Agilent J&W CP-Select 624 Hexane for Best Separation of Solvents and Hexane Isomers

Application Note

Drug Development and Manufacturing, Food Processing and Packaging

Authors

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Abstract

The Agilent J&W CP-Select 624 Hexane GC column, with an optimized G43 stationary phase, separates dichloromethane (DCM) from hexane isomers. This application note shows the separation as applied by pharmaceutical and food processing industries in India. Each column is tested with a special test mix that guarantees good column-to-column reproducibility. As with the specific separation of DCM, the USP <467> specification for the separation of acetonitrile and dichloromethane is in compliance, and tested for in every batch of stationary phase. Column selectivity is compared for two series using a multicomponent mix against two columns from another vendor.

Introduction

The CP-Select 624 Hexane GC column is applicable for:

- Food - hexane extraction solvent in food processing
- Pharma - hexane solvent for purification and process in API bulk manufacturing

To provide quality control over the manufacture of active pharmaceutical ingredients, it is essential to develop highly selective analytical methods. We report here the separation of organic volatile impurities (residual solvents) from hexane isomers as used during extractions from drugs or food. The need for a rapid and reliable method for the determination of residual solvents is significant due to their toxicity in drug substances and drug products, but also in the food processing industry.

Agilent Technologies

The determination of residual solvents in drug substances, excipients, or drug products is known to be one of the most difficult and demanding analytical tasks in the pharmaceutical industry. Furthermore, determination of polar residual solvents in pharmaceutical preparations is still an analytical challenge, mainly because these compounds are difficult to remove from water or polar solvents. Many pharmaceutical products must be analyzed for residual solvents at different stages of their manufacture (raw materials, intermediate products, and final product). Organic solvents, such as ethanol, dichloromethane, hexanes, and heptane, are frequently used in the process. The manufacture of new active pharmaceutical ingredients (APIs) under Good Manufacturing Practice (GMP) conditions requires adequate quality control of the different ingredients used in the synthesis. Therefore, use of organic solvents must be tightly controlled during any GMP synthesis. Headspace gas chromatography is the most favored technique for the analysis of volatiles and semivolatile organics in solid, liquid, and gas samples [1]. Split- and splitless injections from plant extracts are also used.

Industrial producers must follow procedures as described in USP <467> Residual Solvents [2]. Class 2 residual solvents, such as acetonitrile (ACN), dichloromethane (DCM), *n*-hexane, and cyclohexane, should be less than or equal to standard areas according to Procedure A using a USP G43 column (a 624 phase). If the areas are greater, then a test according to Procedure B must be done, with a USP G16 phase (a wax phase).

Fine-tuning of a G43 phase

Columns with a stationary phase of 6% cyanopropyl phenyl and 94% dimethyl polysiloxane film, 624- or G43-type phases (Figure 1), are widely used to analyze residual solvents in pharmaceutical products.

