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TDTS

Thermal Desorption Technical Support

Note 91: Using headspace–thermal desorption (HS–TD) to enhance the measurement of residual solvents in drugs via USP 467

Key Words:

USP 467, residual solvents, headspace, drugs, pharmaceuticals

Introduction

Residual solvents are trace-level volatile organic compounds (VOCs) that are sometimes found in pure drugs and pharmaceutical products. They may be by-products of manufacturing, or can be introduced/formed during packaging and storage (*e.g.* taint or degradation products). It is the responsibility of the manufacturer to ensure that these residues are not present at levels which might impact the quality or safety of their products.

The United States Pharmacopeia standard method 467 (USP 467) categorises residual solvents by class according to their toxicity. Class 1 compounds are carcinogenic, toxic or environmentally harmful, and the use of such compounds in pharmaceuticals should be avoided. However, if they are essential (for example in a particular manufacturing process) levels are tightly controlled, typically to below 10 ppm (Table 1). Class 2 compounds are non-genotoxic carcinogens or have suspected toxicity; concentrations of these compounds should be minimised. Concentration limits in this case range from 20–4800 ppm in pharmaceuticals, depending on specific compound toxicity (Table 1). USP 467 specifies a static headspace sampling method with gas chromatographic (GC) analysis for measuring both Class 1 and Class 2 residual solvent levels in drugs and pharmaceuticals[1].

The innovative and powerful combination of headspace sampling with thermal desorption (HS–TD) retains the principles of USP 467 but allows repeated and selective concentration of larger volumes of headspace vapour over a longer period of time. This dynamic headspace approach improves sensitivity by as much as one or two orders of magnitude, facilitating measurement of the most toxic volatiles at the lowest concentrations of interest.

Additionally, direct thermal desorption with GC/MS provides a complementary alternative to dynamic headspace, useful for obtaining the total VOC content of small samples This approach was further investigated here using a test pharmaceutical spiked with USP 467 solvents.

HS-TD

Conventional static (equilibrium) headspace (HS) sampling involves introducing the drug or pharmaceutical sample into a sealed vial, dissolving or slurrying it with a suitable solvent and allowing equilibrium to be reached between the VOCs in the sample and in the headspace. 1 or 2 mL of headspace vapours are then transferred from the vial to the GC and analysed. Though a fairly simple preparation technique, it has limitations with regards to sensitivity. It is also difficult to optimise the conditions for simultaneous analysis of compounds over a wide volatility range.

Purge and trap is an alternative, 'dynamic' headspace sampling technique whereby gas is bubbled through a sample for a set amount of time. Purged vapours are concentrated on a sorbent trap and analysed by thermal desorption with GC(MS). This offers enhanced sensitivity over static headspace, but sample foaming issues can interfere with results.

In essence, HS-TD combines the principles of both of these techniques. During HS–TD analysis headspace vapours are repeatedly transferred from the sample vial to a sorbent focusing trap in a fully-automated, multi-step approach that optimises sensitivity and volatility range, but without the problem of foaming. Markes' low-cost HS5-TD™ accessory for the UNITY 2™ desorber (Figure 1) facilitates cost-effective HS–TD operation for small sample numbers. UNITY 2 can also be interfaced to several leading brands of automated headspace device for high-throughput operation.

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Figure 1: HS5-TD accessory (left) connects directly to UNITY 2

The headspace sampler (HS5-TD module or alternative automated headspace device) connects directly to the UNITY 2 TD instrument. Headspace vapours are swept from each vial in one or multiple steps and selectively concentrated in the focusing trap of the UNITY 2. Exhaustive extraction of volatiles from the headspace is possible in some cases. Focusing conditions (sorbents, trapping temperature, etc.) are selected prior to analysis so that compounds of interest are retained while unwanted interference, such as water, is selectively purged to vent. After the headspace vapour transfer and concentration step has been completed, the focusing trap of UNITY 2 is dry purged and then heated rapidly (100ºC/sec) with carrier gas flowing in the reverse direction to that used during vapour concentration. Retained analytes are transferred/injected into the GC(MS) analyser as a narrow band for optimum sensitivity.

