

Residual solvents analysis using an Agilent Intuvo 9000 GC system

Technology advantage: simplified dual column analysis with Flow Chip modularity



Introduction

USP <467> defines a method for analysis of residual solvents in pharmaceuticals. The choice of solvent during product manufacturing can improve yield or affect chemical properties of the product synthesized. Solvents do not enhance the product's efficacy, and must be removed as much as possible to meet product specification and good manufacturing practices¹. Therefore, testing for residual solvents during production or purification processes is a necessary aspect of manufacturing.

USP <467> specifies a single column analysis with confirmation on a second system configured with a different analytical column yielding different retention times. With conventional gas chromatographic systems, this analysis requires two separate analytical runs. An Agilent Intuvo 9000 GC system with an inlet splitter for dual flame ionization detection (FID) enables both analyses in a single run, in half the time.

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Experimental

An Intuvo 9000 GC system was equipped with an Agilent 7697A headspace sampler. Class 1, Class 2A, and Class 2B standard solutions were prepared and evaluated according to USP <467> methodology. Class 1 was prepared from an Agilent concentrated ampoule (p/n 5190-0490) by diluting 1 mL of the standard and 9 mL of dimethyl sulfoxide to 100 mL in water (stock 1). Stock 2 was prepared by diluting 1 mL of stock 1 to 100 mL with water. Lastly, 10 mL of stock 2 was diluted to 100 mL with water to make stock 3. Stock 3 was used for headspace vial preparations.

Agilent Class 2A (p/n 5190-0492) and Class 2B (p/n 5190-0513) were prepared by diluting 1 mL of the standard to 100 mL with water for the respective classes.

Headspace vials were prepared by adding 1 mL of each class dilution to 5 mL of water.

Replicate samples were analyzed using the 7697A headspace sampler and the Intuvo 9000 GC system configured with an inlet splitter for dual column, dual detection.

Tables 1 and 2 display instrument parameters almost the same as those used in previous work². To reflect the improvements of column offerings for this application, different columns were chosen: Agilent DB-624 Select Ultra Inert (UI) and Agilent DB-WAX Ultra Inert (UI). Adding the Guard Chip and the bus is unique to the Intuvo 9000 GC system. Due to the cleanliness of the sample introduced, the Jumper Chip was chosen. It was held at the inlet temperature to transfer the sample from the inlet through the Jumper Chip to the inlet splitter. The bus was held at the default setpoint.

Table 1. Instrument conditions are given for the Agilent Intuvo 9000 GC system.

Agilent Intuvo 9000 GC	Setpoint
Oven	40 °C (5 minutes) 15 °C/min to 180 °C (3 minutes)
Split/Splitless inlet	Split 5:1, 140 °C
DB-624 Select Ultra Inert (123-0334UI-INT) 30 m × 320 µm, 1.8 µm	Constant flow 2 mL/min
DB-WAX Ultra Inert (123-7032UI-INT) 30 m × 320 µm, 0.25 µm	Controlled by column 1
FID (front and back)	250 °C
H ₂	30 mL/min
Air	400 mL/min
N ₂ (make up)	25 mL/min
Jumper Chip	140 °C
Bus	200 °C
Front/Back signal	20 Hz

Table 2. Instrument conditions are given for the Agilent 7697A headspace sampler.

Agilent 7697A headspace sampler	Setpoint
Oven	85 °C
Loop	85 °C
Transfer line	100 °C
Vial equilibration time	40 minutes
Injection duration	0.5 minutes
Vial size	10 mL
Vial shaking	Level 2, 25 shakes/min
Vial fill mode	Default (50 mL/min to 15 psi (0.1 minutes))
Vial fill pressure	15 psi
Loop ramp rate	20 psi/min
Loop final pressure	0 psi
Loop equilibration time	0.05 minutes



Agilent 7697A headspace sampler.

Results and discussion

Nine headspace vials were prepared for each solvent standard (Classes 1, 2A, and 2B), and repeatability (area and retention time) was evaluated. Excellent retention time repeatability was achieved on both columns for the three classes evaluated. Most analytes gave a relative standard deviation (RSD) of <0.1 % for retention time repeatability. Area repeatability was also good, giving an RSD of 5 % or better for all analytes on both columns. Most of the analytes were 2 % or better for area RSD. Tables 1–3 give the retention time and area repeatability measurements for the three classes on dual columns.