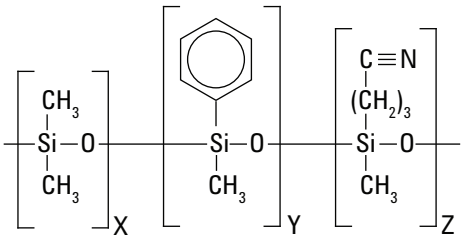


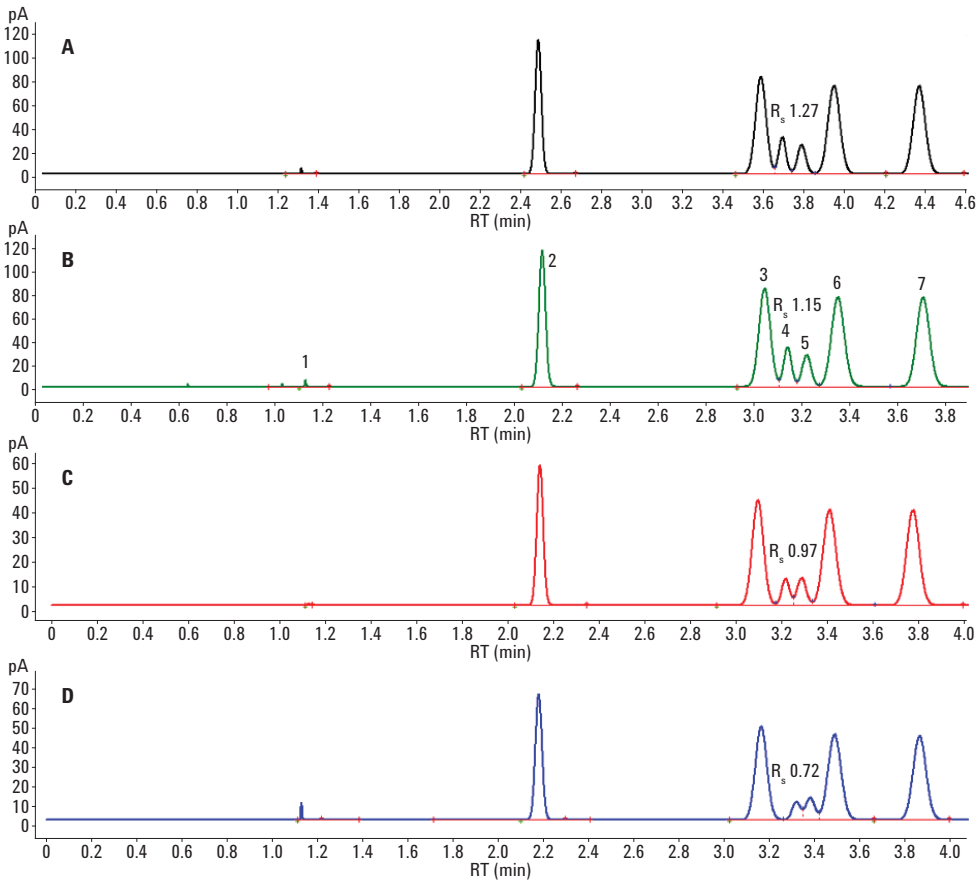
Figure 1. Stationary phase structure of G43, containing 6% cyanopropyl phenyl and 94% dimethyl polysiloxane.

Agilent developed a stationary phase within the range allowed for a G43 phase. The focus was on the most critical separation between closely eluting peaks of 2-methylpentane, acetonitrile, dichloromethane, and 3-methylpentane. The separation is affected by a slight change in composition and needs be optimized, as demonstrated in Figure 2.

Resolution for ACN/DCM needs to be ≥1.0, and ACN and DCM must be separated from the 2-MeC5 and 3-MeC5 hexane isomers. Column B was selected as the best performer.

Hexane isomers and DCM separation

In India, hexane is used as a solvent for extraction and purification in food and pharma. This commercial- or industrial-grade hexane is a mixture of different isomers, and so it is important to have a good selectivity for the separation of dichloromethane (and other solvents) from hexane isomers. Figure 3 shows an example.

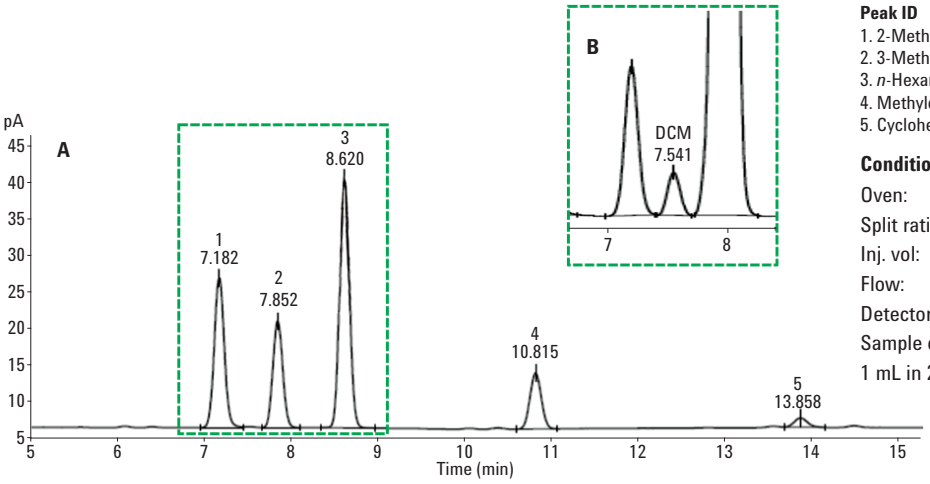


Peak ID
1. Methane
2. *n*-Pentane
3. 2-MeC5
4. ACN
5. DCM
6. 3-MeC5
7. *n*-Hexane

Conditions

Oven: 40 °C
Split injection
Carrier gas: N_2, 38 to 44 cm/s
Column: 30 m × 0.32 mm, 1.8 µm

Figure 2. Four different fine-tuned G43 stationary phases affect the separation of acetonitrile and dichloromethane from hexane isomers.



