Development and validation of an HPLC method to analyze ibuprofen and impurities according to the European Pharmacopoeia

Application Note

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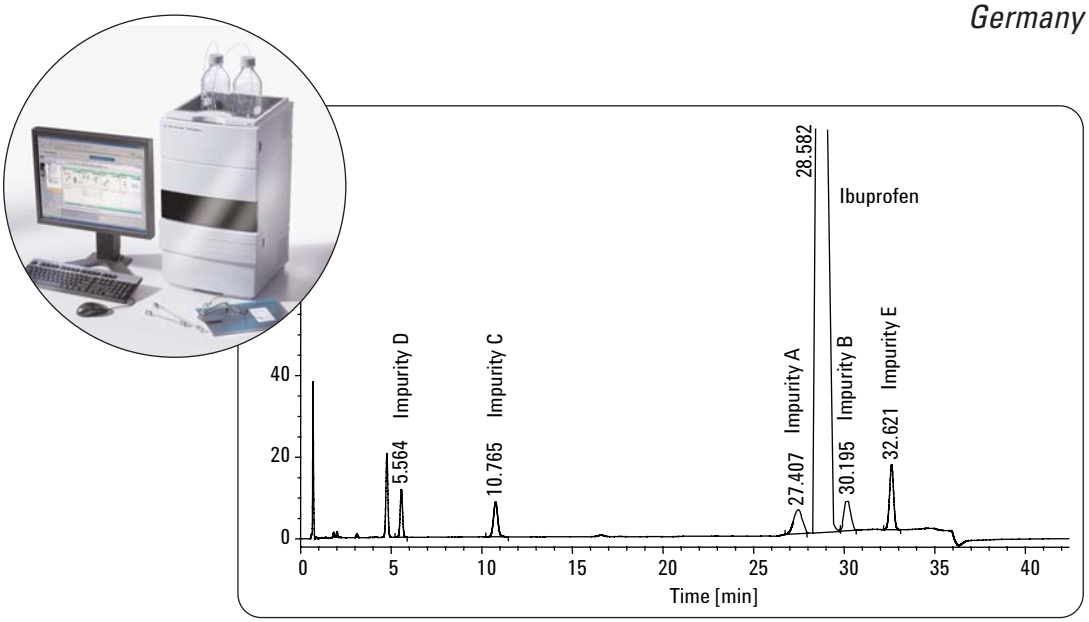

Abstract

This Application Note describes the development of a fast, accurate, and reproducible method to analyze ibuprofen and related impurities according to European Pharmacopoeia (EP) regulations[1], using an Agilent 1120 Compact LC. The experiments described in this Application Note include determination of precision of areas and retention times, as well as chromatographic parameters like resolution and signal-to-noise ratios. The experiments prove precise results from a system that was optimized for everyday productivity, and they fulfill regulatory compliance.

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Introduction

Performing routine testing in a quality control laboratory with standardized methods for active pharmaceutical ingredients (APIs) or final products requires analytical instrumentation with high reliability and ease-of-use, combined with optimal cost-of-ownership.

This Application Note shows how the Agilent 1120 Compact LC[2], a highly robust and reliable instrument for standard LC methodology, can be used effectively in a routine environment to efficiently measure pharmaceutical compounds such as ibuprofen and related impurities.

Ibuprofen impurities A, B, C, D, and E were analyzed on an Agilent 1120 Compact LC system according to methodology described in the EP, and system suitability and performance tests were executed.

Experimental

Equipment

An Agilent 1120 Compact LC system with the following built-in modules was used:

- Gradient pump and vacuum degasser
- Autosampler
- Column oven
- Variable wavelength detector

Preparation of samples

The impurities A, B, C, D, and E were chosen according to the EP. A reference solution was prepared as follows:

Step 1 – Preparation of individual stock solutions of each impurity at 1 mg/mL: 10 mg of each impurity was dissolved in 2 mL acetonitrile and was then diluted to 10 mL with mobile phase A.

Step 2 – Preparation of solution of all impurities at 0.06 mg/mL: 600 µL of each stock solution from step 1 was combined and the mixture was diluted to 10 mL with mobile phase A.

Step 3 – Preparation of reference solution: 20 mg of a certified reference standard (CRS) of ibuprofen was dissolved in 2 mL acetonitrile. Then 1 mL of the impurity solution from step 2 was added and the mixture was diluted to 10 mL with mobile phase A. The final concentrations were 2 mg/mL ibuprofen and 0.006 mg/mL (6 µg/mL) of each of the impurities.

Various dilutions of the reference solution were made to establish calibration curves for ibuprofen and the impurities.

Chromatographic conditions

- Column: Agilent ZORBAX SB-C18, 150 mm x 4.6 mm, 5 µm particle size
- Injection volume: 20 µL, as described in the EP regulations
- Column temperature: 30 °C, as described in the EP regulations
- Detector: 14 µL cell, peak width: 0.1 min (5 Hz), signal: 214 nm

Solvents, gradient, and pump settings

Solvent A was prepared by mixing 340 volumes acetonitrile, 0.5 volumes phosphoric acid, and 600 volumes water; then allowing to equilibrate and diluting to 1000 volumes with water. The procedure is described in the EP regulations. Solvent B was acetonitrile.

The gradient described in the EP regulations was as follows: 0-25 min 100 %A, 25-55 min 85 %B, 55-70 min 85 %B, 70-75 min 100 %A.