Class 1 showed the highest RSDs for area (Table 3). Due to the multistage dilutions for Class 1, the final concentration is the lowest of the three classes evaluated. This dilution results in greater variability, as it is a measurement of the sample preparation reproducibility, instrument precision, and peak integration consistency.

Class 2A resulted in the best area precision (Table 4). This is likely due to the concentration evaluated, which was higher than Class 1 or Class 2B. Class 2B has a wider range of concentrations, which is reflected in the wider range of area RSDs achieved (Table 5). For lower concentration analytes or analytes with a higher degree of asymmetry (*n*-hexane, nitromethane, and 1,2-dimethoxyethane), the area RSDs are slightly higher than those with high responses and symmetric peaks (tetralin). Retention time repeatability was excellent regardless of class, response, or symmetry. Except for nitromethane (0.4 %), all analytes were 0.2 % or better for retention time repeatability, with most analytes even more precise, at <0.1 %.

Table 3. Retention time precision and peak area repeatability for Class 1 compounds evaluated with the inlet splitter on the Agilent Intuvo 9000 GC system.

Compound	Agilent DB-624 RT RSD (%)	Agilent DB-624 area RSD (%)	Agilent DB-WAX RT RSD (%)	Agilent DB-WAX area RSD (%)
1,1-Dichloroethane	0.031 %	3.3 %	0.074 %	2.1 %
1,1,1-Trichloroethane	0.026 %	2.6 %	0.037 %	1.8 %
Carbon tetrachloride	0.11 %	4.8 %	Coelutes with 1,1, 1-trichloroethane	Coelutes with 1,1, 1-trichloroethane
Benzene	0.017 %	2.2 %	0.055 %	1.5 %
1,2-Dichloroethane	0.016 %	3.9 %	0.067 %	1.8 %

Table 4. Retention time precision and peak area repeatability for Class 2A compounds evaluated with the inlet splitter on the Agilent Intuvo 9000 GC system.

Compound	Agilent DB-624 RT RSD (%)	Agilent DB-624 area RSD (%)	Agilent DB-WAX RT RSD (%)	Agilent DB-WAX area RSD (%)
Methanol	0.23 %	1.3 %	0.22 %	1.3 %
Acetonitrile	0.023 %	2.1 %	0.039 %	2.0 %
Methylene chloride	0.018 %	0.98 %	0.033 %	0.78 %
<i>trans</i> -1,2-Dichloroethene	0.016 %	0.85 %	0.023 %	0.72 %
<i>cis</i> -1,2-Dichloroethene	0.012 %	0.76 %	0.039 %	0.78 %
Tetrahydrofuran	0.018 %	0.95 %	Coelutes with methylene chloride	Coelutes with methylene chloride
Cyclohexane	0.011 %	0.96 %	0.013 %	0.96 %
Methylcyclohexane	0.0087 %	0.98 %	0.018 %	0.96 %
1,4-Dioxane	0.012 %	1.9 %	0.025 %	1.7 %
Toluene	0.0073 %	0.81 %	0.029 %	0.80 %
Chlorobenzene	0.0061 %	0.69 %	0.024 %	0.69 %
Ethylbenzene	0.0060 %	0.84 %	0.016 %	0.86 %
<i>m</i> -Xylene	0.0061 %	0.82 %	0.017 %	0.82 %
<i>p</i> -Xylene	Coelutes with <i>m</i> -xylene	Coelutes with <i>m</i> -xylene	0.015 %	0.83 %
<i>o</i> -Xylene	0.0059 %	0.81 %	0.026 %	0.81 %

Table 5. Retention time precision and area repeatability for Class 2B compounds evaluated with the inlet splitter on the Agilent Intuvo 9000 GC system.