Experimental

Headspace sampling

USP 467 divides the target compounds into three mixtures for headspace–GC analysis; Class 1, Class 2 mix A and Class 2 mix B (N.B. A fourth mixture, Class 2 C compounds, is also available but these compounds are not compatible with headspace–GC analysis). The standards for Class 1, 2A and 2B compounds were prepared by diluting the appropriate mixture in dimethyl sulphoxide (DMSO), and then in HPLC-grade water to provide a series of standard solutions at levels equating to 0.05, 0.1, 0.5, 1, 2 and 5 times the concentration limit specified in USP 467. 5 mL of each standard was transferred to a 20 mL headspace vial and capped with a blue PTFE-coated septum.

Vials containing 5 mL of HPLC-grade water were also sealed with a blue silicone PTFE-coated septum and used as blanks.

Additionally, a sample of the pharmaceutical omeprazole was evaluated. One tablet was ground to a fine dust and 0.5 g weighed directly into a 20 mL headspace vial. This was dissolved in 5 mL of DMSO and analysed as below. Another 0.5 g sample of the same tablet was weighed in to a 20 mL headspace vial and spiked with a mix of residual solvents before being dissolved in DMSO to a total volume of 5 mL.

It is not always necessary to test pharmaceutical products to confirm their safety in terms of residual solvents. Where the daily dosage is less than 10 g, the raw materials may be tested and, if they pass, it is assumed the final product will also pass. This approach is termed ‘Option 1’. Table 1 presents a list of the compounds specified in USP 467 and their respective limits in the raw materials. These were calculated by the following equation (assuming 10 g administered daily):

$$\text{Concentration (ppm)} = \frac{1000\ \text{mg/mL} \times \text{PDE}^*}{\text{Dose}}$$

*PDE = Permitted daily exposure: Determined by the Gaylor-Kodell method of risk assessment[2] for Class 1 solvents, the USP procedures for setting exposure limits in pharmaceuticals[3] and the method adopted by IPCS for Assessing Human Health Risk of Chemicals[4] for Class 2 solvents.

Option 2 under USP 467 allows finished pharmaceutical products to be evaluated the same way. Option 2 is applied when some of the raw materials are known to exceed limits on occasion, even though solvent levels in the final product are still within acceptable limits. Option 2 is also applied where more than 10 g of the drug is administered. The above equation can again be used to calculate the acceptable concentrations with a known dose.

The diluted stock solutions of Class 1, 2A and 2B chemicals represent varying proportions of the permitted daily exposure limit (Table 2) assuming 10 g is administered. The limit level for each compound is represented by the ‘x 1’ solution.

Solvent	PDE (mg/day)	Concentration limit (ppm) assuming 10g administered per day
Class 1		
Benzene	0.02	2
Carbon tetrachloride	0.04	4
1,2-Dichloroethane	0.05	5
1,1-Dichloroethene	0.08	8
1,1,1-Trichloroethane	15	1500
Class 2		
Acetonitrile	4.1	410
Chlorobenzene	3.6	360
Chloroform	0.6	60
Cyclohexane	38.8	3880
1,2-Dichloroethene	18.7	1870
1,2-Dimethoxyethane	1	100
N,N-Dimethylacetamide	10.9	1090
N,N-Dimethylformamide	8.8	880
1,4-Dioxane	3.8	380
Hexane	2.9	290
Methanol	30	3000
Methylbutylketone	0.5	50
Methylcyclohexane	11.8	1180
Methylene chloride	6	600
Nitromethane	0.5	50
Pyridine	2	200
Tetrahydrofuran	7.2	720
Tetralin	1	100
Toluene	8.9	890
Trichloroethylene	0.8	80
Xylene†	21.7	2170

Table 1: Residual solvents (Classes 1 and 2) with their USP 467-specified concentration limits

