EVALUATION OF HYDROGEN AS A CARRIER GAS IN THE ANALYSIS OF RESIDUAL SOLVENTS IN PHARMACEUTICALS BY HS_GC_MS

Dr Diane Turner, Anthias Consulting Ltd.
Richard Stokes, Anthias Consulting Ltd.
Dr Geraint Morgan, The Open University

Contact: Gerard Catchpole – gerard.catchpole@vici.ch

INTRODUCTION

Residual solvents and organic volatile impurities (OVIs) in pharmaceuticals can result from the manufacturing process of the active pharmaceutical ingredients (API) and the final product. The level of residual solvents can also be affected by the packaging, storage and transportation of pharmaceutical products. All drug substances, excipients and drug products must be monitored and controlled for safety, their effect on crystalline form, solubility and stability[1].

One of the most common methods used is the United States Pharmacopeia (USP) Method <467> which closely follows the International Conference on Harmonisation (ICH) Q3C guideline Q3C (R6) on impurities: guideline for residual solvents[2]. Residual solvents have been classified by the ICH into three main classes based on their risk:

- Class 1: solvents are considered hazardous and should be avoided in the manufacturing process due to toxicity or environmental impact.
- Class 2: solvents should be limited in their use due to potential toxicity.
- Class 3: solvents are considered less toxic and pose a lower risk to human health.

Static headspace sampling followed by gas chromatography (GC) separation with either flame ionisation detection (FID) or hyphenation with a mass spectrometer (MS) is very common in Quality Control (QC) laboratories for the low-level determination of residual solvents in pharmaceutical manufacturing facilities. The most common carrier gas used for this application is helium. However, helium is a limited resource and, as such, is expensive with increasing costs and reducing availability, especially in developing countries. The necessity for this analysis, however, is increasing with higher consumption of pharmaceutical products and more products on the market; therefore, reducing the sample analysis time is important with the increasing need for higher sample throughput. As more scientific evidence gathers on the toxicity of chemicals like residual solvents, there is always a potential for a reduction in concentration limits and therefore the need for more sensitive analysis methods. To summarise, there is a need for more sensitive methods, enabling a higher throughput of samples while reducing costs and making the analytical methods accessible to all.

Helium (He), is one of the three most common gases used as the mobile phase in GC, alongside nitrogen (N_2) and hydrogen (H_2). How good each of these gases is at separating against the analysis time can be summarised by the van Deemter plot (Figure 1). The smaller the Height Equivalent of a Theoretical Plate (HETP) the better the column efficiency, whereas, higher linear velocities equate to shorter run times. Although nitrogen produces the best separations, this is only possible at low

VICI AG International address: Parkstrasse 2, CH-6214 Schenkon, Switzerland
web: www.vicidbs.com email: sales@vicidbs.com phone: +41 41 925-6200

velocities resulting in longer run times. The most common carrier gas, helium, is widely used as it is inert and although not producing such a good separation as nitrogen can be used at higher velocities. Hydrogen is becoming more common in GC methods, as although there are concerns over safety, high background signal due, for example, to higher column bleed and potential reactions with analytes, it produces better separation over a larger velocity range than helium and can result in faster analyses with better signal to noise ratios. The use of gas generators can alleviate some of the hydrogen concerns by producing high quality gas on demand.

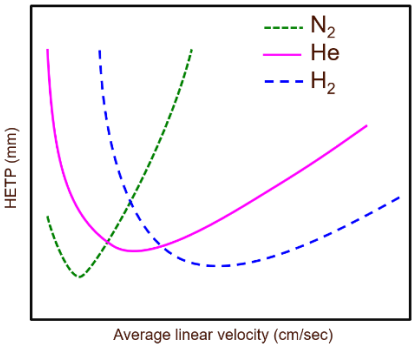


Figure 1: Representation of the van Deemter plots for N_2, He and H_2

AIMS

The aim of this white paper is to optimise and produce a robust and repeatable method for the analysis of residual solvents in pharmaceuticals, using GC-MS and generated hydrogen carrier gas. Then, evaluate the use of hydrogen as the carrier gas for the separation and detection of an ICH Class 2 Mix of solvents in paracetamol tablets.

