

Analyzing Iohexol by Compendial Method Produces Excellent Precision with Shallow Gradients Using an Agilent 1260 Infinity III LC



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Abstract

Compendial methods of pharmacopoeias are the primary guidance for the analysis of pharmaceuticals. Some of these methods require conditions such as shallow gradients that are challenging for the applied liquid chromatography (LC) hardware. The Agilent 1260 Infinity III LC was designed with these requirements in mind and delivers highly precise retention times even under demanding conditions. This application note demonstrates the performance for the compendial analysis of iohexol. The method is further optimized using the same system to reduce analysis time and solvent consumption to work more efficiently and sustainably while maintaining system suitability requirements of the compendial method.

Introduction

Compendial methods found in different pharmacopoeias provide validated analytical procedures for many pharmaceuticals. These compendial methods are widely used, e.g., for quality control and impurity tests in the pharmaceutical production workflow. When introduced in a new laboratory or applied to new hardware for the first time, each method needs to be verified under "actual conditions of use".¹ To verify a method, pharmacopoeias usually describe system suitability parameters that need to be met, e.g., minimum resolution between two analytes. For a reliable determination of these parameters, LC hardware needs to deliver precise and reproducible results.

An example for a compendial method is the analysis of iohexol and its related substances.² Iohexol (Figure 1) is used as an X-ray contrast agent.³ The polar nature of the molecule causes poor retention of the main compound and its impurities on reversed-phase stationary phases. For this reason, the separation method employs a long, shallow gradient starting at highly aqueous conditions, which is demanding for any HPLC system.

This application note demonstrates the analysis of iohexol and two known impurities, applying the method described in the European Pharmacopoeia (Ph. Eur.) to a quaternary LC system. The compendial method will then be adjusted to UHPLC conditions to minimize solvent consumption and time spent per analysis. System suitability parameters, such as resolution and retention time stability, will be monitored and reported to evaluate the quality of both methods.

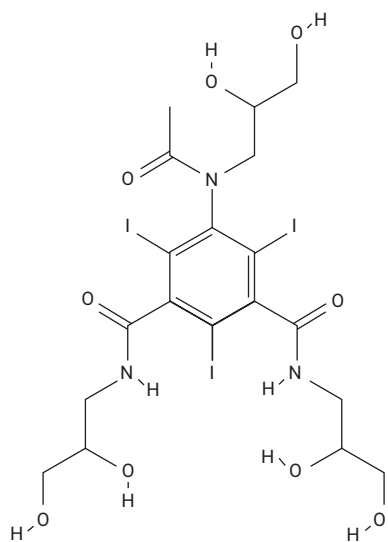


Figure 1. Chemical structure of iohexol.

Experimental

Instrumentation

The 1260 Infinity III LC consisted of the following modules:

- Agilent InfinityLab Assist Upgrade (G7178A), consisting of InfinityLab Assist Interface (G7179A) and InfinityLab Assist Hub (G7180A)
- Agilent 1260 Infinity III Quaternary Pump (G7111B)
- Agilent 1260 Infinity III Vialsampler (G7129A) with integrated column compartment (ICC) with 3 μ L heat exchanger (option #063) and sample thermostat (option #101)
- Agilent 1260 Infinity III Variable Wavelength Detector (G7114A) with 10 mm standard flow cell (option #018)

Columns

- Agilent ZORBAX Eclipse Plus C18, 4.6 $\times$ 250 mm, 5 μ m (part number 959990-902)
- Agilent InfinityLab Poroshell 120 Aq-C18, 3 $\times$ 150 mm, 2.7 μ m (part number 693675-742)

Software

Agilent OpenLab CDS revision 2.7 or later versions

Chemicals

Agilent InfinityLab Acetonitrile (ACN) Gradient Grade for LC (part number 5191-5100*) was used throughout the experiments. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 μ m membrane point-of-use cartridge (Millipak). Analytical-grade sodium hydroxide was purchased from VWR (Darmstadt, Germany).