†Usually 60% *m*-xylene, 14% *p*-xylene, 9% *o*-xylene with 17% ethyl benzene

	ppb	ppb	ppb	ppb	ppb	ppb
Class 1	**x 5**	**x 2**	**x 1**	**x 0.5**	**x 0.1**	**x 0.05**
Benzene	439	176	88	44	9	4
Carbon tetrachloride	1584	634	317	158	32	16
1,2-Dichloroethane	1566	627	313	157	31	16
1,1-Dichloroethene	2400	960	480	240	48	24
1,1,1-Trichloroethane	3300	1320	660	330	66	33
	ppm	**ppm**	**ppm**	**ppm**	**ppm**	**ppm**
Class 2	**x 5**	**x 2**	**x 1**	**x 0.5**	**x 0.1**	**x 0.05**
Acetonitrile	161	64	32	16.1	3.2	1.6
Chlorobenzene	200	80	40	20	4	2
Chloroform	44	18	9	4.4	0.9	0.4
Cyclohexane	1527	611	305	152.7	30.5	15.3
1,2-Dichloroethene	614	246	123	61.4	12.3	6.1
1,2-Dimethoxyethane	45	18	9	4.5	0.9	0.5
N,N-Dimethylacetamide	489	196	98	48.9	9.8	4.9
N,N-Dimethylformamide	415	166	83	41.5	8.3	4.2
1,4-Dioxane	196	79	39	19.6	3.9	2
Hexane	95	38	19	9.5	1.9	0.9
Methanol	1204	481	241	120.4	24.1	12
Methylbutylketone	20	8	4	2	0.4	0.2
Methylcyclohexane	462	185	92	46.2	9.2	4.6
Methylene chloride	398	159	80	39.8	8	4
Nitromethane	28	11	6	2.8	0.6	0.3
Pyridine	98	39	20	9.8	2	1
Tetrahydrofuran	307	123	61	30.7	6.1	3.1
Tetralin	50	20	10	5	1	0.5
Toluene	381	153	76	38.1	7.6	3.8
Trichloroethylene	58	23	12	5.8	1.2	0.6
Xylene	929	372	186	92.9	18.6	9.3

Table 2: Dilutions of the Class 1 and 2 compounds showing their relative concentrations in the prepared solutions

Analytical conditions

HS-TD

Instrument configuration:	HS5-TD + UNITY 2
Cold trap:	Air Toxics Analyser (U-T15ATA-2S)
HS vial septa:	Blue silicone PTFE
Pre-purge:	1 min (10 mL/min to split)
Sample cycles:	1 (3 for N,N-dimethylformamide and N,N-dimethylacetamide standards)
Pressurise:	1 min
Sampling:	1.5 min (30 mL/min to trap)
Equilibration:	1 min
Flush sample:	2 min
Post sampling purge:	1 min (10 mL/min)
Pre-trap fire purge:	1 min (40 mL/min)
Trap low temperature:	25°C
Trap heating rate:	Maximum
Trap high temperature:	300°C
Trap high time:	1 min
Split:	10 mL/min
Flow path:	140°C
Vial temperature:	85°C

N.B. Trap and temperature were selected to retain volatiles of interest whilst eliminating water extracted from the headspace.

GC

Column:	DB-624, 30 m x 0.32 mm x 1.8 µm
Pressure:	4.18 psi @ 35°C
Column flow (calculated):	2 mL/min
Mode:	Constant flow
Oven program:	35°C (20 min) then 10°C/min to 190°C
Total run time:	35.5 min

MS

Quad temperature:	150°C
Source temperature:	230°C
Full scan range:	30–200 amu

Direct desorption

A PTFE liner (P/N C-PL010) was packed with a small amount of conditioned quartz wool and then 0.5 g ground omeprazole was weighed directly into the liner. The liner was then packed with more conditioned quartz wool to secure the sample in position before it was directly desorbed using the conditions below.