EXPERIMENTAL

A residual solvents stock standard was prepared by diluting 1000 µL of the European Pharmacopeia/ICH Class 2 Mix A (PN36229, Restek, Belfont, PA, USA) to 50 mL with methanol. An internal standards stock solution at a concentration of 20 ppm was prepared by diluting 800 µLof 8260A Internal Standard Mix (PN 30241, Restek, Belfont, PA, USA) to 100 mL with methanol.

Calibration standards and blanks were prepared by adding 5 ± 0.05 g of VOC free sand (Supelco, Bellefonte, PA, USA), 1.4 ± 0.1 g sodium chloride (Sigma-Aldrich, St Louis, MO, USA) and 5 ± 0.1 mL of Milli-Q water to a 20 mL headspace vial using a 4-decimal place BP211D high sensitivity balance (Sartorius AG Gottingen, Germany). To this, 100 µL of the internal standard stock solution and 0 (for a blank), 1, 5, 10, 25, 100 or 250 µL of the residual solvent stock solution was added, respectively, and the vial immediately capped. Paracetamol samples were prepared by crushing the tablet with a pestle and mortar then adding to a tared 20 mL headspace vial and making up to a final weight of 5 ± 0.05 g with VOC free sand. Then, 1.4 ± 0.1 g sodium chloride, 5 ± 0.1 mL of Milli-Q water and 100 µL of the internal standard stock solution was added and the vial immediately capped.

The analyses were performed on an Agilent 7890 GC with 5975C XL inert MSD (Agilent Technologies Inc., Santa Clara, CA, USA) and a CTC Analytics CombiPal autosampler (CTC Analytics AG, Zwingen, Switzerland). Hydrogen carrier gas was supplied by a VICI DBS NM Plus hydrogen generator. The method was optimised so as to use the lowest temperatures thus minimising any reactions with hydrogen. The vials were equilibrated at 70 °C for 45 mins, at 750 rpm, with 10 s on

VICI AG International address: Parkstrasse 2, CH-6214 Schenkon, Switzerland
web: www.vicidbs.com email: sales@vicidbs.com phone: +41 41 925-6200

and 2 s off. 1 mL of the headspace was injected at 440 µL/s into the GC inlet, held at 120 °C. A 2 mm internal diameter liner was installed and operated with a split ratio of 5:1. The hydrogen carrier gas was set to constant flow at 2 mL/min. Separation was performed on an Rxi-624Sil MS column (30 m x 0.25 mm x 1.4 µm film thickness) (PN 13868, Restek, Belfont, PA, USA). The GC oven was programmed from 30 °C, with a 0.5 min hold, to 145 °C at 15 °C/min. The total run time was 8.17 mins. Compounds eluted through a heated transfer line held at 150 °C into the MS with the ion source set at 200 °C and the quadrupole at 150 °C. A BFB tune was used with a gain factor of 1.02 and the MS was scanned from 30 to 200 u with a threshold of 20 and a sampling rate of 3. The headspace syringe was purged with nitrogen for 3 minutes after sample analysis.

RESULTS & DISCUSSION

Procedural blanks were analyzed before and after the calibration, the replicate Calibration Standard Level 2 standards, the replicate Calibration Standard Level 4 standards and between each sample type to check for carryover. No carryover was found.

Extracted calibration standards were prepared and analyzed at six different levels. A chromatogram of extracted Calibration Standard Level 5 is shown in Figure 2 and shows excellent separation and peaks shapes of the residual solvents. Calibration ranges for each residual solvent can be seen in Table 1. For the lowest concentration residual solvent, 1,1,2-trichloroethylene, this was from 0.32 to 80 ppb and for the highest concentration residual solvent, cyclohexane, this was from 15.52-3880 ppb. The peaks were normalized against the internal standards, fluorobenzene or chlorobenzene-d5, each present at a concentration of 400 ppb. The correlation coefficients for all residual solvents can be seen in Table 1; whilst, the calibration curves can be seen in Figure 3. All residual solvents showed linear regression curves across 2.5 orders of magnitude. All correlation coefficients were >0.999, except for 3 of the most volatile compounds and these had correlation coefficients of >0.998.