Compound	Agilent DB-624 RT RSD (%)	Agilent DB-624 area RSD (%)	Agilent DB-WAX RT RSD (%)	Agilent DB-WAX area RSD (%)
Hexane	0.021 %	4.4 %	0.039 %	4.5 %
Nitromethane	0.42 %	4.9 %	0.018 %	2.5 %
Chloroform	0.0099 %	4.8 %	0.026 %	3.2 %
1,2-Dimethoxyethane	0.013 %	3.6 %	0.089 %	3.6 %
Trichloroethane	0.010 %	3.4 %	0.028 %	3.1 %
Pyridine	0.020 %	1.7 %	0.034 %	1.7 %
2-Hexanone	0.0081 %	2.7 %	0.020 %	0.74 %
Tetralin	0.0078 %	2.1 %	0.016 %	2.1 %

The dual column analysis achieved with the 7697A headspace sampler and Intuvo 9000 GC system also provided excellent chromatographic performance and demonstrated the advantages of running both columns simultaneously. In Class 1, carbon tetrachloride shows adequate signal-to-noise to meet USP <467> requirements (Figure 1).

Both the DB-624 Select UI and DB-WAX UI columns show excellent peak symmetry and similar responses for both columns. The separation between 1,1,1-trichloroethane and carbon tetrachloride on DB-624 Select UI, and lack thereof on DB-WAX UI (Figure 2) provide an example of the usefulness of evaluating both columns simultaneously.

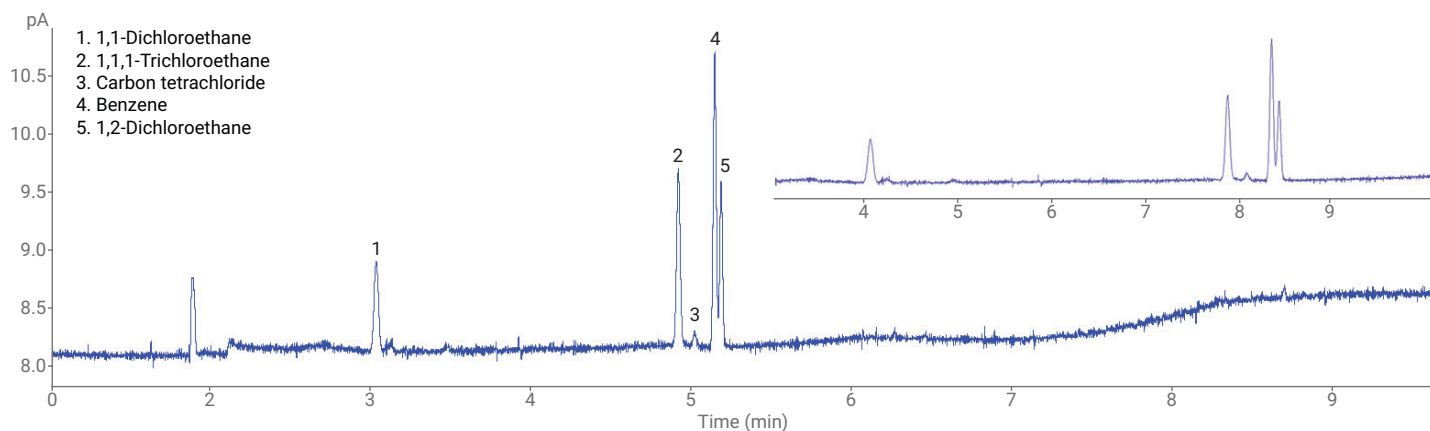


Figure 1. Analysis of Class 1 residual solvents on an Agilent DB-624 Select UI column demonstrates excellent chromatography and meets signal-to-noise requirements for all solvents, including carbon tetrachloride.

