

# Headspace Liquid Microextraction

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The authors describe the use of a new sampling method for gas chromatography termed single-drop microextraction (SDME). Here, SDME is applied to residual solvents analysis, both in manual and automated modes, including the first published application with a commercial autosampler.

Headspace sampling for gas chromatographic (GC) analysis has many advantages, the most important of which is the elimination of many interferences from the sample matrix. In addition to the standard methods for headspace sampling, the technique of solid-phase microextraction (SPME) has found wide acceptance, because it is simple and inexpensive. More recently, single-drop microextraction (SDME) has been evaluated as an alternative to SPME. In this straightforward technique, a microdrop of solvent is suspended from the tip of a conventional microsyringe and is then immersed in a sample solution in which it is immiscible or suspended in the headspace above the sample. The original application used an 8  $\mu\text{L}$  drop of *n*-octane in an aqueous sample,<sup>1</sup> and only a fraction of this drop was analysed subsequently by GC. Later, a smaller drop was used (1 or 2  $\mu\text{L}$ ) and all of it was injected,<sup>2</sup> a technique that was replicated by a second laboratory.<sup>3</sup> To date, there are more than a dozen publications<sup>4–13</sup> that describe the use of the SDME method, which has also been called liquid-phase microextraction, solvent microextraction, liquid–liquid microextraction or for headspace sampling, headspace solvent microextraction or headspace liquid-phase microextraction. SDME also has been included in three literature reviews.<sup>14–16</sup>

Headspace SDME is similar to traditional headspace sampling in that volatiles are sampled from the vapours above the sample, thus avoiding interferences from

the sample matrix. A variety of methods and specialized equipment is available for this purpose. An alternative technique, headspace SPME, has the further advantage of improved detectivity, which results from the use of a coated fibre chosen for its selectivity for the analyte. In headspace SDME, the fibre used in SPME is replaced by a liquid microdrop that can also be chosen for its selectivity. The first papers appeared in 2001,<sup>17,18</sup> and only five others have followed.<sup>19–23</sup> The range of reported analyses includes alcohols, chlorobenzenes, trihalomethanes, and BTEX (benzene, toluene, ethylbenzene, and xylenes).

The extracting solvents that have been used for SDME are listed in Table 1. A wide

range of solvents and polarities is possible. For headspace sampling, the boiling point of the solvent should be high to avoid significant evaporation during sampling. Because that requirement is often necessary for residual solvents analysis as performed in the pharmaceutical industry, headspace SDME is a natural application. Reference 24 provides further information for those not familiar with residual solvents analysis. Methods for the analysis of residual solvents in pharmaceutical products are presented in the *United States Pharmacopeia* (USP)<sup>25</sup> and include direct liquid injection as well as headspace methods. Recent publications can be consulted to compare the older methods

**Table 1:** Extracting solvents reported in the literature.

Liquid–liquid SDME	Headspace SDME
<i>n</i> -Hexane	<i>n</i> -Octane
<i>n</i> -Octane	<i>n</i> -Decane
Isooctane	Tetradecane
Cyclohexane	<i>n</i> -Hexadecane
<i>n</i> -Hexadecane	Toluene
Toluene	<i>o</i> -Xylene
Chloroform	Cyclohexane
Butylacetate	1-Octanol
Diisopropyl ether	Benzyl alcohol
	Ethylene glycol
	Diethylphthalate

**Table 2:** Residual solvents analysed.

Class 2 solvents	Class 3 solvents	Other solvents
Chloroform*	Acetic acid	Water*
Cyclohexane*	Acetone*	
Methylene chloride	1-Butanol*	
Hexane*	Ethanol*	
Methanol*	Ethyl acetate*	
Toluene*	Ethyl ether	
	Heptane	
	Methylethyl ketone	
	1-Propanol*	
	2-Propanol*	
	Tetrahydrofuran*	

\*Quantitatively analysed

with headspace SDME: a static headspace method is presented in reference 26, and references 27 and 28 describe residual solvents analyses by the competing method, SPME.