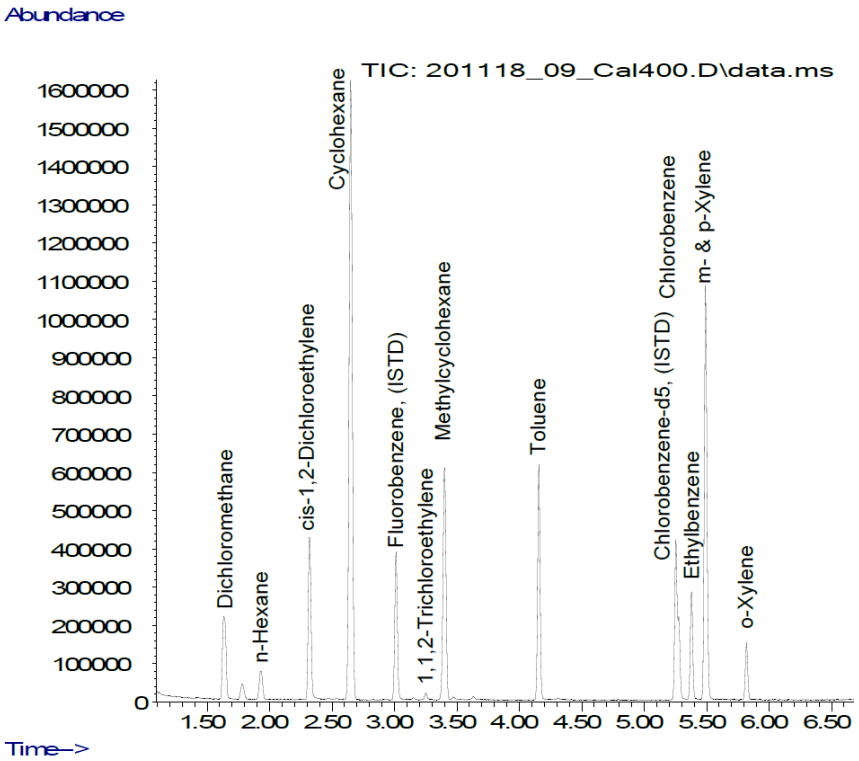
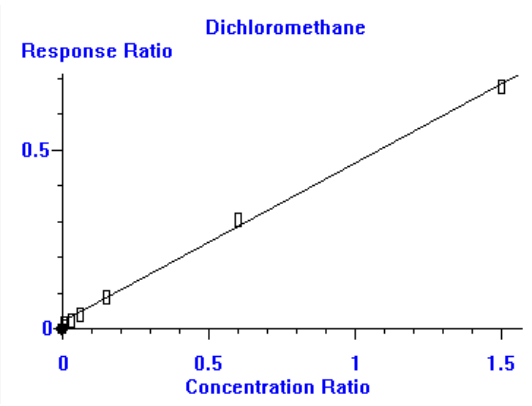
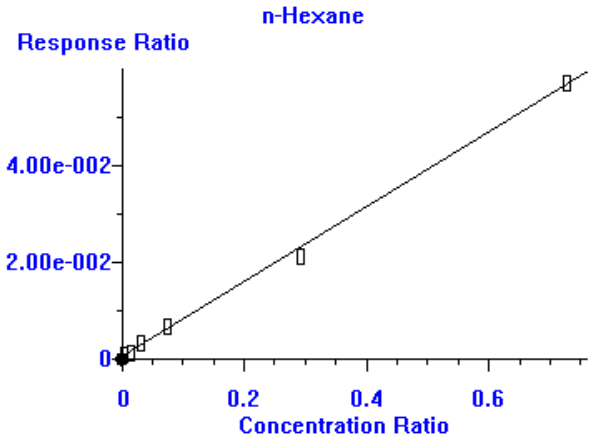
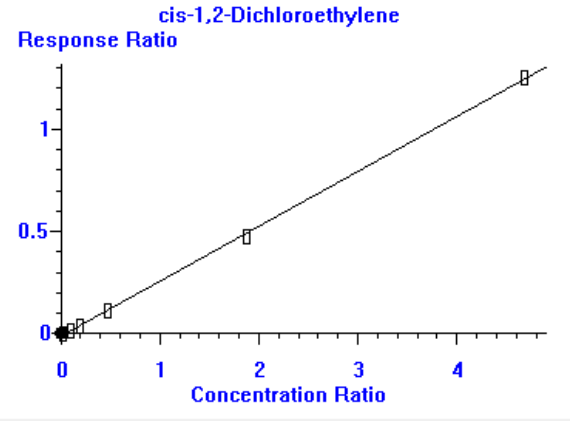