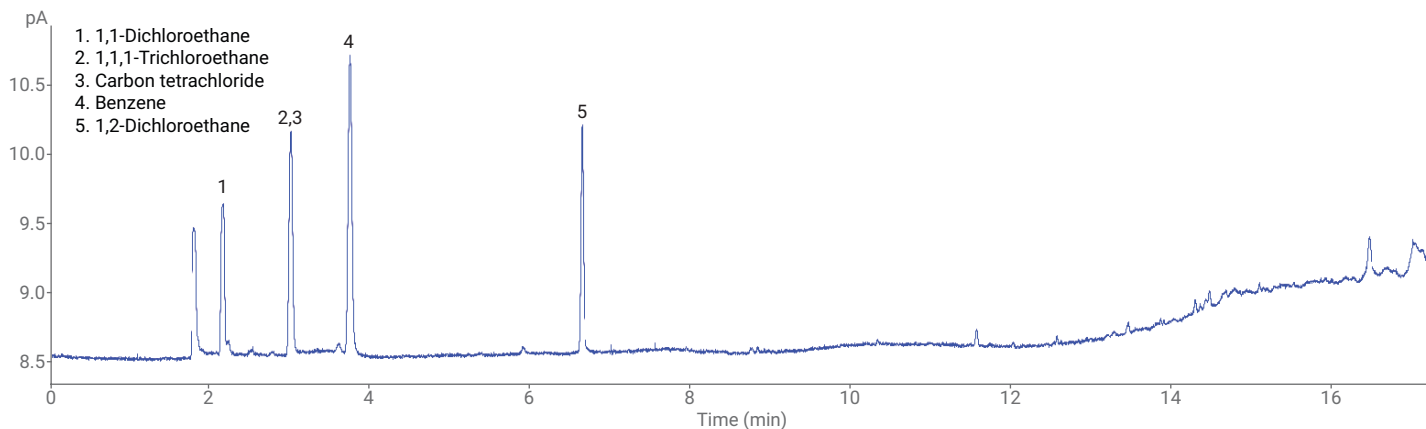


Figure 2. Analysis of Class 1 compounds on an Agilent DB-WAX UI yields the same elution order as an Agilent DB-624 Select UI column, although retention times have shifted, as expected.

Class 2A also demonstrated excellent peak symmetry for the solvents evaluated. Significant elution order changes were observed between the DB-624 Select UI and DB-WAX UI columns, as expected from different stationary phases (Figures 3 and 4). Contrary to Class 1 solvents, where better resolution for critical analytes (carbon tetrachloride) was achieved on the DB-624 Select UI column, the

DB-Wax UI column provided better resolution of xylene isomers included in Class 2A. This highlights an advantage of running both columns simultaneously. The DB-624 Select UI, which shows better performance for Class 1, and the DB-WAX UI, which shows better performance for Class 2A, can be run on one system, providing optimal chromatography for both classes.

Class 2B provides the final example of the benefits of using a dual column ensemble for USP <467> residual solvents. Nitromethane yields a low response and a nonsymmetric peak shape on the DB-624 Select UI column (Figure 5, peak 2). However, on the DB-WAX UI column, the peak shape and response is improved (Figure 6). This is also reflected in the improvement in area RSD for nitromethane between the DB-624 Select UI and DB-WAX UI columns.

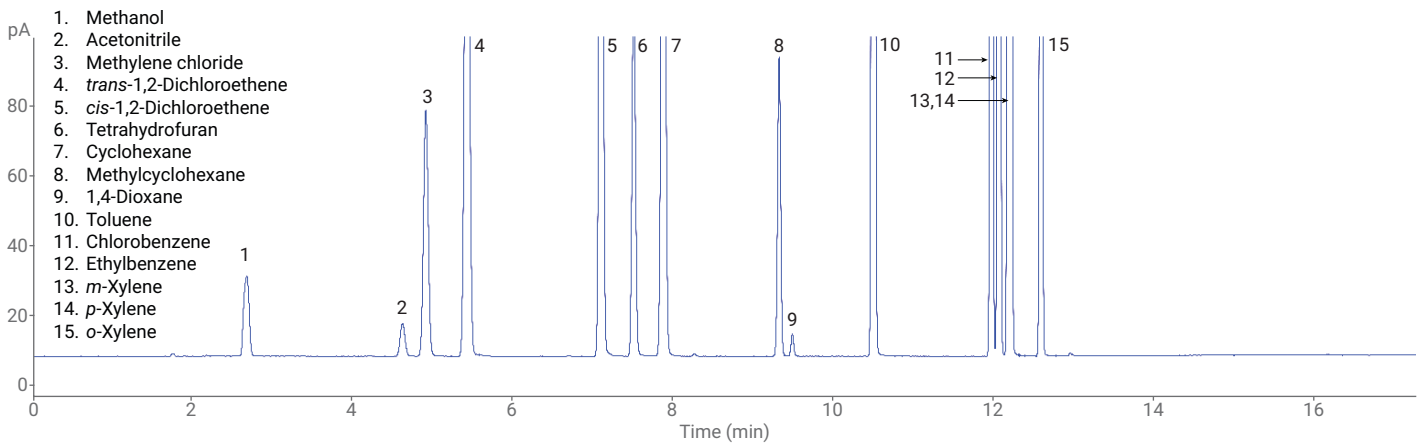


Figure 3. Analysis of Class 2A on an Agilent DB-624 Select UI column shows excellent peak shape.