* Only available in select countries.

Sample

Iohexol Ph. Eur. reference compound, iohexol impurity A Ph. Eur. compound, and iohexol impurity J Ph. Eur. compound were purchased from Merck (Darmstadt, Germany). A mixed solution of all compounds was prepared in 1 mM aqueous sodium hydroxide solution at a final concentration of 75 μ g/mL.

Method settings

Chromatographic conditions were prescribed by the iohexol Ph. Eur. monograph², see Table 1.

To optimize the separation with respect to run time and solvent consumption, the method was adjusted and transferred to UHPLC conditions. All changes applied were within allowable adjustments given in the harmonized chapter 621 of the United States Pharmacopeia (USP)⁴ and 2.2.46 of the Ph. Eur.⁵ More details on the permitted ranges and formulas required to transfer the method can be found in another application note.⁶ The optimized method (Table 1) was applied to a column of the same packing as required by the Ph. Eur. monograph but holding superficially porous particles optimized for highly aqueous conditions.

Table 1. Chromatographic conditions of the Ph. Eur. method and optimized method.

Parameter	Value	
	Ph. Eur. Method	Optimized Method
Mobile Phase	A: Water B: Acetonitrile	A: Water B: Acetonitrile
Flow Rate	1 mL/min	0.788 mL/min
Gradient	Time (min) %B 0 1 60.00 13 60.01 99	Time (min) %B 0 1 19.44 13 19.45 99
Stop Time	67 min	23 min
Post Time	8 min	4 min
Injection Volume	10 µL	2.55 µL
Temperature	25 °C	25 °C
UV Detection	254 nm 5 Hz data rate	254 nm 10 Hz data rate

Results and discussion

A standard solution comprising iohexol and its impurities A and J was separated using the Ph. Eur. compendial method. All compounds eluted in the first half of the chromatogram, see Figure 1. The two impurities are well separated from the main component. Due to endo-exo isomerism, iohexol exhibits two peaks that are not expected to be separated under the conditions of the compendial method.² Resolution between impurity A and iohexol was 5.5, meeting the system suitability requirements as defined by Ph. Eur.² (no less than 5.0). Impurity J was also baseline separated from iohexol. This compendial method specifies a gradient slope of 0.2%/minute over 60 minutes, which poses a challenge to any gradient pump. Despite this setting, the relative standard deviation (RSD) of retention time (RT) for eight consecutive injections was 0.083% or lower for all compounds, see Table 2. These numbers demonstrate the high composition precision of the 1260 Infinity III Quaternary Pump.

Table 2. Peak properties of eight replicate injections of the sample using the compendial method.

Compound	RT (min)	Relative RT	Resolution (EP)	RT RSD (%)
Impurity A	15.865	0.83	—	0.069
Iohexol Endo	18.096	0.95	5.5	0.083
Iohexol Exo	19.041	1.00	2.2	0.078
Impurity J	20.685	1.09	3.8	0.059