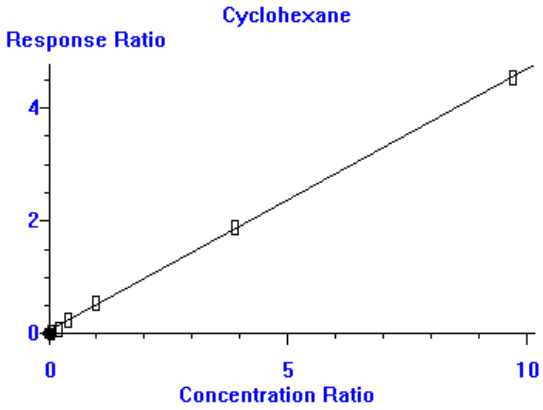
Figure 2: Total ion chromatogram (TIC) of extracted Calibration Standard Level 5 acquired in full scan mode containing 0.032-3.88 ppm of residual solvents by HS-GC-MS

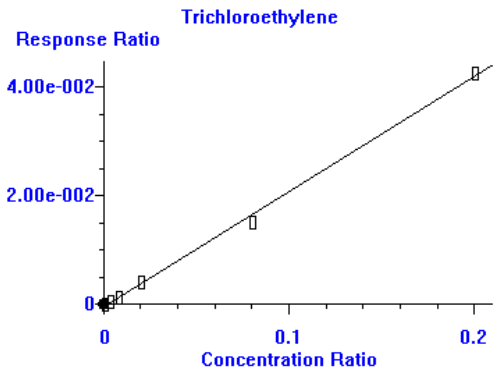
VICI AG International address: Parkstrasse 2, CH-6214 Schenkon, Switzerland
web: www.vicidbs.com email: sales@vicidbs.com phone: +41 41 925-6200

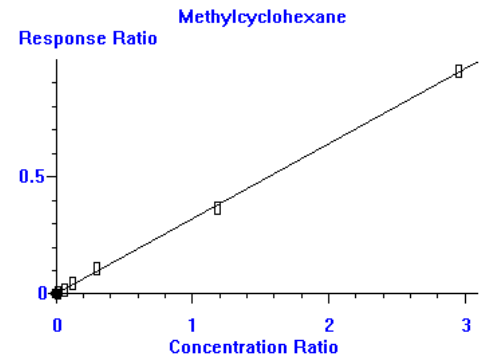












VICI AG International address: Parkstrasse 2, CH-6214 Schenkon, Switzerland
web: www.vicidbs.com email: sales@vicidbs.com phone: +41 41 925-6200

