Development, validation, and comparison of an HPLC method to analyze paracetamol and related impurities according to the European Pharmacopoeia (EP) and USP using the Agilent 1120 Compact LC and the Agilent 1200 Series LC system

Application Note

Pharmaceuticals

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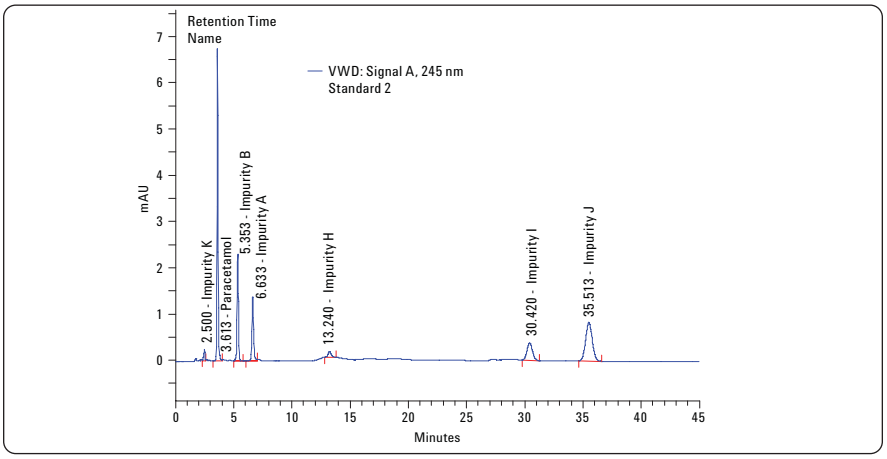

Abstract

This Application Note compares the results of the development of an accurate and reproducible method to analyze paracetamol and related impurities according to European Pharmacopoeia (EP) and USP regulations[1], using an Agilent 1120 Compact LC and a 1200 Series LC system. The experiments described in this Application Note include the determination of the precision of areas and retention times, as well as chromatographic parameters such as resolution and signal-to-noise ratios. The well-suited Agilent ZORBAX StableBond RP8 column shows good selectivity for this application.

The results of these experiments prove that the Agilent 1120 Compact LC is a reliable instrument for routine testing that is able to fulfill the requirements of the European and U.S. regulations and can produce results comparable to those of a standard Agilent 1200 Series LC system.

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Introduction

The analytical instrumentation for routine analysis of samples with standardized LC methods, especially for quality control testing, have several requirements. Foremost are high reliability, ease of use, and an optimal cost of ownership.

This Application Note shows how the Agilent 1120 Compact LC, in comparison with a standard Agilent 1200 Series LC system, works as a highly robust and reliable instrument for standard LC methodology and can be used effectively in a routine environment to efficiently measure pharmaceutical compounds, such as paracetamol and related impurities.

According to the EP regulations, paracetamol impurities A, B, F, H, I, J, and K were analyzed on both LC systems, and system suitability and performance tests were executed.

Experimental

Instrumentation

An Agilent 1120 Compact LC system and a standard Agilent 1200 Series LC system with the following configurations were used:

Configuration of the 1120 Compact LC	Configuration of the 1200 Series system
Gradient pump and vacuum degasser	Quaternary pump and vacuum degasser
Auto sampler	Standard autosampler
Column oven	Column compartment
Variable wavelength detector	Diode array detector
Software: EZChrom Elite Compact 3.3	Software: ChemStation B.04.01

Preparation of samples

The reference solution was prepared as follows, in accordance with EP regulations. 5 mg of paracetamol and 5 mg of each impurity were dissolved in methanol and diluted to 20 mL with the same solvent. 1 mL of the solution was diluted to 250 mL with mobile phase. The substances to be checked were paracetamol and impurities K, A, B, H, I, J, and F.

