Impurity Profiling with the Agilent 1200 Series LC system

Part 5: QA/QC Application Example Using a Fast LC method for Higher Sample Throughput

Application Note

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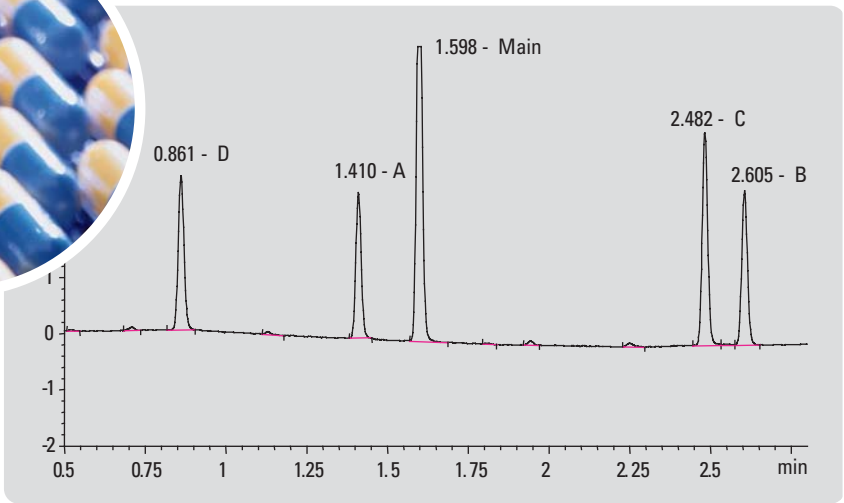

Abstract

Analytical QA/QC departments are faced with increasing amounts of samples and increasing demands regarding data quality and reliability. Typically, for one sample run, 10 to 15 ancillary runs have to be performed to ensure correctness of qualitative and quantitative data. Fast LC methods, which have been thoroughly validated, can help to increase sample throughput significantly without compromising data quality. This Application Note illustrates how the Agilent 1200 Series Rapid Resolution (RRLC) system in combination with sub-2-µm-particle columns and fast LC methods can assist in increasing sample throughput and decreasing costs per analysis by a factor of 3 to 4.

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Introduction

Having developed a fast analytical LC method, and after validation of this fast method, the next step is to transfer this method to the QA/QC laboratory. Typically, detailed standard operation procedures (SOPs) are available to ensure that the risk for errors and misunderstandings is as small as possible. Following is an example for a standard operation procedure including a system suitability check, sequencing and pass criteria for the obtained results. The Agilent 1200 Series Rapid Resolution LC system was used to perform sequencing and reporting of system suitability checks and analysis of samples and calibration mixtures. The chromatographic conditions are based on a fast LC method developed and validated in previous notes (see references 1 and 2). Cycle times of approximately 5 minutes should allow a significant increase in sample throughput, and consequently also reduce costs. This Application Note introduces a standard operating procedure (SOP) and three samples evaluated following the SOP.

Standard operating procedure (SOP) for the determination of a main compound and four impurities, version 1.1, September 10, 2006

The standard operating procedure was developed for one main compound and its four impurities. The run time is as short as 2.8 min and the cycle time from injection to injection is approximately 5 minutes.

1.0 Instrumentation

An Agilent 1200 Series Rapid Resolution LC system is recommended with the following modules:

1.1 Agilent 1200 Series binary pump SL and vacuum degasser
1.2 Agilent 1200 Series high-performance autosampler
1.3 Agilent 1200 Series thermostatted column compartment SL
1.4 Agilent 1200 Series DAD SL for up to 80 Hz operation
1.5 Data acquisition and evaluation software: Agilent ChemStation B.02.01.SR1, Integration: autointegration and the advanced integration mode should be used for better integration results.
1.6 ZORBAX SB C-18 columns with internal diameters of 4.6 mm and lengths of 50 mm, packed with 1.8-µm particles should be used.