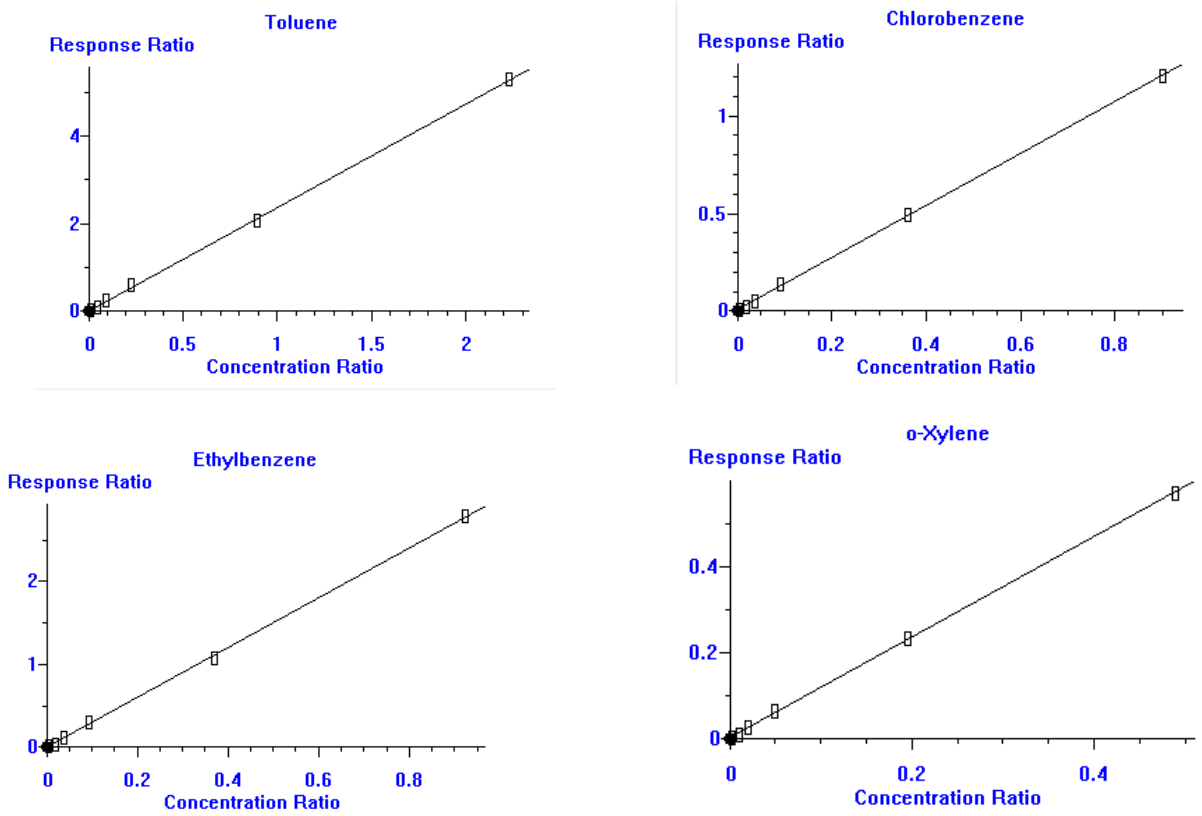


Figure 3: Calibration curves for all residual solvents

The Limit of Detection (LOD) was determined by analysing 6 replicate extracted calibration standards at Calibration Level 2 with a concentration of 1.6 - 77.6 ppb for each residual solvent. Concentrations were determined from the calibration curves, from these the standard deviation was calculated and multiplied by the students t-test for 99 % statistical confidence for n-1. The LOD for each residual solvent can be seen in Table 1 and these range from 0.66 to 20.8 ppb.

Table 1: The retention times, calibration range, correlation coefficients, LODs , repeatability and accuracy determined for the residual solvents.

Compound name	Retention time (min)	Calibration range* (ppb)	R^2	LOD** (ppb)	Repeatability (% RSD)***	Accuracy (%)****
Dichloromethane	1.640	2.4-600	0.998242	3.31	5.90	103.5-121.5
n-Hexane	1.934	1.16-290	0.998508	1.87	5.27	81.0-99.0
cis-1,2-Dichloroethylene	2.326	7.48-1870	0.999731	4.91	1.85	81.5-96.2
Cyclohexane	2.656	15.52-3880	0.999281	20.80	2.46	91.4-105.4
1,1,2-Trichloroethylene	3.254	0.32-80	0.998035	0.66	3.59	82.7-102.6
Methylcyclohexane	3.407	4.72-1180	0.999431	5.06	2.83	92.3-104.9
Toluene	4.163	3.56-890	0.999628	3.47	2.37	99.3-107.6
Chlorobenzene	5.284	1.44-360	0.999755	1.10	1.83	91.6-103.9
Ethylbenzene	5.385	1.48-369	0.999586	0.97	3.02	94.0-103.0
m- & p-Xylene	5.498	6.42-1606	0.999612	7.29	1.48	100.7-110.1
o-Xylene	5.824	0.78-195	0.999654	0.68	3.58	96.9-103.8

VICI AG International address: Parkstrasse 2, CH-6214 Schenkon, Switzerland
web: www.vicidbs.com email: sales@vicidbs.com phone: +41 41 925-6200

Where:
* = 6 point,
** = Calibration Standard Level 2, n=6, concentration STDEV x 3.365,
*** = Calibration Standard Level 4, n=6, (STDEV x Mean) x 100) of peak areas
**** = Calibration Standard Level 4, n=6, %recovery = (conc/known conc) x 100%

The repeatability and accuracy were both determined from the analysis of 6 replicate extracted calibration standards at Calibration Standard Level 4 with a concentration of 8 - 388 ppb for each residual solvent. The repeatability was determined from the area of each peak, without normalisation against the internal standards and the results are presented in Table 1. These show low % RSD for a headspace method with values of 1.48 – 5.90 %, the highest values being those of the two most volatile residual solvents. The accuracy was calculated by determining the concentration of each compound for each replicate using their calibration curves, then comparing to the known concentration to determine the recoveries, the lowest and highest recoveries are shown in Table 1.