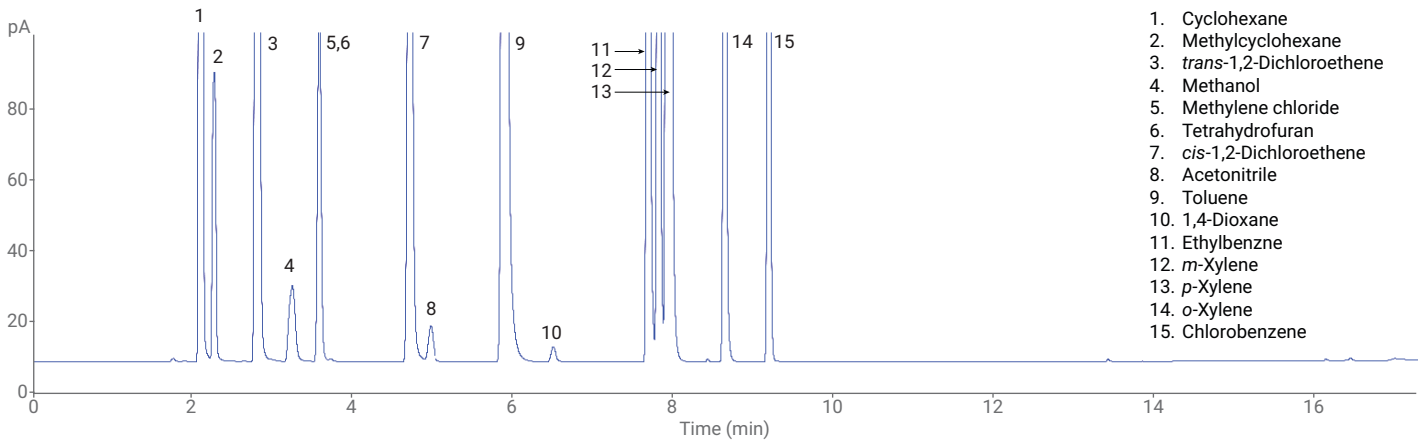


Figure 4. Analysis of Class 2A on an Agilent DB-WAX UI column demonstrates elution order changes.

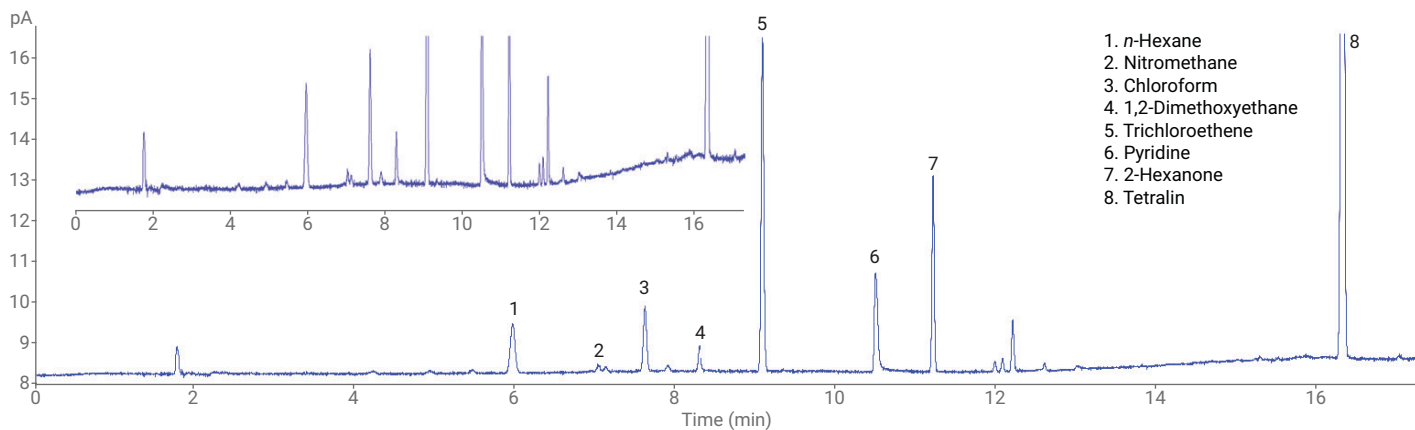


Figure 5. Analysis of Class 2B residual solvents on an Agilent DB-624 Select UI column demonstrates excellent peak shape across a range of responses.