Chromatographic conditions

Column	Agilent ZORBAX StableBond-C8, 4.6 x 250 mm, 5 µm
Mobile phase	Mix together 375 mL of a 17.9 g/L solution of disodium hydrogen phosphate, 375 mL of a 7.8 g/L solution of sodium dihydrogen phosphate, and 250 mL of methanol containing 6 mL of a 400 g/L solution of tetrabutylammonium hydroxide in methanol
Pump settings	No gradient (in accordance with EP regulations)
Stop time	45 min
Flow rate	1.5 mL/min, isocratic
Injection volume	20 µL
Column compartment temp.	35 °C
Detector	
1120 LC system	14 µL
1200 Series system	13 µL
Peak width	0.1 min (5 Hz)
Signal	245 nm

System suitability and performance test

The EP regulations for paracetamol require system suitability testing with a reference solution, as described in Preparation of samples, above. The testing included the following limits:

Resolution	4.0 minimum between peaks to impurity K and to paracetamol
Signal-to-noise ratio	50 minimum for the peak due to impurity J
Relative retentions (paracetamol)	Impurity K = 0.8
	Impurity F = 3
	Impurity J = 7

No special regulations for paracetamol come from the USP. However, according to USPC Official 8/1/08, General Chapter <621> (Chromatography, System Suitability, p. 28) if there are no special requirements in the monographs, the data of five replicate injections should have a relative standard deviation of less than 2% for each calculated parameter.

From these above-mentioned requirements and to check and compare the chromatographic performance of both LC systems, the following parameters were tested and the limit settings below were fulfilled:

• Precision of areas must be < 2% RSD

• Precision of retention times must be < 0.5% RSD

• Resolution must be > 4 for impurity K and Paracetamol

• Signal-to-noise ratio must be > 50 for impurity J

With these limits and settings for testing, the samples in Table 1 were prepared and analyzed.

Sample	Purpose	Number of injections
Blank solution	Verify baseline stability and identify artifacts	2
Control sample	Verify sensitivity and resolution for reference solution	6
Suitability sample	Verify precision of areas and retention times for reference solution	10

Table 1
Setup for testing.

Results and discussion

Figure 1 shows a chromatogram achieved with the 1200 Series system and ChemStation, whereas Figure 2 shows the chromatogram yielded with the 1120 Compact LC and EZChrom Elite Compact. The data for both chromatograms, shown in Tables 2 and 3, are very similar.

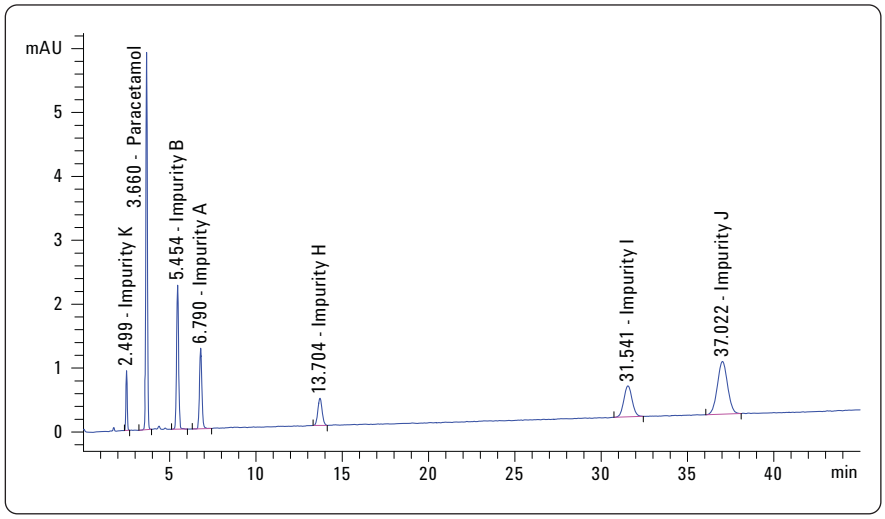

Figure 1
Example chromatogram of paracetamol and impurities with the Agilent 1200 Series system.