Analytical conditions

TD

Instrument configuration:	UNITY 2
Cold trap:	Air Toxics Analyser (U-T15ATA-2S)
Pre-purge:	1 min (10 mL/min split)
Primary desorb temp:	100°C
Desorption:	10 min (50 mL/min to trap)
Pre-trap fire purge:	1 min (10 mL/min)
Trap low:	-30°C
Trap heating rate:	Maximum
Trap high:	300°C
Trap high time:	5 min
Split:	10 mL/min
Flow path:	200°C

GC/MS

As previous.

Results

All chromatograms displayed in this report were reprocessed using ClearView™. ClearView is a GC/MS reprocessing package from ALMSCO International (a division of Markes) which uses sophisticated 'dynamic background compensation' (DBC) algorithms to distinguish between chromatographic peaks and background/baseline anomalies. It reprocesses stored GC/MS and LC/MS data files, eliminating background ions from the total ion chromatogram (TIC), thereby improving sensitivity, spectral purity and peak integration. ClearView is able to discriminate between peak and background signal, even if the same mass ions are present in both (See TDTS 83 and 85 for more information.)

N.B. Original data files for this work are available on request.

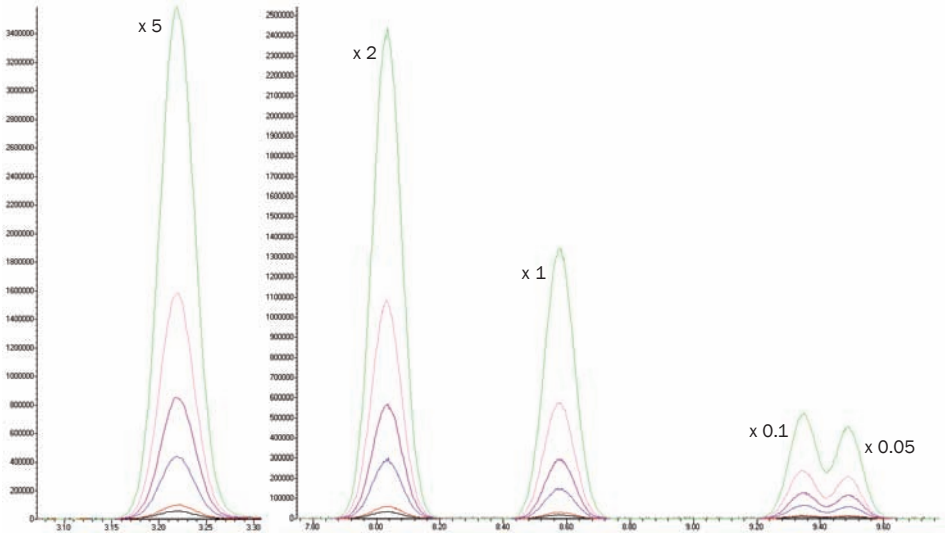

Figure 2: HS–TD–GC/MS analysis of the Class 1 residual solvents at six dilution levels (x 0.05, x 0.1, x 0.5, x 1, x 2 and x 5) of the USP 467 concentration limit

HS–TD

Figure 2 shows the chromatogram for the five Class 1 residual compounds at six dilutions (x 0.05, x 0.1, x 0.5, x 1, x 2 and x 5) of the recommended concentration limits (ppm) specified in USP 467 (Table 1).

USP 467 states that, to verify system suitability for the analysis of Class 1 compounds, the standard solution (x 1) must have a 1,1,1-trichloroethane signal-to-noise ratio ≥ 5:1, and all other peaks in the solution must have a signal-to-noise ratio ≥ 3:1.

Actual signal to noise ratios for the x 0.05 dilution, as obtained from the ClearView-processed chromatograms, were 60:1 for 1,1,1-trichloroethane and between 16:1 and 120:1 for other compounds. This greatly exceeds USP 467 suitability guidelines and allows the detection of toxic residues at much lower levels than is normally possible with conventional static HS methods.

