Chromatography Technical Note AS133

Method Development Considerations for Automated Headspace Solvent Micro-Extraction (aHSME) using a Gerstel Multipurpose Sampler (MPS2)

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Introduction

The need for increased sensitivity and selectivity is a constant challenge facing many industries. One such technique that is becoming more popular amongst analytical scientists to overcome these challenges in a variety of sample types, particularly complex, dirty matrices is Single Drop Micro-Extraction (SDME).

SDME involves suspending a microdrop (typically 1-3µl) of solvent from the tip of a syringe in either a liquid sample, or the headspace above it. The intent is that analytes in the liquid sample or headspace migrate into the microdrop of solvent resulting in an enriched sample that can be retracted back into the syringe and injected directly onto a Gas Chromatography or Liquid Chromatography system for analysis.

This application note will focus on Headspace Solvent Micro-Extraction (HSME) for the analysis of common extractable/leachable substances in aqueous based sample systems. This technique can be described as occurring over 4 stages;

1. Sample Incubation - analytes are driven into the headspace
2. Enrichment - analytes are extracted into microdrop during the exposure period
3. Solvent Recovery - the microdrop containing the analytes is retracted back into syringe
4. Injection - Direct injection onto GC System.

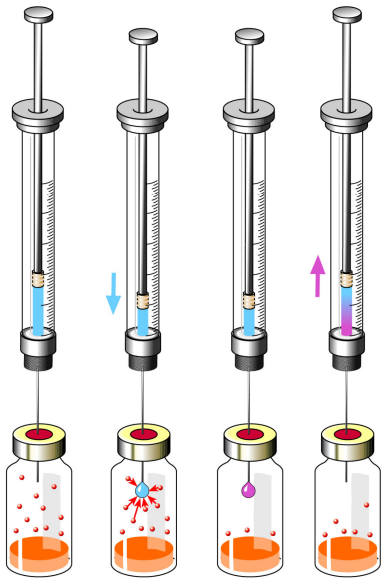

Figure 1: Schematic of Headspace Solvent Micro-Extraction

Method Development Considerations

Syringe: - Recovery of the drop back into the barrel of the syringe requires the needle tip to have a relatively large surface area. Hamilton 10ul syringes with a 26s gauge and a number 2 point style have been shown to be suitable for this purpose.

Extraction Solvent: - Choice of solvent is a critical aspect of the HSME procedure and subsequent analysis. Some important considerations when selecting the microdrop extraction solvent include:

- Solubility of target analytes in solvent to ensure analyte recovery
- Purity and boiling point of solvent to prevent target analyte interference during chromatographic analysis
- Boiling point of solvent to prevent evaporation during the extraction process
- Suitability for GC analysis

For these reasons, high molecular weight hydrocarbons such as 1-octanol, Ethyl Decanoate, 1-Bromopentadecane and n-Hexadecane are ideal extraction solvents.

To date, HSME has been conducted using a manual procedure, which has the potential to introduce variation and prevents large numbers of samples being run efficiently. As such, a partnership between GSK, Anatune and Gerstel developed an automated solution for performing this analysis (see Chromatography Technical Note, Number AS126).

The purpose of this application note is to use this procedure to further our understanding of this technique and the key experimental parameters associated with it.

Solvent Choice

One of the key benefits of HSME is the ability to tailor the extraction solvent to specific analytical challenges or scenarios. Figure 2 illustrates the effect solvent choice can have on analyte recovery using n-hexadecane & 1-bromopentadecane.

1-bromopentadecane was found to be a more effective extraction solvent than n-hexadecane across a broad range of substances with varying volatility. Benzyl alcohol and ethyl decanoate were also assessed, but found to be even less effective than n-hexadecane.

For the more volatile analytes, only 1-bromopentadecane was effective at extracting these from the headspace above 5ml of a model 2µg/ml aqueous sample. For the less volatile analytes, 1-brompentadecane extracted approx 5-10 times more than n-hexadecane.

The recovery of polar analytes was low for all extraction solvents evaluated most probably due to their reluctance to partition into the gas phase in the first place and then extraction into solvents that are relatively non-polar in

nature. This will be further assessed and discussed in a later application note.