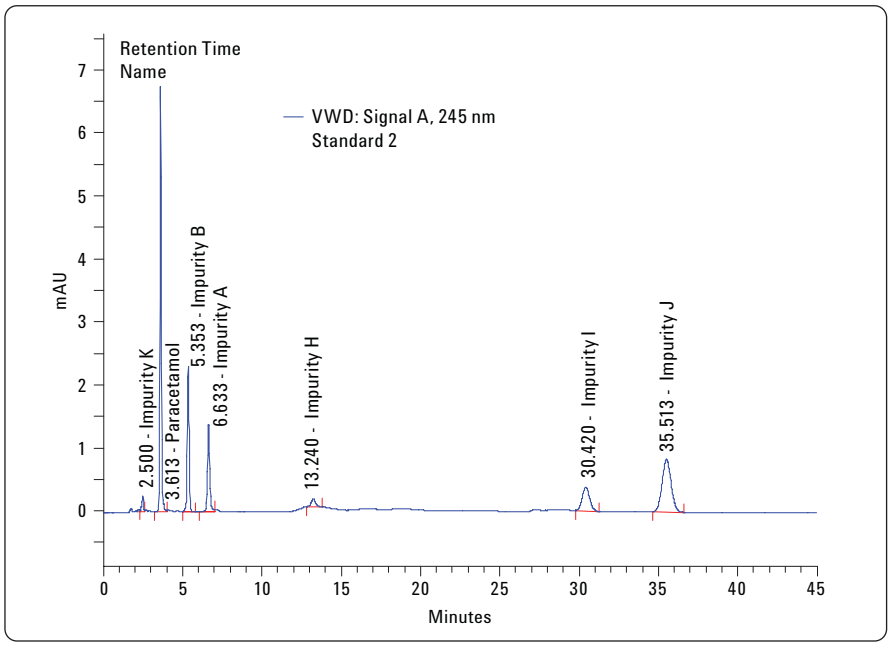

Figure 2
Example chromatogram of paracetamol and impurities with the Agilent 1120 Compact LC.

As shown in Figures 1, 2, and 3, the resolution and relative retention meet the EP requirement for both paracetamol and impurity F.

The results of the control sample, shown in Table 2, fulfill all criteria. The sensitivity was given for all peaks and resolution was achieved for all relevant compounds of the mixture. Not only for impurity K and paracetamol, but for all other relevant peaks the resolution was greater than 4, showing very good selectivity for the ZORBAX StableBond RP8 material and the good performance of the system. The data for peak symmetry (not shown) for all peaks ranged from 0.88–1.02.

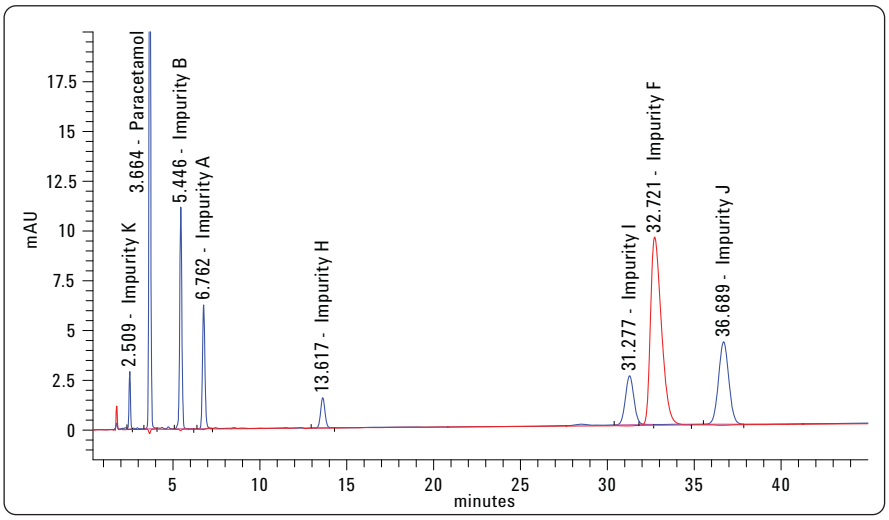

Figure 3
Chromatogram with all relevant impurities.