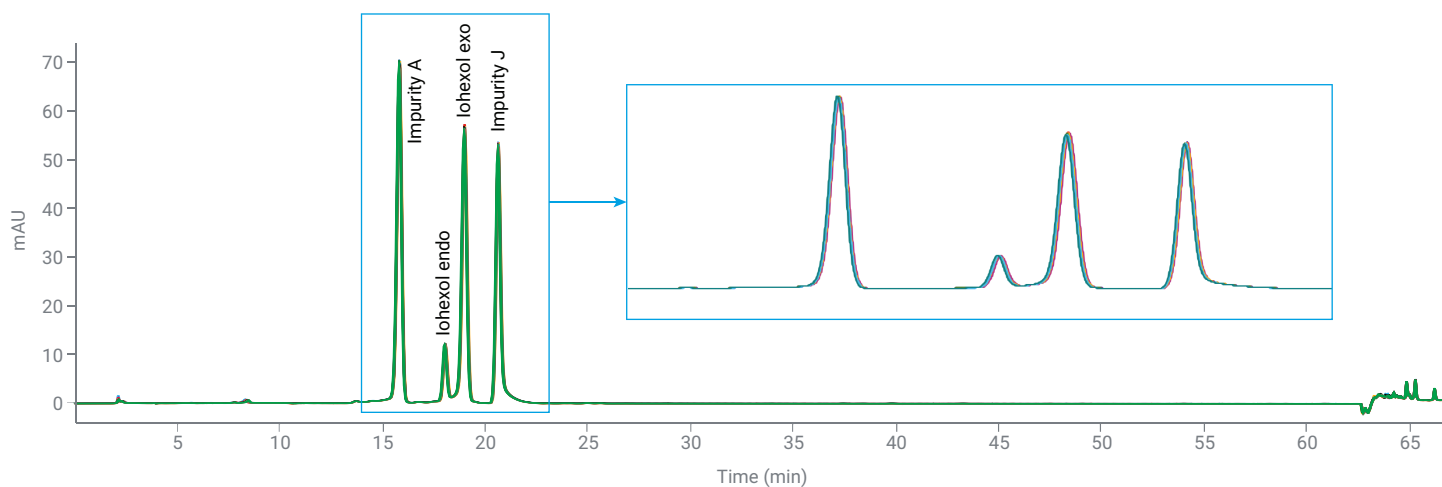


Figure 2. Chromatogram overlay (N = 8) of the separation using the compendial method.

To reduce solvent consumption and analysis time, the method was optimized and transferred to a shorter, narrow-bore column with smaller particles. Flow rate, gradient, and injection volume were adjusted to the new dimensions. All calculations and adjustments were made within the permitted limits for LC gradient elution described in chapter 2.2.46 of the Ph. Eur.⁵ The column was changed from fully porous C18 particles to superficially porous, 100% aqueous-compatible C18 particles. These Poroshell 120 Aq-C18 particles feature a C18 modification with proprietary end-capping that makes them more resistant to phase dewetting than traditional C18 phases.⁷ Phase dewetting occurs when nonpolar stationary phases (such as C18) are operated under highly aqueous conditions. This may cause retention time shifts and peak distortion.

Table 3. Peak properties of eight replicate injections of the sample using the optimized method

Compound	RT (min)	Relative RT	Resolution (EP)	RT RSD (%)
Impurity A	5.793	0.82	—	0.044
Iohexol Endo	6.763	0.96	6.8	0.033
Iohexol Exo	7.068	1.00	2.1	0.032
Impurity J	7.380	1.04	2.2	0.026

The optimized method shortened the run time from 75 minutes (including column flush and re-equilibration) to 27 minutes, equaling a 64% reduction. The sample was analyzed eight times under the adjusted conditions. Figure 2 shows a chromatogram overlay of all runs. The four compounds were again well-separated, and eluted in the first half of the gradient. Resolution between impurity A and iohexol increased to 6.8. Impurity J was baseline separated from the exo isomer of iohexol, albeit with lower resolution than before. RT precision was again very high, with RSDs even lower than in the original method, see Table 3. The system suitability requirements of the compendial method with respect to minimum resolution were fulfilled, indicating that the adjusted method is suited for analysis.

Under the adjusted parameters, the analysis was carried out in 34% of the time, using less than 29% of the solvent of the original method, while maintaining system suitability requirements and RT precision on a high level.