In addition to screening for the presence of Class 1 or 2 solvents, USP 467 suggests a subsequent headspace analysis to be carried out for quantification. However, data from the initial HS–TD (Figure 2) demonstrated excellent linearity for all Class 1 compounds across the specified concentration range (Figure 3), indicating that quantification can be comfortably acheived in the preliminary analysis. No additional quantification step is required. The degree of linearity obtained in this study also demonstrates the suitability of Markes' HS5-TD technology for residual solvent analysis.

Figure 4 shows the chromatography obtained for Class 2A residual compounds at five different dilutions of the concentration limit specified in USP 467.

Figure 5 shows the linearity achieved for each compound in the 2A mixture, demonstrating the excellent performance of HS–TD, even at 5% of the concentration limit specified in USP 467.

Figure 6 shows the chromatograms for Class 2B residual compounds at five dilutions of the concentration limit specified in USP 467.

N,N-dimethylformamide and N,N-dimethylacetamide were obscured in the Class 2B chromatograms, therefore these compounds were run seperately at the five dilution levels. The resulting chromatogram is shown in figure 7.

Figure 8 again shows exceptional linearity over the concentration range for class 2B compounds including N,N-dimethylformamide and N,N-dimethylacetamide.

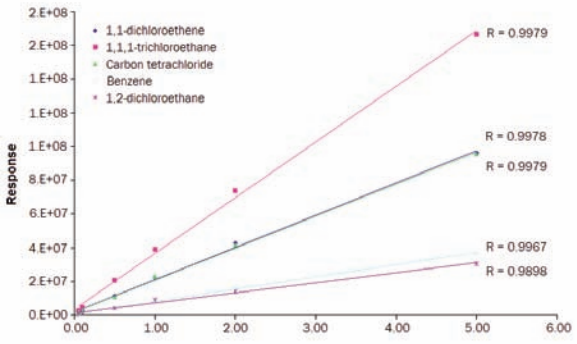

Figure 3: Linearity of HS–TD–GC/MS analysis of Class 1 residual solvents

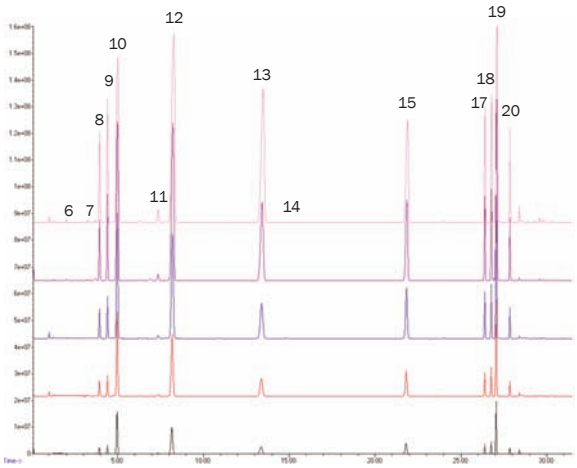

Figure 4a: HS–TD–GC/MS analysis of the Class 2A standard at five different dilutions (x 0.05, x 0.1, x 0.5, x 1 and x 2) of the USP 467 concentration limit

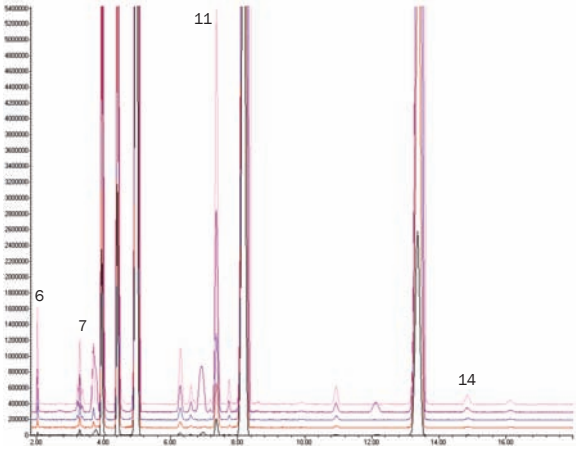

Figure 4b: Enlarged view showing HS–TD–GC/MS analysis of the Class 2A standard at five dilutions, showing the minor components 6, 7, 11 and 14