Compound	Retention time (minutes) Agilent 1200 Series LC system	Retention time (minutes) Agilent 1120 Compact LC	Resolution Agilent 1200 Series LC system	Resolution Agilent 1120 Compact LC
Impurity K	2.498	2.507	–	–
Paracetamol	3.659	3.620	7.19	7.03
Impurity B	5.454	5.353	9.22	9.04
Impurity A	6.792	6.633	5.75	5.51
Impurity H	13.716	13.240	19.52	18.35
Impurity I	31.562	30.387	26.25	24.89
Impurity J	37.066	35.480	5.47	5.03

Table 2
Results for control sample: retention times and resolution.

The criterion for the signal-to-noise ratio for impurity J was fulfilled for both systems. With the Agilent 1200 Series system the average value was found to be 61.4 and with the Agilent 1120 Compact LC system, it was 63.9 for the reference solution.

Table 3 shows the areas and retention time precision results of the main compound and the impurities of the suitability sample. The reliability and precision of the Agilent 1200 Series and the Agilent 1120 Compact LC system were proven. For all components the criteria for precision of retention times and areas were fulfilled, so that both systems can be used for QC methods.

Comparing the results for the suitability sample, it was found that the precision of retention times was nearly the same. Only few deviations were observed. The same was seen with the precision of areas. Both systems provide for the same injector and detector performance, independent of the hardware.

	Agilent 1200 Series LC system			Agilent 1120 Compact LC		
Compound	**Retention time (min)**	**RSD RT n = 25**	**RSD Area n = 10**	**Retention time (min)**	**RSD RT n = 25**	**RSD Area n = 10**
Impurity K	2.498	0.127	0.656	2.507	0.177	0.690
Paracetamol	3.659	0.048	0.545	3.620	0.111	0.342
Impurity B	5.454	0.061	0.263	5.353	0.135	0.352
Impurity A	6.792	0.154	0.215	6.633	0.206	0.348
Impurity H	13.716	0.22	0.831	13.240	0.311	0.636
Impurity I	31.562	0.278	0.599	30.387	0.371	0.681
Impurity J	37.066	0.324	0.404	35.480	0.386	0.258

Table 3
Suitability sample: precision of retention times and areas.

Conclusion

The Agilent 1120 Compact LC is designed for users who need the highest reliability, ease of use, and lowest cost of ownership for standard LC methodology in a QA/QC environment in medium to small companies. The comparison with the standard 1200 Series LC system shows very similar results for these applications.

To prove precise results from a system 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 and signal-to-noise ratios.

As shown in Table 2, the resolution of all peaks was found to be greater than 4.0, with a signal-to-noise ratio of more than 60 (> 50 required) with both systems for the relevant compound. The calculated signal-to-noise ratios prove the sensitivity of the system and show that the instrument can be operated according to the requirements in a quality control environment.

The results of Table 3 show that all criteria for the precision of the determination (areas and retention times) are fulfilled. All criteria related to EP and USP requirements are fulfilled, and the determination of paracetamol and its impurities can also be done with the reliable Agilent 1120 Compact LC system.

All results explicitly show the applicability of the 1120 Compact LC system for drug testing in QA/QC departments due to reduced costs per system and improved simplicity of use. In addition to the instrument capabilities, the new version of the EZChrom Elite Compact software allows full control of the Agilent 1120 Compact LC, with a wide range of features for data analysis and reporting of the results.

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 the relevant components with the reference sample) are a result of the low pump pulsation. The high precision of the injector is shown with very similar results for area precision compared to the standard LC system.

The results for peak symmetry show the good selectivity and performance of Agilent column technology as well as the very good flow design of the LC systems, with no band broadening or peak distortion.

Reference

1. European Pharmacopoeia 5.0, 2184–5

www.agilent.com/chem/1200

June 15, 2010
Publication Number 5990-3739EN

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