2.0 Preparation of samples

2.1 Sample preparation: 1 mg/mL of the sample (main compound and four impurities (A,B,C,D) is dissolved in water and 5 µL of this solution are injected.
2.2 System suitability sample: A stock solution is prepared with the following concentrations: main compound: 10 µg/mL, impurities A, B, C and D: 5 µg/mL each.
2.3 Calibration mixture: Two calibration mixtures are prepared. Each calibration mixture should contain: main compound: approximately 1 mg/mL, impurity A, B, C, and D: 0.1 % level.
2.4 Control sample: The same concentration of impurities as the system suitability sample, but main compound concentration should be 1 mg/mL.

3.0 Chromatographic conditions

3.1 Column
50 x 4.6 mm ZORBAX SB C-18, 1.8 µm for 600 bar operation
3.2 Pump
Solvent A: Water + 0.2 % TFA and Solvent B: ACN + 0.16 % TFA
Gradient: 17 to 45 % B in 2.8 min, holdover 0.2 min,
Stop time: 3 min
Post time: 1 min
Flow rate: 2.2 mL/min
3.3 Autosampler
Injection volume: 5 µL, wash 10 s for exterior of needle
3.4 Thermostatted column compartment
Temperature: 30 °C
3.5 Detector
13-µL cell, Peak width = > 0.03 min, Slit width: 8 nm, sSgnal: 270/10 nm, ref. 500/100 nm

4.0 Performance tests

Several tests are needed to qualify a sample as passed or failed. Calibration mixtures and control samples have to be used as well as blank samples containing a solvent which is also used for sample dilution. Table 1 is a compendium of requirements to determine precision, sensitivity, resolution and other method performance parameters for the samples.

	Sample	Purpose	Number of injections
4.1	Blank solution (pure sample solvent)	Verify baseline stability and identify artifacts	2 to 3
4.2	Control sample	Verify sensitivity and resolution	1
4.3	Calibration mixture 1	Verify stability of response	3
4.4	Calibration mixtures 2	Verify stability of response and correctness of calibration mixture 1	3
4.5	System suitability sample	Verify precision of areas and retention times, resolution, peak width, k´ and signal to noise ratio	6
4.6	Sample	Quantitation of impurities and main compound and determination of precision of areas and RT	3

Table 1
Tests required to qualify samples as passed or failed.

Based on the requirements defined in the table in section *4.0 Performance tests*, the following sequence table was set up in the Agilent ChemStation software. Figure 1 shows an example chromatogram for system suitability testing.

Line	Location	Sample name	Method name	Inj/ location	Sample type	Data file	Injection vol.
1	P1-E-01	water	IMPURITIES1	3	Sample	Water1_1	5
2	P1-C-03	Suitability	IMPURITIES1	6	sample	Suita_1	5
3	P1-E-01	water	IMPURITIES1	2	Sample	Water1_2	5
4	P1-C-04	Calib1	IMPURITIES1	3	Calibration	Calib1_1	5
5	P1-E-02	water	IMPURITIES1	2	Sample	Water2_1	5
6	P1-C-05	Calib2	IMPURITIES1	3	Calibration	Calib2_1	5
7	P1-E-02	water	IMPURITIES1	2	Sample	Water2_2	5
8	P1-E-08	control	IMPURITIES1	1	sample	Control_1	5
9	P1-E-03	water	IMPURITIES1	2	Sample	Water3_1	5
10	P1-D-03	Sample1	IMPURITIES1	3	Sample	Sample1_1	5
11	P1-E-03	water	IMPURITIES1	2	Sample	Water3_2	5
12	P1-D-04	Sample2	IMPURITIES1	3	Sample	Sample2_1	5
13	P1-E-04	water	IMPURITIES1	2	Sample	Water4_1	5
14	P1-D-05	Sample3	IMPURITIES1	3	Sample	Sample3_1	5
15	P1-E-04	water	IMPURITIES1	2	Sample	Water4_2	5
16	P1-C-04	Calib1	IMPURITIES1	3	Calibration	Calib1_2	5
17	P1-E-05	water	IMPURITIES1	2	Sample	Water5_1	5
18	P1-C-04	Calib2	IMPURITIES1	3	Calibration	Calib2_2	5

Table 2
Examples of sequences with system suitability test samples at the beginning and calibration and further samples in the following lines. In between the sample and calibration runs, pure water must be injected.