Peak ID
1. 2-Methylpentane
2. 3-Methylpentane
3. *n*-Hexane
4. Methylcyclopentane
5. Cyclohexane

Conditions

Oven: 40 °C
Split ratio: 1:5
Inj. vol: 1 mL
Flow: N_2, 1 mL/min, constant
Detector: FID
Sample conc: 100 mg/mL
1 mL in 20 mL headspace vial, heated 30 min at 80 °C

Figure 3. Chromatogram from an Agilent customer using headspace analysis with an Agilent J&W CP-Select 624 Hexane, 30 m × 0.32 mm, 1.8 µm GC column (p/n CP9111). A) A typical hexane isomeric solvent used for extraction in India. B) The same hexane solvent with dichloromethane separated from the isomers. Two other hexane isomers (2,2-dimethylbutane and 2,3-dimethylbutane) were not identified.

Carrier gas conditions

Columns of the 624 type are usually coated with thicker liquid phase films to provide retention and improved resolution for the more volatile solvents that elute early in GC analysis. Figure 4 illustrates the H-ū curves for a 30 m × 0.32 mm, 1.8 µm 624-type column using different carrier gases. When the column is used according to USP <467> conditions at 35 cm/s linear velocity of helium or nitrogen, helium provides extra efficiency. However, nitrogen clearly provides the highest plate count or efficiency at the optimum linear velocity compared to helium or hydrogen, with an increase of approximately 50% for the tested phase at 40 °C and 1.8 µm film thickness. Nitrogen is a cheaper carrier gas, and when used at its optimum can also provide separation advantages for close-eluting compounds. However, higher column efficiency comes at the expense of longer analysis times because of the relatively low optimum gas velocity for nitrogen of approximately 8 cm/s.

Experimental

An Agilent 7890A GC with split/splitless inlet and FID was used, with an Agilent 7693 Autosampler equipped with a 10 µL syringe. See figure captions for detailed conditions.