Use of the EP gradient would lead to a runtime of 85 minutes. To achieve high resolution at short retention times with the column required by EP, the gradient time was reduced accordingly until all peaks were eluted (in 42.5 minutes including backflushing) with the same gradient slope, so that the same elution could be achieved as with the long gradient.

The final gradient and pump parameters were as follows:

- Gradient: 0-25 min 100 %A, 25-35 min 28.3 %B, 35.1-42.5 min 100 %A
- Stop time: 42.5 min
- Post time: 10 min (usually not necessary with the chosen conditions)
- Flow rate: 2 mL/min

System suitability and performance test

In accordance with Q3A(R) Impurities in New Drug Substances[3], the following parameters must be tested and the limit settings below must be fulfilled:

- Precision of areas must be < 2 % RSD.
- Precision of retention times must be < 0.5 % RSD.
- Resolution must be > 1.5 for all peaks.
- Signal-to-noise ratio must be > 50 for all peaks.
- Calibration for impurities and main peak must be linear without dilution.

With these requirements for testing, the samples shown in table 1 were prepared and analyzed.

An Agilent publication was used as a reference for this work.[4]

Results and discussion

The figure on the cover page shows an example chromatogram of ibuprofen and its impurities. The results of the control sample are shown in table 2. At the lowest calibration concentration, all criteria were fulfilled. The required sensitivity was obtained for all peaks and resolution > 1.5 was achieved for all compounds in the mixture.

Table 3 shows the precision of the areas and retention times for the main compound and the impurities in the suitability sample. The reliability and precision of the Agilent 1120 Compact LC system was proven. For all components, the criteria for precision of retention times and areas were fulfilled, showing that the system can be used for QC methods.

The results of all calibration runs are summarized in table 4. Linearity is shown from the lowest calibration concentration (10 % of the concentration of the reference solution) to the highest concentration (150 % of the concentration of the reference solution). No dilution of the main compound was necessary and no enrichment was required to detect the impurities.

The average calculated limit of detection (with S/N of 10) for all compounds was 0.10 µg/mL by using this method.

Sample	Purpose	Number of injections
Blank solution	Verify baseline stability and identify artifacts	2
Calibration mixture 1-6	Verify stability of response and correctness of calibration, linearity	6 of each
Control sample	Verify sensitivity and resolution for lowest calibration sample	6
Suitability sample	Verify precision of areas and retention times for reference solution	6

Table 1
Setup for testing system suitability and performance.

	Retention time (min)	Amount	Resolution	S/N
Impurity D	5.573	0.6 µg/mL	3.89	56.9
Impurity C	10.783	0.6 µg/mL	16.29	223.7
Impurity A	27.516	0.6 µg/mL	27.82	128.8
Ibuprofen	28.874	0.2 mg/mL	1.98	5300
Impurity B	30.231	0.6 µg/mL	2.61	195.2
Impurity E	32.625	0.6 µg/mL	5.49	407.6

Table 2
Results for control sample: resolution and signal-to-noise (S/N) ratio.

Compound	Retention time (min)	Amount (µg/mL)	RSD RT n=6	RSD area n=6
Impurity D	5.573	6	0.225	0.089
Impurity C	10.783	6	0.267	0.535
Impurity A	27.516	6	0.222	0.159
Ibuprofen	28.874	2,000	0.448	0.061
Impurity B	30.231	6	0.142	0.236
Impurity E	32.625	6	0.078	0.088

Table 3
Suitability sample: precision of retention times and areas. (RSD = relative standard deviation; RT = retention time).

	m	b	Residual standard deviation	r
Impurity D	14872.3	11189.7	2778.9	1
Impurity C	19818.7	3086.1	19463.5	0.9999
Impurity A	24614.7	-40068	24769	0.99989
Ibuprofen	6417010	689238	895680.5	1
Impurity B	19969.4	-13173	7292	0.99999
Impurity E	35314.4	-19975.5	5740.8	1

Table 4
Calibration (Setting "Ignore Origin", y=mx+b, 0.6 to 9 µg/mL for impurities and 0.2 to 3 mg/L for ibuprofen).

The disregard limit according to EP for each impurity is 0.05 times the area of ibuprofen in a reference solution that has a concentration of 20 µg/mL for ibuprofen and 1 µg/mL for each of the impurities. The results of the control sample showed a signal-to-noise ratio of > 50 at 0.6 µg/mL, so the requirements for limit of detection were fulfilled.

The excellent results shown here reflect a system that was designed for robust operation. The high performance of the new pump is strongly demonstrated by the good results for the precision of the retention times. Also, the high S/N ratios (> 50 for each component down to the disregard limit) are a result of the low pump pulsation. The high precision of the

injector is shown with the good correlation results (r>0.999) during calibration, which is also proof of very low carryover.

Conclusion

The Agilent 1120 Compact LC is designed for users in medium- to small-size companies who need the highest reliability and ease-of-use, as well as the lowest cost-of-ownership for standard LC methodology in a QA/QC environment.

In order to prove precise results from a system that was optimized for everyday productivity, and to fulfill regulatory compliance, the experiments in this Application Note included determination of precision of areas and retention times, as well as chromatographic parameters like resolution, linearity, and signal-to-noise ratios. The results show that the instrument is within the limits of the regulatory requirements for a quality control environment.

The results show explicitly the applicability of the 1120 LC system for pharmaceutical testing in QA/QC departments. In addition to the instrument capabilities, the new version of the Agilent EZChrom Elite Compact software allows the full control of the Agilent 1120 Compact LC with a wide range of features for data analysis and reporting of the results.

References

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www.agilent.com/chem/1120

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