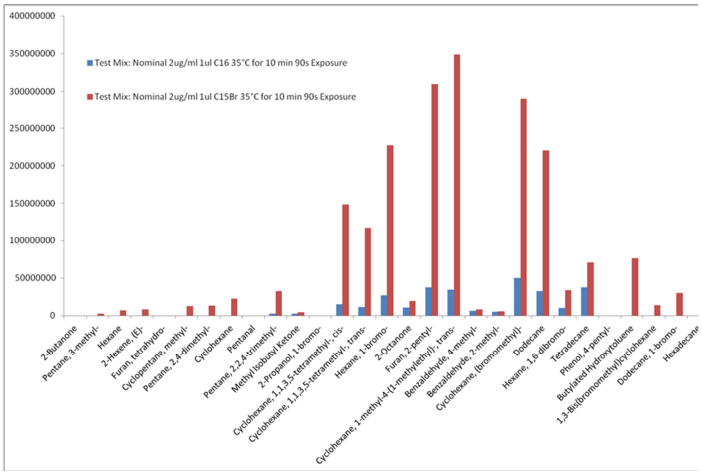

Figure 2: n-Hexadecane vs 1-Bromopentadecane

Incubation Temperature

The incubation temperature is a critical parameter affecting the partitioning of analytes between the sample, headspace and extraction solvent. Table 1 illustrates that at lower incubation temperatures, highly volatile analytes are recovered to a greater extent. For less volatile analytes, it is a balancing act between driving these into the headspace and migration into the extraction solvent.

Table 1: Effect of Incubation Temperature on analyte recovery

Compound	Peak Area Ratio (35°C)	Peak Area Ratio (80°C)	% Increase / Decrease
Pentane, 3-methyl-	0.074	0.032	129
Hexane	0.180	0.076	136
2-Hexene, (E)-	0.197	0.087	128
Cyclopentane, methyl-	0.277	0.126	121
Pentane, 2,4-dimethyl-	0.289	0.128	127
Cyclohexane	0.432	0.204	111
Pentane, 2,2,4-trimethyl-	0.614	0.280	119
Methyl Isobutyl Ketone	0.015	0.021	-31
Cyclohexane, 1,1,3,5-tetramethyl-, cis-	2.036	1.160	76
Cyclohexane, 1,1,3,5-tetramethyl-, trans-	1.593	0.917	74
Hexane, 1-bromo-	2.234	1.647	36
2-Octanone	0.079	0.185	-57
Furan, 2-pentyl-	2.964	2.385	24
Cyclohexane, 1-methyl-4-(1-methylethyl)-, trans-	4.094	2.696	52
Benzaldehyde, 4-methyl-	0.033	0.060	-46
Benzaldehyde, 2-methyl-	0.019	0.041	-53
Cyclohexane, (bromomethyl)-	2.166	2.291	-5
Dodecane	1.116	1.158	-4
Hexane, 1,6-dibromo-	0.194	0.193	0
Tetradecane	0.231	0.302	-23
Butylated Hydroxytoluene	0.312	0.568	-45
1,3-Bis(bromomethyl)cyclohexane	0.090	0.117	-23
Dodecane, 1-bromo-	0.079	0.130	-39

Precision

The precision of the technology was assessed using 6 replicate samples incubated at 35°C for 10 minutes and recovered using 1ul of 1-bromopentadecane suspended for 90s. Table 2 shows the mean Peak Area Ratio's (PARs) using Toluene as an Internal Standard and demonstrates acceptable precision for trace analysis of extractable/leachable compounds.

Table 2: Precision of aHSME

Compound	Mean Peak Area Ratio (n = 6)	%RSD
Pentane, 3-methyl-	0.064	17
Hexane	0.153	18
2-Hexene, (E)-	0.169	17
Cyclopentane, methyl-	0.238	17
Pentane, 2,4-dimethyl-	0.250	15
Cyclohexane	0.374	17
Pentane, 2,2,4-trimethyl-	0.542	13
Methyl Isobutyl Ketone	0.016	5
Cyclohexane, 1,1,3,5-tetramethyl-, cis-	1.961	8
Cyclohexane, 1,1,3,5-tetramethyl-, trans-	1.527	8
Hexane, 1-bromo-	2.157	6
2-Octanone	0.086	8
Furan, 2-pentyl-	2.903	5
Cyclohexane, 1-methyl-4-(1-methylethyl)-, trans-	4.081	6
Benzaldehyde, 4-methyl-	0.037	10
Benzaldehyde, 2-methyl-	0.021	11
Cyclohexane, (bromomethyl)-	2.232	4
Dodecane	1.210	6
Hexane, 1,6-dibromo-	0.201	12
Tetradecane	0.258	10
Butylated Hydroxytoluene	0.363	15
1,3-Bis(bromomethyl)cyclohexane	0.100	11
Dodecane, 1-bromo-	0.090	11

Conclusion

Sample enrichment is an important consideration in GSKs approach to solving complex analytical challenges associated with the risk posed by substances leaching from materials used to manufacture, deliver or package a pharmaceutical product.

For Large Volume Parenteral (LVP) pharmaceuticals, it has become increasingly more challenging to align leachable methods to the Safety Concern Threshold (SCT) and Genotoxic Impurities guidance. Furthermore, pharmaceutical formulations, due to their increasing complexity, require selective extraction techniques that pre-clean the sample prior to analysis.

As such, the additional sensitivity (4ng per ml of sample) and selectivity that can be gained from HSME makes this an attractive choice for analysts developing quantitative leachable methods for complex biopharmaceutical and parenteral applications.

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