To test the method, three different types of paracetamol tablets were analysed in duplicate. The results are presented in Tables 2 and 3.

Table 2: Samples analysed for residual solvents

Paracetamol tablet	Weight (including any capsule) (g)	Residual solvents determined	Concentration in the tablet (ppm)
Sample 1 500 mg	0.61544	None	-
Sample 1 500 mg	0.62166	None	-
Sample 2 500 mg	0.54913	None	-
Sample 2 500 mg	0.54392	None	-
Sample 3 500 mg	0.63041	n-Hexane below calibration	-
Sample 3 500 mg	0.65067	n-Hexane 1.82 ppb	0.0028

No residual solvents were identified by the method for Samples 1 or 2 paracetamol tablets. In the Sample 3 tablets a very low concentration, of n-hexane was determined. The determined value is far below the ICH limit for hexane of 290 ppm.

As no residual solvents were determined in the Sample 2 tablets, another sample of this tablet was prepared, it had a weight of 0.55404 g, and was spiked with calibration standard 4 and analysed as a matrix spike, the results can be seen in Table 3.

VICI AG International address: Parkstrasse 2, CH-6214 Schenkon, Switzerland
web: www.vicidbs.com email: sales@vicidbs.com phone: +41 41 925-6200

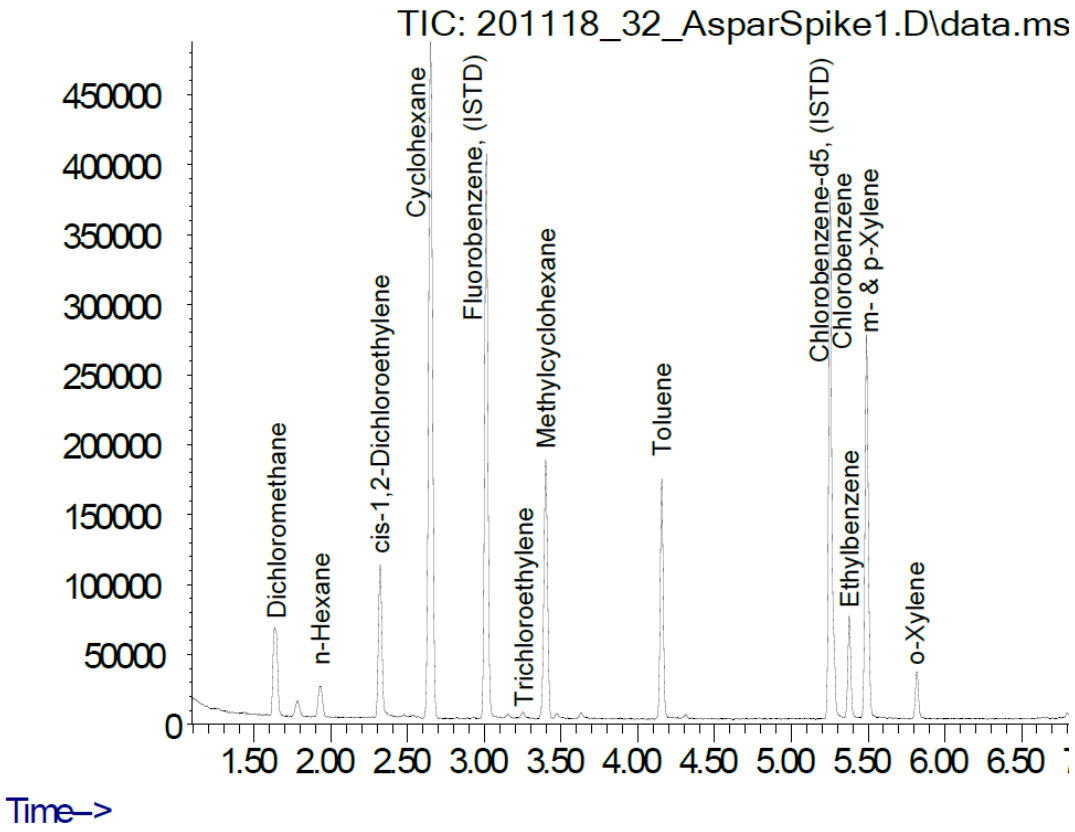


Figure 4: Sample 2 500 mg paracetamol tablet spiked at Calibration Standard Level 4

Table 3: Matrix spike results for Sample 2 500 mg sample spiked at Calibration Standard Level 4.