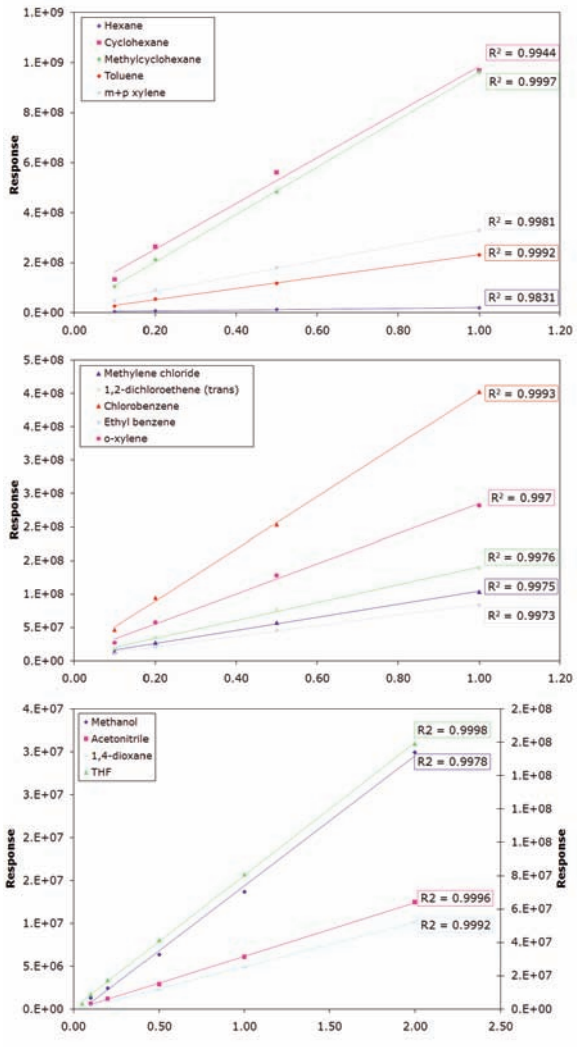

Figure 5: Linearity of HS–TD–GC/MS analysis of the Class 2A residual solvents over the concentrations of interest, in USP 467

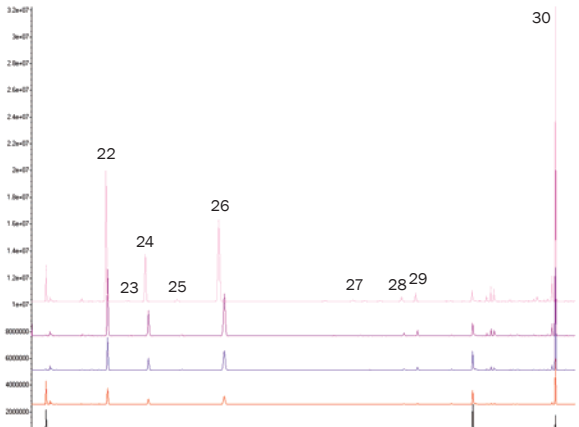

Figure 6a: HS–TD–GC/MS analysis of the Class 2B standard at five different dilutions (x 0.05, x 0.1, x 0.5, x 1 and x 2) of the USP 467 concentration limit

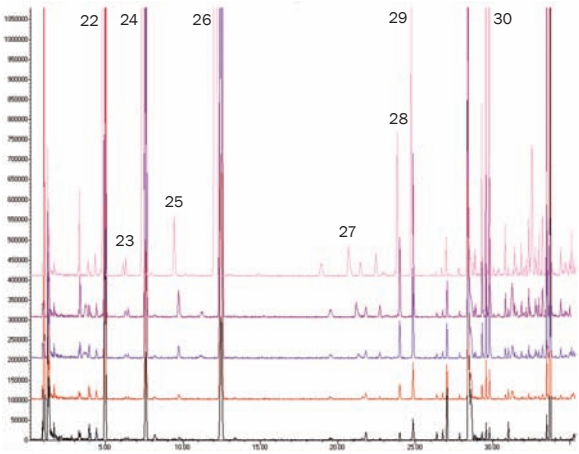

Figure 6b: Close-up display of the HS–TD–GC/MS analysis of the Class 2B standard at each dilution level showing the minor components 22, 23, 25, 27, 28 and 29