The International Conference on Harmonization (ICH), formed in 1997 to create worldwide uniformity of policy in the pharmaceutical industry, identified four classes of residual solvents<sup>28</sup> that must be monitored in pharmaceutical formulations. Class 1 solvents have unacceptable toxicity and are to be avoided during pharmaceutical manufacture. They are not routinely analysed, although confirmation of their absence could be part of the development process. Class 2 solvents are less toxic, Class 3 are "of lower risk to human health",<sup>29</sup> and Class 4 solvents are those for which no adequate toxicological data were found. These last three classes of solvents are the ones most commonly analysed. Solvents that we have analysed using headspace SDME are listed in Table 2.

In this paper, we report our preliminary headspace SDME results using N-methyl-pyrrolidone, an ICH Class 2 solvent. N-methylpyrrolidone has been used as a solvent for residual solvents analysis by Crowther<sup>30</sup> because it is miscible with water, able to solubilize many pharmaceutical products and is eluted after most of the other residual solvents because of its relatively high boiling point (202 °C). We chose it as the extracting drop solvent as well as the sample solvent, thereby eliminating concern about drop

evaporation during extraction,<sup>21</sup> because the headspace is already saturated with it. The use of N-methylpyrrolidone precludes its determination in residual solvent samples, as well as that of any other residual solvents that are higher boiling and are eluted with or after N-methylpyrrolidone. We obtained 99.5%

N-methyl- pyrrolidone from Burdick & Jackson (Muskegon, Michigan, USA). Experiments were performed manually and with a commercially available GC autosampler (Table 3).

Parameters that have been considered for SDME include the following: size of drop; shape of needle tip; temperature of

Figure 2: Automated apparatus: CTC Combi PAL installed on an Agilent GC-MS system.



Table 3: Instrumental conditions.

Item	Manual Injection	Automated Injection
Gas chromatograph	HP 5890	Agilent 6890
Detector	FID	MS (Wilmington, DE), Agilent 5973
Column	30 m widebore	30 m HP-5 MS
Data System	SP 4290 integrator Thermo (Sunnyvale, California, USA)	PC with Agilent ChemStation software
Microsyringe	Hamilton 7002, 2 µL and 7105, 5 µL. Type: sample contained in needle; point #2	Hamilton 1701, 10 µL, gas tight Needle point #2
GC Temperatures (°C)		
Injection	250	250
Detector	250	280
GC Split ratio	5:1	10:1
Automation	N/A	CTC Analytics Combi PAL Sample vial, 20 mL Sample size, 10 mL Wash with NMP, 3 times, 6 µL Sample temp.: 60 °C Stirring rate: 750 rpm Equil. time: 30 min. Drop volume: 1.0 µL Filling Strokes: 3 Rate of drop expulsion: 0.2 µL/s Extraction time: 5 min. Drop volume retracted: 1.2 µL

Figure 1: A 1 µL droplet of extracting solvent on the tip of a #2 bevelled microsyringe needle.



## Sample Preparation Perspectives

sampling; equilibration time; sampling (extraction) time; effect of stirring and ratio of headspace volume to sample volume. Our choices were made based on the reports in the literature, and our instrumentation is listed in Table 3.

### Manual Procedures

Samples were prepared in N-methylpyrrolidone with appropriate internal standard, and 10 mL of sample was placed in 20 mL headspace vials in a water bath kept at 60 °C and not stirred. A 1- or 2 µL volume of N-methylpyrrolidone was suspended approximately midway in the headspace, as shown in Figure 1. After a defined period of time, usually four or five minutes, the drop was withdrawn back into the syringe past the original volume mark and injected into the gas chromatograph. Table 3 provides some further details. Following the GC run and the analyte data acquisition, the column was heated rapidly to 300 °C to elute the N-methylpyrrolidone (postrun). A random sampling of ICH solvents from Classes 2 and 3 was run to evaluate the method (see Table 2). Limits of detection were estimated at less than 1 ppm.