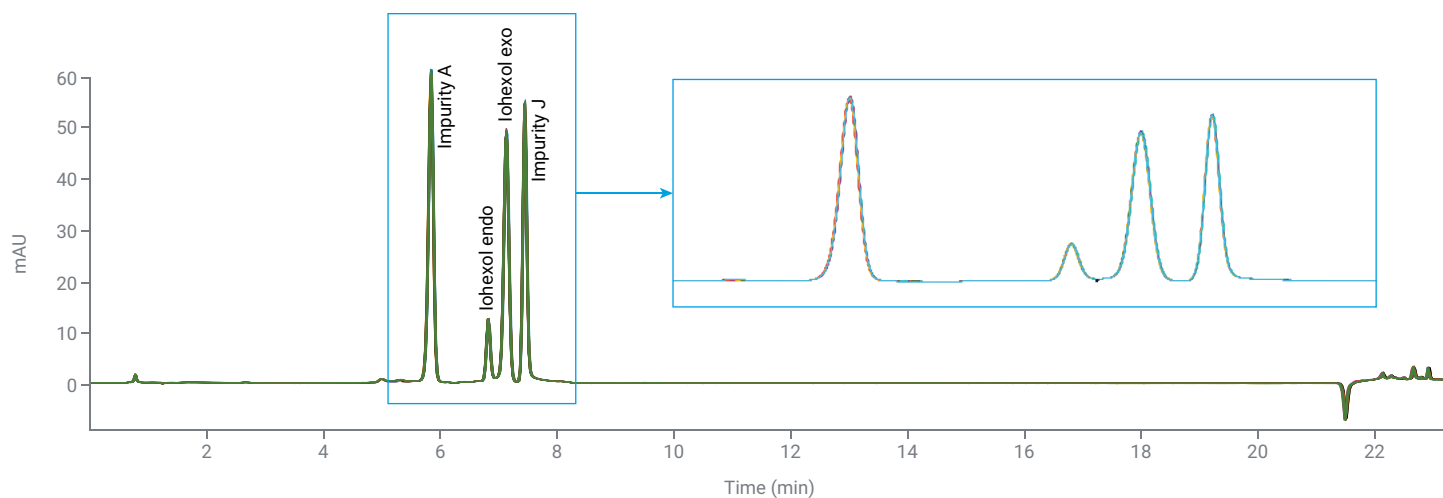


Figure 3. Chromatogram overlay (N = 8) of the separation under optimized conditions.

Conclusion

A compendial method using a shallow binary gradient was applied to separate iohexol and two impurities. Ph. Eur. system suitability parameters were passed, with retention time RSDs $\leq 0.083\%$ (N = 8). The original 75-minute method was optimized and transferred to UHPLC conditions within the boundaries dictated by the harmonized chapter 621 of the USP⁴ and 2.2.46 of the Ph. Eur.⁵ The optimized method maintained the resolution achieved with the original method, improved RT RSDs to $\leq 0.044\%$, and reduced analysis time and solvent consumption by 66 and 71%, respectively. These numbers demonstrate that the Agilent 1260 Infinity III LC delivers highly reproducible results, even under challenging shallow gradient conditions of compendial methods, and at the same time offers the flexibility to optimize these methods to save time and operate more sustainably.

References

1. The United States Pharmacopoeia. General chapter <1226> Verification of Compendial Procedures, **2019**. https://doi.usp.org/USPNF/USPNF_M870_03_01.html (accessed 2024-06-17).
2. The European Pharmacopoeia 11.0, Iohexol. 01/2017:1114.
3. National Drug Code Directory, United States Food and Drug Administration.
4. The United States Pharmacopoeia. General chapter <621> Chromatography, **2023**. https://online.uspnf.com/uspnf/document/1_GUID-6C3DF8B8-D12E-4253-A0E7-6855670CDB7B_8_en-US (accessed 2024-06-17).
5. The European Pharmacopoeia 11.0, Chapter 2.2.46, Chromatographic Separation Techniques.
6. Schipperges, S.; Naegele, E. Reduce the Cost per Injection for Your USP Compendial Method. *Agilent Technologies application note*, publication number 5994-5897EN, **2023**.
7. Fu, R.; Wei, T. Analysis of Polar Compounds Using an Agilent InfinityLab Poroshell 120 Aq-C18 Column with Improved and Reliable Performance. *Agilent Technologies application note*, publication number 5994-5555EN, **2022**.