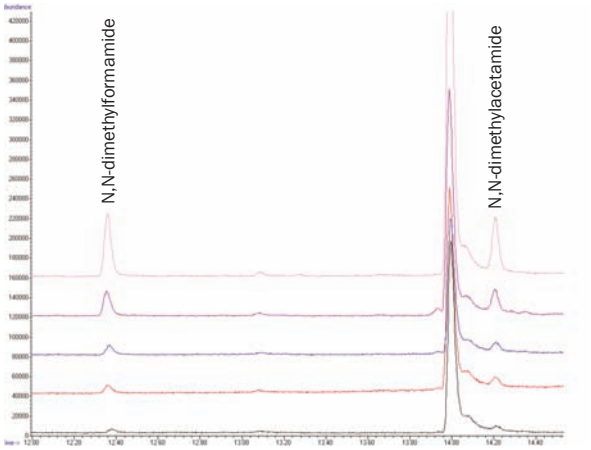

Figure 7: HS–TD–GC/MS analysis of N,N-dimethylformamide and N,N-dimethylacetamide at five different dilutions (x 0.05, x 0.1, x 0.5, x 1 and x 2) of the USP 467 concentration limit

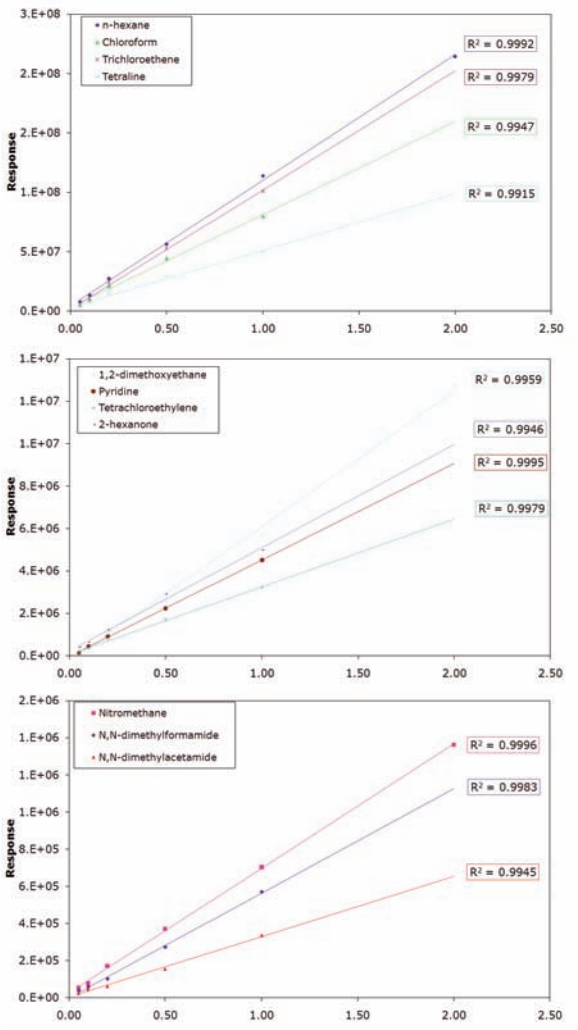

Figure 8: Class 2B residual solvents at various dilutions of the concentration limits specified in USP 467

Figure 9 shows the results obtained from running the two test omeprazole samples; one as sourced and the other spiked with several Class 1 and 2 residual solvents at the x 1 dilution level. The chromatogram for the unspiked pharmaceutical shows small peaks for some Class 2 residual solvents, e.g. 1,2-dichloroethane and methanol, amongst other non-regulated VOCs, plus the DMSO used for dilution. HS-TD-GC/MS analysis of the spiked sample illustrates the excellent sensitivity and broad applicability of the HS-TD technique, including detection of Class 1 compounds, e.g. benzene and carbon tetrachloride, at the lowest levels of interest.