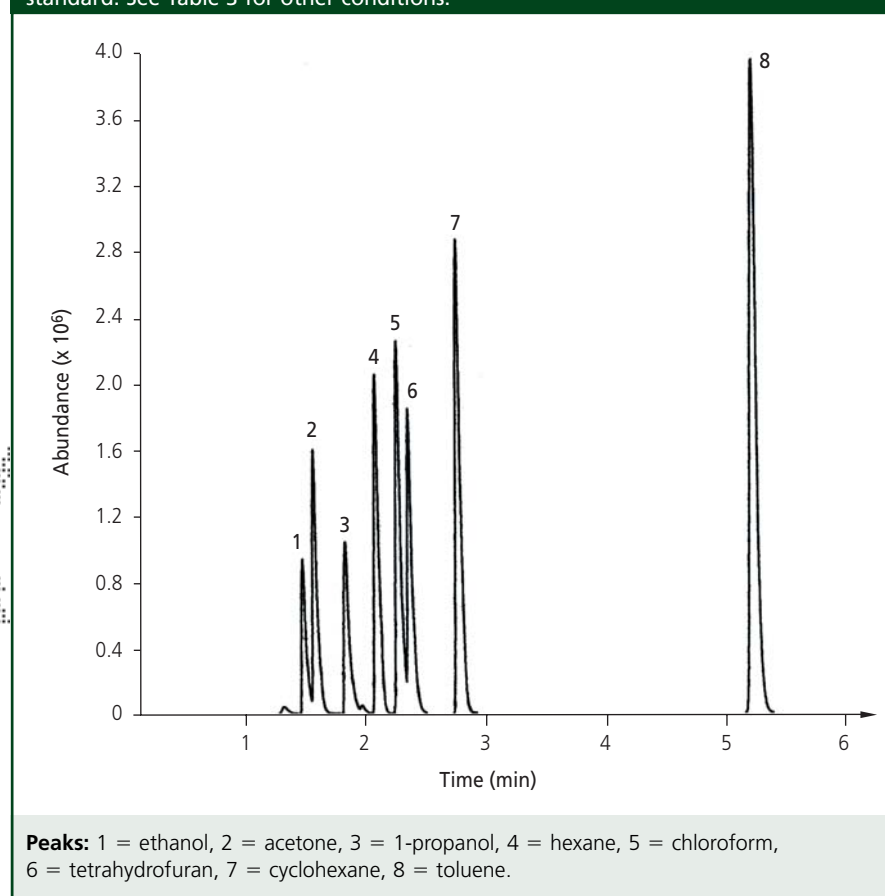
A quantitative analysis of methanol was run using 1-butanol as the internal standard. Analysis of the data showed that it is necessary to use an internal standard with this method because the relative standard deviation (RSD) was reduced from 8.8% without an internal standard to 2.7% with an internal standard. Some workers have put the internal standard in the extracting drop and this procedure was also tested using ethylbenzene as the internal standard. This change did not improve the precision of the method. Apparently, this internal standard was sufficiently volatile so that a variable

amount was lost during extraction at the elevated sampling temperature, resulting in poor precision. No attempt was made to find a better internal standard for use in the extracting drop.

A commercial sample was prepared and supplied by Ortho Clinical Diagnostics (Raritan, New Jersey, USA) to provide a

comparison between our headspace method and the conventional *USP* direct liquid injection method for the determination of methanol. The headspace SDME method provided an RSD of 1.5% and had a lower limit of quantitation (1.8 ppm) than the direct liquid injection method, presumably due to the elimination

**Figure 3:** Typical GC-MS chromatogram with total ion current output. Conditions: MS detection: total ion range 30–200 Da; temperature program: isotherm of 35 °C for 8 min, programmed at 50°/min to 150 °C, then 300 °C postrun for 6 min. No internal standard. See Table 3 for other conditions.