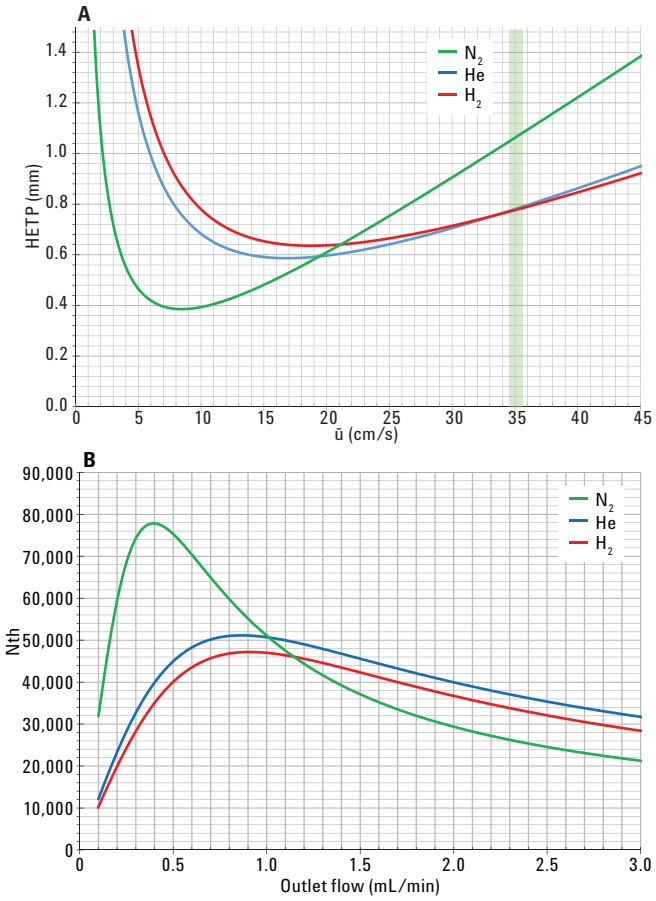


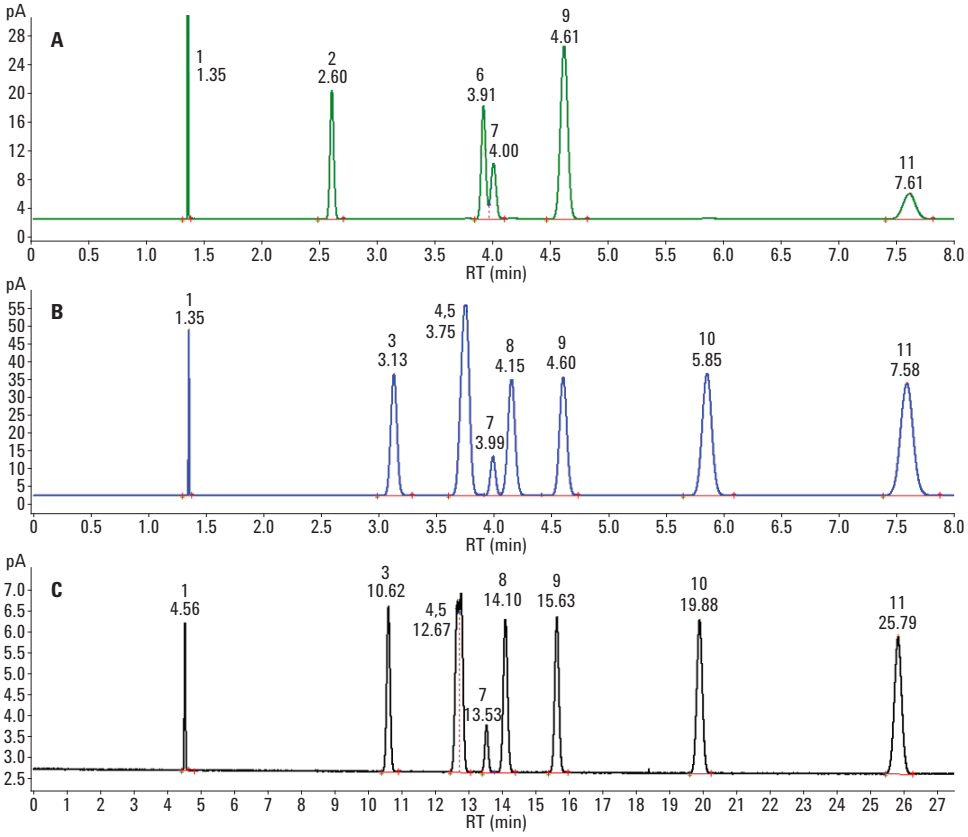
Figure 4. Van Deemter curves (A) for a thick film 30 m × 0.32 mm, 1.8 µm G43 column at 40 °C using helium (He), hydrogen (H_2), and nitrogen (N_2) as carrier gas. The green area is the 35 cm/s average linear velocity as specified in the USP <467> method for helium and nitrogen. This gives the best efficiency for these gasses but lower efficiency when using nitrogen. When nitrogen is used at optimum velocity (B), a higher efficiency can be obtained but analysis times will increase. For helium and nitrogen, an outlet flow of approximately 2.2 mL/min corresponds to 35 cm/s.

Results and Discussion

After the tuning of the G43 phase was completed and optimized, some example chromatograms were run to show reproducibility, quality control, and performance for several important solvents used in commercial applications.

Hexane isomers separated from DCM and ACN

Figure 5 shows the key application. As well as dichloromethane, acetonitrile was also separated from the hexane isomers and DCM with reasonable resolution.



Peak ID

1. Methane
2. *n*-Pentane
3. 2,2-Dimethylbutane
4. 2,3-Dimethylbutane
5. 2-Methylpentane
6. Acetonitrile
7. Dichloromethane
8. 3-Methylpentane
9. *n*-Hexane
10. Methylcyclopentane
11. Cyclohexane

Conditions

Inlet:	250 °C
Detector:	FID 250 °C
Split ratio:	1:100, 20 Hz

Figure 5. Separation of hexane isomers, acetonitrile, and dichloromethane at 40 °C using nitrogen as the carrier gas on an Agilent J&W CP-Select 624 Hexane, 30 m × 0.32 mm, 1.8 µm GC column (p/n CP9111). A) A mixture of acetonitrile and dichloromethane at 37 cm/s, R_s 1.12. B). A mixture of hexane isomers and dichloromethane at 37 cm/s. C) Same as B, except that the mixture was recorded at the optimum average linear velocity 8.4 cm/s, giving increased resolution, but also a longer runtime. The components were dissolved in the late-eluting solvent toluene (not shown).

Reproducibility comparison using a multicomponent mix

Because monitoring other residual solvents is also important in pharmaceutical and food processes, we assessed their elution and compared them to another vendor's 624 columns (Figure 6). Besides peak order, the Agilent columns also showed excellent reproducibility. For comparison, the mix was recorded at optimal resolution using nitrogen as carrier gas.

The Agilent columns from two series showed excellent reproducibility. However, the columns from another vendor showed different selectivity, and for one of them (C) ACN and DCM were not sufficiently separated according to USP <467>.

The two CP-Select 624 Hexane columns from different series exhibited excellent column-to-column reproducibility over the entire range of solvents, with near identical selectivity. The columns from the other vendor show a variable solvent separation pattern, indicating inconsistent liquid phase polarity and column manufacturing.