Direct desorption

Results from direct desorption of a sample of unspiked omeprazole were compared to those from the HS–TD analysis (figure 10). Desorption temperatures, gas flows and times can be selected to mimic HS(–TD) data or to obatin more complete (exhaustive) extraction as in this case. Direct desorption is limited with respect to sample size (maximum typically <500 mg), making it unsuitable for inhomogeneous samples, however it provides an invaluable analytical option for measuring residual solvents in prototype drugs (where limited material is available) and for quantitative analysis of volatiles in insoluble drugs, which are difficult to analyse by HS(–TD).

Discussion and conclusions

The excellent linearity obtained for all Class 1 and 2 compounds at the various dilutions specified in USP 467 shows that HS–TD–GC/MS allows reliable quantitative determination of residual solvents in drugs at the levels require. The HS–TD method has also been shown to significantly exceed USP 467 sensitivity requirements.

This study further demonstrates that one HS–TD method can be used to qualify and quantify a wide variety of residual solvents in drugs/pharmaceuticals.

Sampling of a real-world pharmaceutical preparation further validates the Markes' HS–TD system for this method as no 'matrix interference' was observed in the spiked or unspiked samples.

The utility of the complementary direct desorption capability of the Markes' HS–TD analytical platform has also been demonstrated.

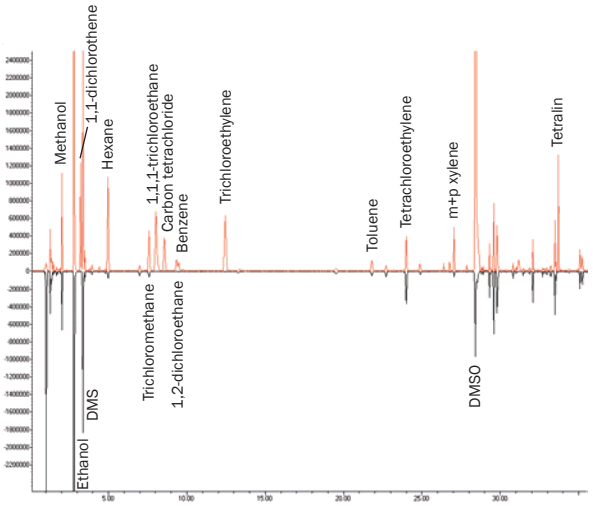

Figure 9: HS–TD–GC/MS analysis of residual solvents in the pharmaceutical preparation omeprazole. Plain sample (black trace) and spiked sample (red trace)

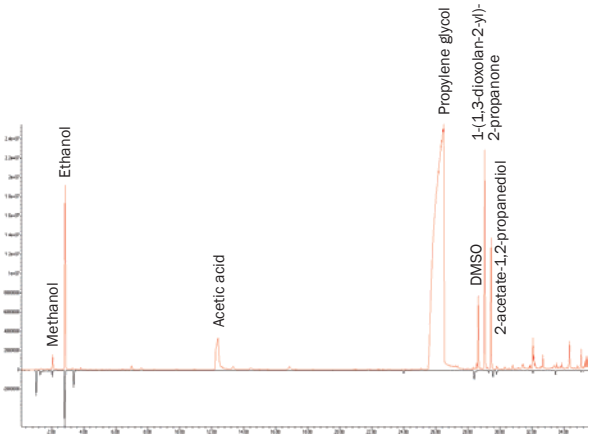

Figure 10: HS–TD–GC/MS analysis of unspiked omeprazole (black trace) overlaid with the direct desorption of 500 mg unspiked omeprazole at 100 °C (red trace)

Trademarks

HS5-TD™ and UNITY 2™ are trademarks of Markes International Ltd., UK

ClearView™ is a trademark of ALMSCO International, UK

References

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Applications were performed using the stated analytical conditions. Operation under different conditions, or with incompatible sample matrices, may impact the performance shown.

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