**Table 4:** Over-the-counter products screened for residual solvents.

Name	Manufacturer	RS found
Tylenol PM caplets, extra strength	McNeil	None
Aspirin tablets	Rite Aid	None
Aspirin tablets (old)	Harrison	None
Centrum advanced formula multivitamin tablets, dietary supplement	Whitehall-Robbins	Ethanol
Centrum Herbals: ginseng	Whitehall-Robbins	Ethanol, isopropanol
Excedrin tablets	Bristol-Myers	None
Empirin analgesic tablets	Burroughs Wellcome	None
Formula V dietary supplement tablets	Solgar	Triethylamine

of matrix interferences (including solids) present in that method. The headspace SDME method had a superior signal-to-noise ratio and no carryover between samples was detected.

Clearly, headspace SDME is an acceptable manual method for this analysis. It should be a desirable method for academic instructional laboratories. However, for routine industrial use, it was necessary to investigate the possibility of automation.

### Automated Analyses

The similarity of operation between SDME and SPME suggests that autosamplers that can be used for SPME should also work with SDME. Preliminary results using a 2  $\mu$ L drop of hexadecane for BTEX analysis (similar to reference 20) using a CTC Analytics Combi PAL autosampler (Zwingen, Switzerland), and a standard 10  $\mu$ L Hamilton (Reno, Nevada, USA) microsyringe confirmed this supposition. A Single Magnet Mixer from Chromsys LLC (Alexandria, Virginia, USA) was used with the autosampler to permit temperature-controlled extractions while stirring the sample.

A similar autosampler was installed on a GC-mass spectrometry (GC-MS) system at our university for a preliminary study of its application to residual solvents analysis. The equipment used, including part of the software macro for sampling, is included in Table 3. The equipment is shown in Figure 2.

Several experiments were run to test the equipment and confirm its applicability to the residual solvents analysis.

Approximately 200 samples were run over a two-week period. During this time, there were no mechanical failures, although a few of the chromatograms contained faulty data, indicating that some drops might have fallen off the needle. Overall, automation of the headspace SDME process was deemed successful and reasonably robust.

A reproducibility study of the determination of ethanol using 1-butanol as the internal standard resulted in RSDs between 1.9 and 4.2% at the 50 ppm level. The conditions were very similar to those used in the manual study: a 1  $\mu$ L drop was extracted at 60  $^{\circ}$ C for 5 min. Next, a calibration of eight solvents was run using three standards at each of five levels (from 10 to 1000 ppm) but without an internal standard, approximating the worst-case quantitation. A typical chromatogram obtained from these experiments is shown in Figure 3, and a calibration curve is shown in Figure 4.

Despite the absence of an internal standard, most  $R^2$  values exceeded 0.995 and limits of detection were estimated at sub-parts-per-million levels (for example, ethanol at 0.08 ppm). Contrary to our experience with manual operation, it would appear that, in some cases, this analysis can be performed successfully

without an internal standard when a commercial apparatus is used.

Eight over-the-counter drugs were screened for residual solvents content. Centrum's Ginseng Herbal was found to contain 2-propanol, so it was analysed using a 50 ppm standard solution and 1-propanol as the internal standard. We calculated 836 ppm of 2-propanol with an RSD of 3.3%. The qualitative results for residual solvents analysis for the eight drugs is shown in Table 4.

The final experiment was an attempt to use this method for the determination of water, because it can be detected by MS. Water could not be detected by the flame ionization detector used in the manual study. These data were acquired by selected-ion monitoring (SIM), whereas the previous results were by full scans. The internal standard was 1-propanol, and a typical chromatogram is shown in Figure 5. The very pure N-methylpyrrolidone we used had a label purity of, 500 ppm water, so the preparation of standards in it was

Figure 4: Calibration curve for ethanol in N-methylpyrrolidone.

