 0.06 µg/L and 0.7 µg/L. Table 2 shows this recovery data from replicate SPE extractions, using an internal standard.

Amount spiked (µg/l)	0.06080	0.70400
Amount detected (µg/l)	0.05734	0.71908
	0.05721	0.71908
	0.06000	0.70449
	0.05628	0.72256
	0.05641	0.72204
Mean	0.05745	0.70135
SD	0.0015	0.035856
% RSD	2.61	5.11
% Recovery	94.49	99.62

Table 2 Recovery data for Metaldehyde from extracted water samples using ITSP

Conclusion

With Severn Trent Water running 100 samples a week for metaldehyde and Viapath doing 200 Vitamin D analyses over the same period, and multiple other applications not shown here, ITSP (Smart SPE) can be considered a versatile and robust sample preparation technique for both GC/MS and LC/MS in multiple laboratory environments.