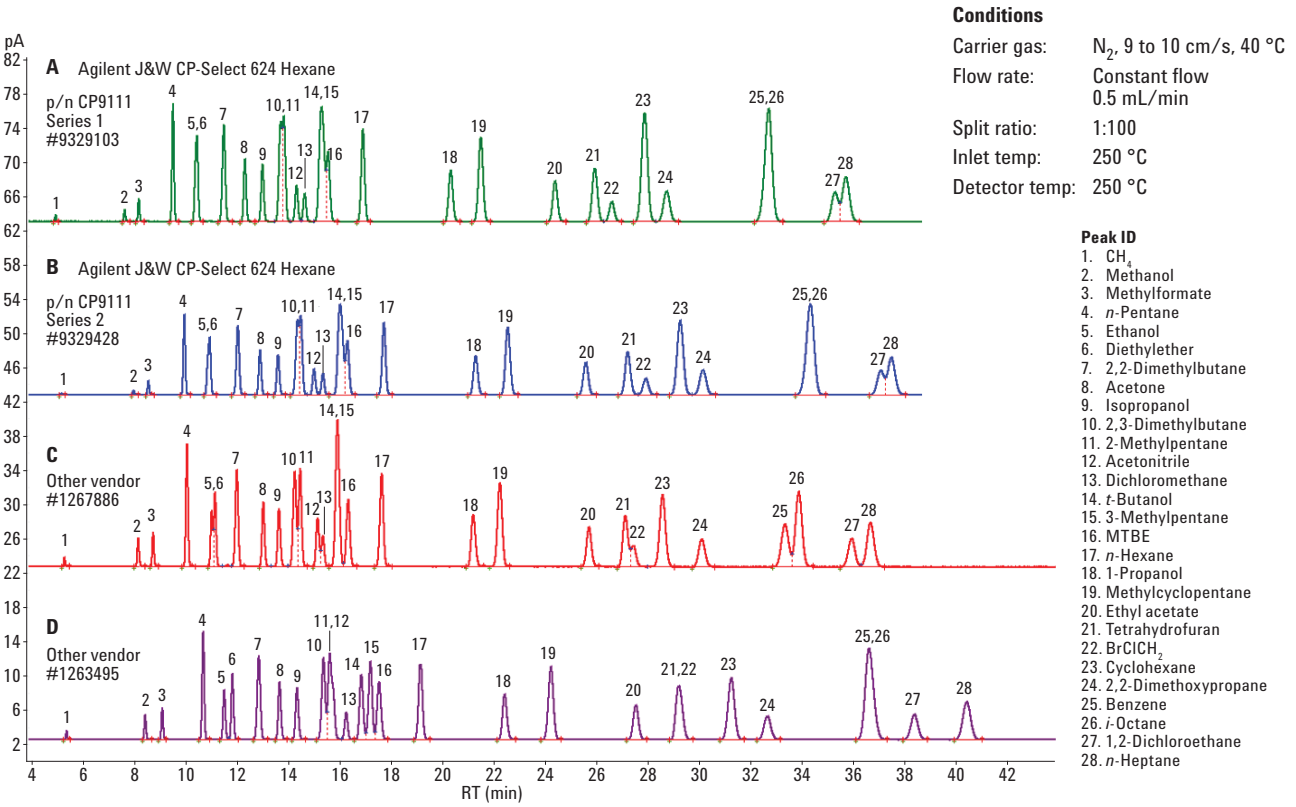
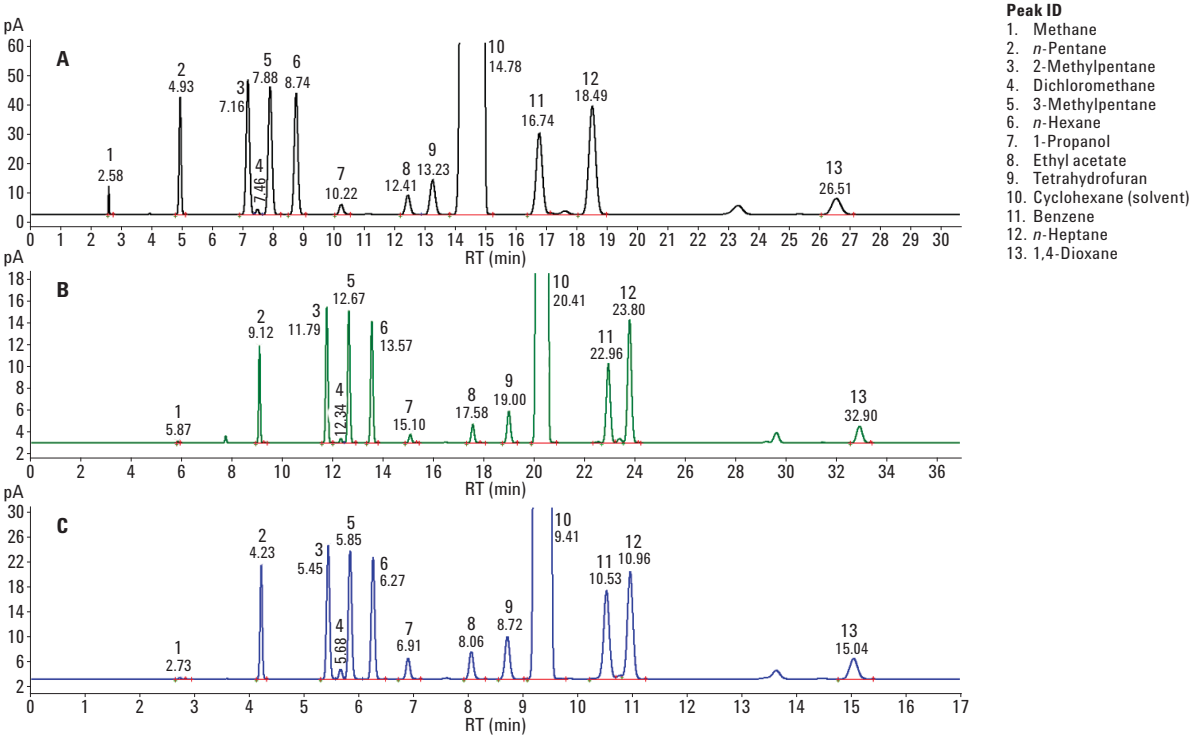