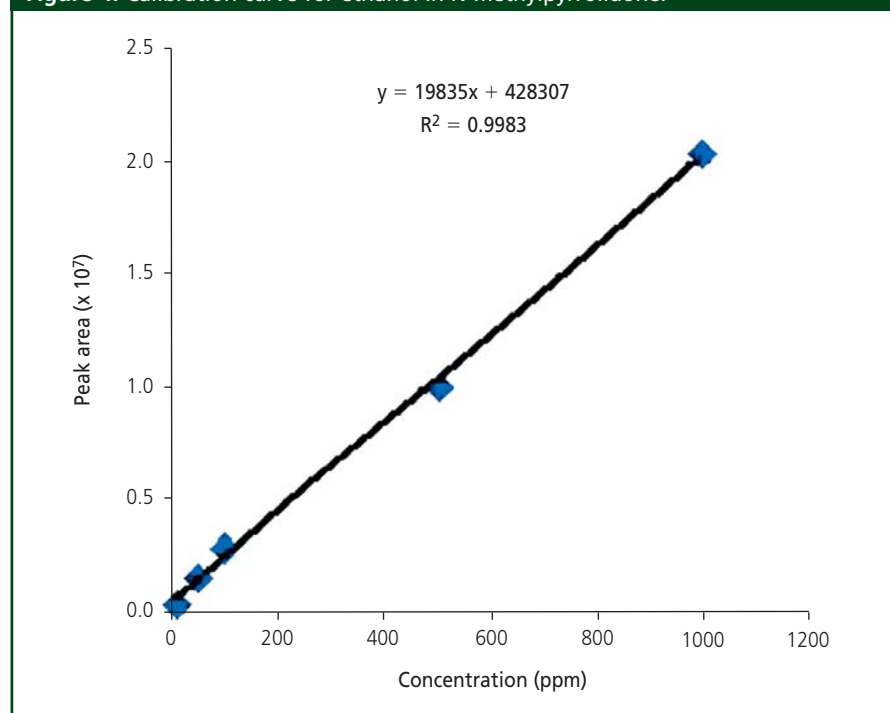
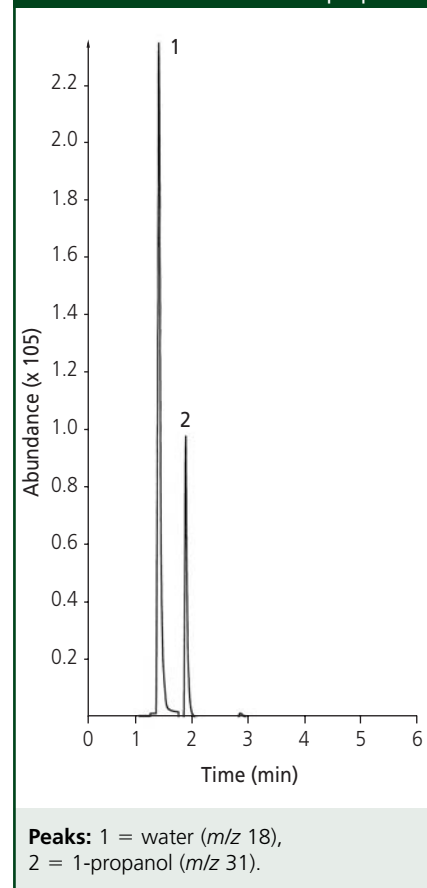


Figure 5: Determination of water by GC-SIM-MS. Conditions are the same as for Figure 3 except for selected ions monitored instead of total ion current, and an internal standard of 1-propanol.



analogous to a standard-addition analysis for water. The calibration was nonlinear at the upper standard of 500 ppm, but assuming that linearity extended at least up to 200 ppm, the calibration curve had an  $R^2$  of 0.9988. The level in N-methylpyrrolidone was found to be 139 ppm with an estimated limit of detection of 0.08 ppm.

### Conclusions

While these headspace SDME results are only preliminary and no attempt was made to optimize any of the variables, the data presented clearly indicate that automated headspace SDME is a method worthy of further investigation. Applications beyond residual solvents to forensic analysis of drugs, environmental analysis of volatile organics, analysis of aromas and flavours in foods and cosmetics and trace water analysis seem ideal.

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