Compound name	Retention time (min)	Spiked concentration (ppb)	Determined concentration (ppb)	Recovery (%)
Dichloromethane	1.637	60.0	61.28	102.1
n-Hexane	1.927	29.0	27.69	95.5
cis-1,2-Dichloroethylene	2.320	187.0	181.79	97.2
Cyclohexane	2.650	388.0	432.24	111.4
1,1,2-Trichloroethylene	3.253	8.0	8.16	102.0
Methylcyclohexane	3.398	118.0	129.27	109.6
Toluene	4.155	89.0	106.37	119.5
Chlorobenzene	5.275	36.0	36.47	101.3
Ethylbenzene	5.376	36.9	39.31	106.5
m- & p-Xylene	5.490	160.6	175.95	109.6
o-Xylene	5.816	19.5	19.51	100.0

Good recoveries were determined for all residual solvents which closely followed recovery values determined in the method validation, the exception is toluene and further investigation would be required.

VICI AG International address: Parkstrasse 2, CH-6214 Schenkon, Switzerland
web: www.vicidbs.com email: sales@vicidbs.com phone: +41 41 925-6200

CONCLUSIONS

The method developed using generated hydrogen as the carrier gas for the HS-GC-MS determination of residual solvents in pharmaceuticals shows very good potential in speed and separating capacity. No adverse effects were observed concerning possible reaction of hydrogen carrier gas with analytes or the stationary phase of the GC column. The purity of the carrier gas as provided by the VICI DBS NM Plus hydrogen generator was fully satisfying. Changing from helium supported by pressure bottles to on-site generated hydrogen proved to be feasible without troubles and allowed steady operation with reduced efforts for system maintenance and eliminated safety issues.

The calibration curves were linear over a wide concentration range, taking this application far below the current required limit of detection; therefore, in terms of sensitivity this application should be useable far into the future, even if concentration limits are reduced. In addition, further sensitivity could simply be gained by running the MS in Selected Ion Monitoring (SIM) mode, rather than scan mode as demonstrated.

The last target compound, o-xylene eluted in less than 6 minutes, much earlier than conventional methods using helium, where it elutes in just under 14 minutes or at up to 28 minutes, depending on the method followed. This optimised method is therefore nearly five times faster than existing methods. The carrier gas flow rate was limited by the pumping capacity of the MS vacuum, if an FID was utilised for this application higher flow rates, in-line with the van Deemter curve for hydrogen, could be used resulting in even shorter run times. Excellent separation of the target compounds was achieved (except for the usual co-elution of m- and p-xylene) along with excellent peak shape, due to the use of hydrogen as a carrier gas. Other residual solvents could also be easily added to this application and still result in a much faster run time than conventional helium-based methods. Therefore, the use of hydrogen as a carrier gas enables the analysis time to be far reduced along with giving excellent separation, peak shape and sensitivity.

As well as the analytical advantages, there are also safety aspects that are addressed when comparing hydrogen to helium. The amount of stored gas in a generator is very small, compared to the high pressure (up to 200 bar), heavy, cumbersome cylinders that helium is supplied in. A VICI DBS generator will shut down in the event of a leak, therefore removing the danger of the lower explosive limit being reached.

With the price of helium constantly increasing, and variation in supply, this white paper shows that there is no reason why hydrogen should not be considered as an alternative to helium as the carrier gas in GC-MS applications, in particular for **analysis of residual solvents in pharmaceuticals by HS-GC-MS**.

REFERENCES

1. European Medicine Agency, ICH guideline Q3C (R6) on impurities: guideline for residual solvents EMA/CHMP/ICH/82260/2006 [9 August 2019]
2. USFDA Q3C – Tables and List Guidance for Industry [June 2017] ICH Revision 3

VICI AG International address: Parkstrasse 2, CH-6214 Schenkon, Switzerland
web: www.vicidbs.com email: sales@vicidbs.com phone: +41 41 925-6200