Figure 6. Chromatogram of a multicomponent mixture on two Agilent J&W CP-Select 624 Hexane columns (A and B, p/n CP9111), and two columns from another vendor (C and D).

Quality control with Agilent J&W CP-Select 624 Hexane

Each CP-Select 624 Hexane column is QC tested using a critical test mix with stringent specifications. These include resolution, Kovats retention index, selectivity, and general specifications such as film thickness, asymmetry, and efficiency to guarantee column-to-column reproducibility. To also guarantee minimal batch differences when switching to a new polymer batch of stationary phase, a special procedure is applied to check more separations, such as ACN and DCM.

Figure 7 shows some example QC test chromatograms for three different dimensions of the column. The wide bore column (0.32 mm id) has a higher efficiency compared to a megabore column. Because DCM already separates from hexane isomers using 60 °C as test temperature, a shorter QC test method is possible. For the megabore column (0.53 mm id), a 40 °C test temperature was set. This provided sufficient resolution to accurately calculate column parameters.

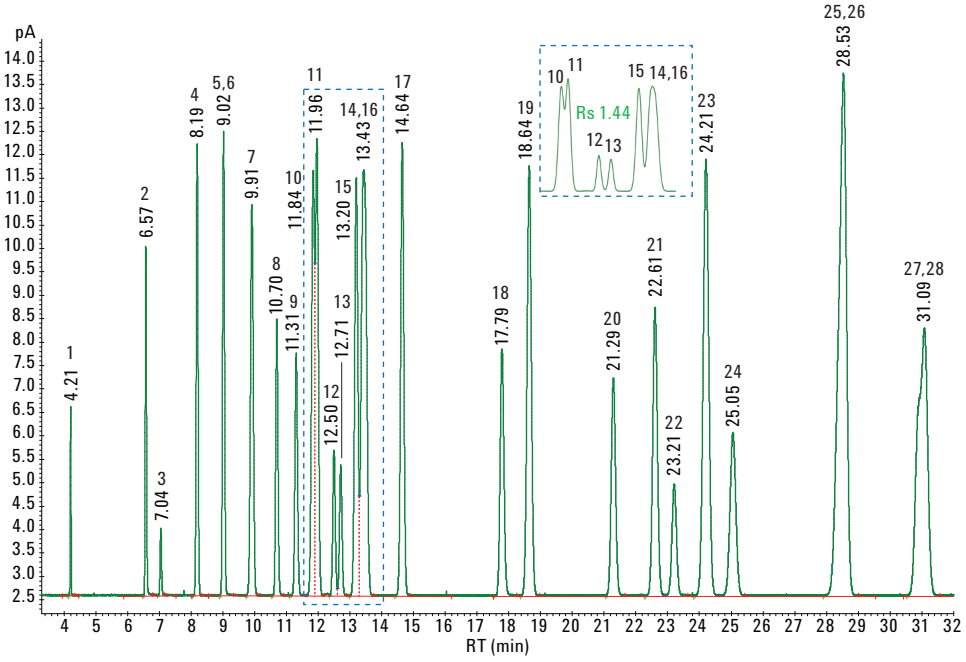


Peak ID
1. Methane
2. *n*-Pentane
3. 2-Methylpentane
4. Dichloromethane
5. 3-Methylpentane
6. *n*-Hexane
7. 1-Propanol
8. Ethyl acetate
9. Tetrahydrofuran
10. Cyclohexane (solvent)
11. Benzene
12. *n*-Heptane
13. 1,4-Dioxane

Figure 7. QC test chromatograms for three sizes of Agilent J&W CP-Select 624 Hexane GC column. A) 30 m × 0.53 mm, 3 µm (p/n CP9113) at 40 °C. B) 60 m × 0.32 mm, 1.8 µm (p/n CP9112) at 60 °C. C) 30 m × 0.32 mm, 1.8 µm (p/n CP9111) at 60 °C. Test mix 83 (0.01%), split ratio 1:100 1 µL, hydrogen carrier gas, FID.