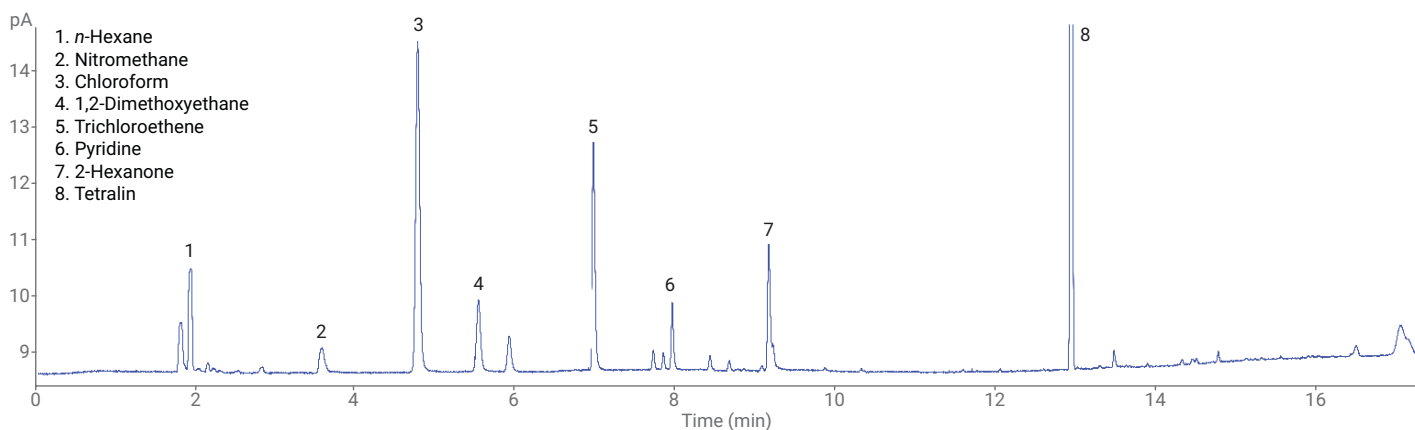


Figure 6. Analysis of Class 2B residual solvents on an Agilent DB-WAX UI column shows improved peak shape for nitromethane.

Conclusions

The Agilent Intuvo 9000 GC system equipped with the Agilent 7697A headspace sampler configured as a dual column, dual FID system delivers excellent repeatability. The results achieved with this system are consistent with previously published results for USP <467> on conventional single column GC systems^{2,3}. Area precision for nine headspace vials was 5 % or better, and retention time repeatability was 0.2 % or better.

Evaluating USP <467> residual solvents with a dual column ensemble offers more advantages. While Class 1 residual solvents showed the best resolution on the Agilent DB-624 Select UI columns, the xylene isomers in Class 2A were fully resolved on the Agilent DB-WAX UI columns. By configuring an Intuvo 9000 GC with an inlet splitter, both columns can be run simultaneously to take advantage of the chromatographic improvements achieved with two stationary phases. This configuration

eliminates the need to run the samples on two systems with two different columns, potentially cutting the total analysis time in half.

The 7697A headspace sampler combined with the Intuvo 9000 GC system configured with an inlet splitter for dual column dual FID analysis provides a robust and straightforward way to analyze USP <467> residual solvents while decreasing analysis time.

References

1. USP 40, general chapter USP <467> Residual Solvents, <https://hmc.usp.org/sites/default/files/documents/HMC/GCs-Pdfs/c467.pdf> (accessed October 2017).
2. Firor, R. L. Analysis of USP <467> residual solvents with improved repeatability using the Agilent 7697A Headspace Sampler, *Agilent Technologies Application Note*, 5990-7625EN, August **2012**.
3. B. Tienport, F. David, P. Sandra. Analysis of USP <467> residual solvents using the Agilent 7697A Headspace Sampler with the Agilent 7890B Gas Chromatograph, *Agilent Technologies Application Note*, 5991-1834EN, January **2013**.

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