Extra separation when using a 60 m wide bore Agilent J&W CP-Select 624 Hexane column

Some analysts need extra separation power to resolve solvents that are difficult to separate from the matrix. In this case, a 60 m wide bore column can be used. The increased resolution is shown in Figure 8. Helium delivers a shorter analysis time with improved resolution compared to a 30 m column.



Peak ID

1. CH_4
2. Methanol
3. Methylformate
4. *n*-Pentane
5. Ethanol
6. Diethylether
7. 2,2-Dimethylbutane
8. Acetone
9. Isopropanol
10. 2,3-Dimethylbutane
11. 2-Methylpentane
12. Acetonitrile
13. Dichloromethane
14. *t*-Butanol
15. 3-Methylpentane
16. MTBE
17. *n*-Hexane
18. 1-Propanol
19. Methylcyclopentane
20. Ethyl acetate
21. Tetrahydrofuran
22. $BrClCH_2$
23. Cyclohexane
24. 2,2-Dimethoxypropane
25. Benzene
26. *i*-Octane
27. 1,2-Dichloroethane
28. *n*-Heptane

Figure 8. Separation of a multicomponent mix on an Agilent J&W CP-Select 624 Hexane, 60 m × 0.32 mm, 1.8 µm GC column (p/n CP9112). Helium carrier gas, constant flow 1.64 mL/min, 24 cm/s, 40 °C, giving shorter analysis time and acceptable separation compared to a 60 m column using nitrogen at optimal conditions.

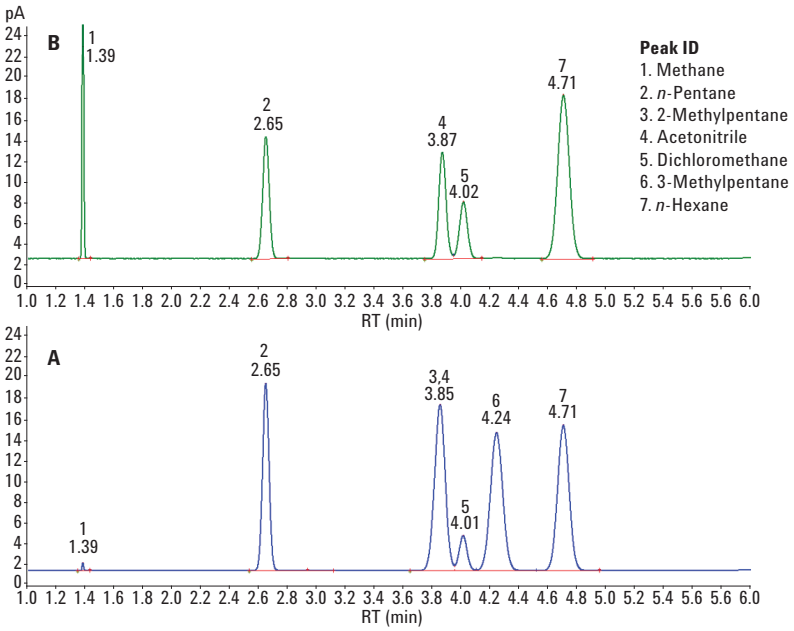


Figure 9. A) A separation of dichloromethane from hexane isomers using a megabore Agilent J&W CP-Select 624 Hexane, 30 m × 0.53 mm, 3 µm (p/n CP9113) GC column. B) Overlay with a standard ACN and DCM showing the coelution of ACN with 2-methylpentane. Helium carrier gas, 36 cm/s, showing a good resolution for ACN and DCM, R_s 1.48 (USP <467> specification ≥1.0).

Megabore Agilent J&W CP-Select 624 Hexane using helium as carrier gas

In the updated USP <467> guideline (May 2015), the megabore dimension is used with helium as the carrier gas. Figure 9 shows an example. Because of an increased selectivity for ACN and DCM, the ACN peak now coelutes with 2-methylpentane.

GC/MS analysis using a volatiles US EPA 624 mix

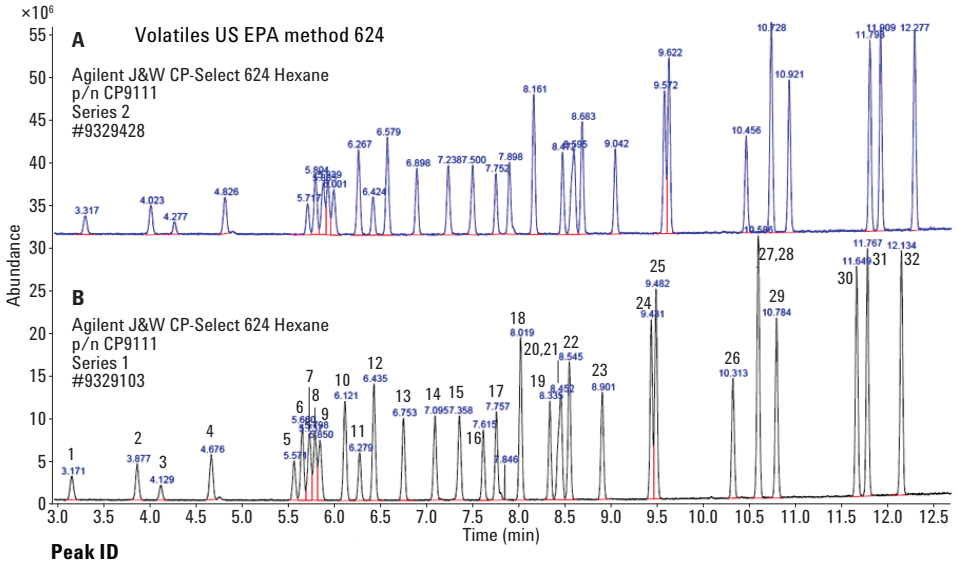
In addition to the specific GC/FID application, CP-Select 624 Hexane can also be used with GC/MS. Figure 10 shows a 624 volatiles mix on two different series of p/n CP9111. The same selectivity is shown for different series, whereas the column from another vendor showed different chromatograms (data not shown).

Conclusions

The Agilent J&W CP-Select 624 Hexane column is dedicated to the separation of dichloromethane and hexane as applied in India. Every column is individually tested and guaranteed for this application, providing absolute assurance of consistency of performance of every column for the separation of dichloromethane from hexane isomers.

References

1. Gupta, A.; Singh, Y.; Srinivas, K.; Jain, G.; Sreekumar, V. B.; Prasad Semwal, V. Development and validation of a headspace gas chromatographic method for the determination of residual solvents in arterolane (RBx11160) maleate bulk drug. *J. Pharm. Bioallied Sci*. **2010**, *2*, 32-37.
2. Anon. USP <467> Residual Solvents, USP 38 Chemical Tests, page 309-324, May 1, 2015. United States Pharmacopeia, Rockville, MD, USA.



Peak ID

1. 1,1-Dichloroethene
2. Dichloromethane
3. *trans*-1,2-Dichloroethene
4. 1,1-Dichloroethane
5. Bromochloromethane
6. Chloroform
7. 1,1,1-Trichloroethane
8. Pentafluorobenzene
9. Carbon tetrachloride
10. Benzene
11. 1,2-Dichloroethane
12. Fluorobenzene
13. Trichloroethene
14. 1,2-Dichloropropane
15. Bromodichloromethane
16. 2-Chloroethyl vinyl ether
17. *cis*-1,3-Dichloropropene
18. Toluene
19. *trans*-1,3-dichloropropene
20. 2-Bromo-1-chloropropane
21. Tetrachloroethene
22. 1,1,2-Trichloroethane
23. Dibromochloromethane
24. Chlorobenzene
25. Ethylbenzene
26. Bromoform
27. 1,4-Dichlorobutane
28. 4-Bromofluorobenzene
29. 1,1,2,2-Tetrachloroethane
30. 1,3-Dichlorobenzene
31. 1,4-Dichlorobenzene
32. 1,2-Dichlorobenzene

Figure 10. Volatiles US EPA method 624 on two series of Agilent J&W CP-Select 624 Hexane, 30 m × 0.32 mm, 1.8 µm (p/n CP9111) showing excellent reproducibility. Agilent 7890A GC/5977 MS, oven 40 °C (3'), 15 °C/min >230 °C, MSD *m/z* 35 to 260, constant flow 1.3 mL/min, helium split 1:20 1 µL. Standard mixtures of diluted standards from Restek; 624 Internal Standard Mix (p/n 30023), 624 Surrogate Standard Mix (p/n 30243), Volatiles MegaMix, EPA Method 624 (p/n 30497).

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