5.0 Test limits

5.1 System suitability test

To test whether the system is still fulfilling the method requirements, a solution between 4 and 10 µg/mL of the main compound and impurities A, B, C and D is prepared. This solution is injected every day prior to the first analysis.

The following parameters must be tested. The following limit settings have to be fulfilled:

5.1.1 Precision of areas must be < 2 % rsd.

5.1.2 Precision of retention times must be < 0.5 % rsd.

5.1.3 Resolution must be > 2 for all peaks.

5.1.4 Maximum peak width must be < 0.08 min at half height.

5.1.5 k´ must be 5 < k' < 25.

5.1.6 Signal-to-noise ratio must be > 50 for all peaks.

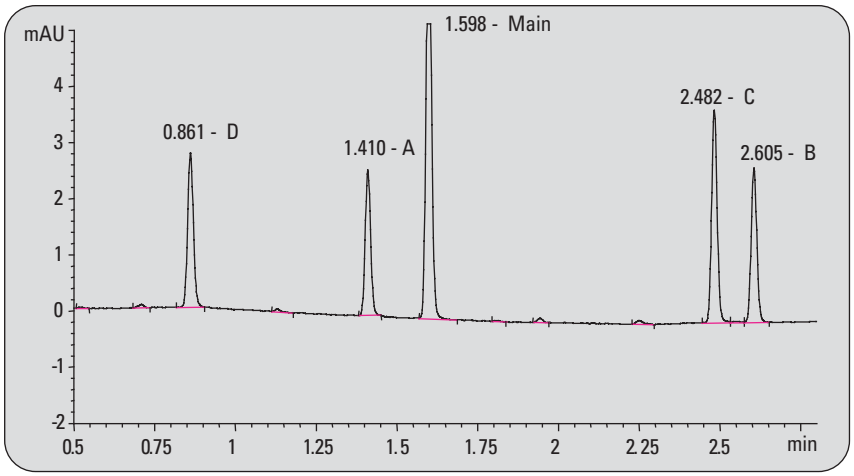

Figure 1
Analysis of sample used for suitability tests.

The results of the system suitability sample shown in figure 1 are summarized in table 3. All limit criteria are fulfilled.

The calibration run results are summarized in table 4. All limit criteria are fulfilled.

The control sample results are summarized in table 5. All limit criteria are fulfilled.

Compound	Amount	RSD RT	RSD Area	Resolution	PW	K´	S/N
A	4.9 µg/mL	0.142	0.155	9.48	0.018 min	11.43	75.3
B	4.5 µg/mL	0.062	0.483	3.88	0.019 min	21.95	79.3
C	5.1 µg/mL	0.069	0.399	6.76	0.019 min	20.88	109.7
D	4.4 µg/mL	0.246	0.201	-	0.019 min	6.61	79.8
Main compound	9.99 µg/mL	0.107	0.160	6.13	0.018 min	13.09	109.7

Table 3
System suitability test results.

5.2 Calibration runs

Evaluation of the calibration runs ensures that the obtained sample run results are reliable. The precision of retention times and areas of the calibration runs must be evaluated.

5.2.1 The precision of area must be < 5 % rsd above the 0.03 % level for all impurities.

5.2.2 Precision of area must be < 20 % rsd below the 0.03 % level for all impurities.

5.2.3 The precision of area must be < 1 %rsd for the main compound.

5.2.4 The precision for retention times should be < 0. 5% rsd.

Compound	Amount	% level	RSD RT	RSD Area
A	1.016 µg/mL	0.102 %	0.142	0.155
B	0.976 µg/mL	0.098 %	0.062	0.790
C	1.026 µg/mL	0.103 %	0.069	0.804
D	0.927 µg/mL	0.093 %	0.246	0.201
Main compound	1 mg/mL	100 %	0.107	0.166

Table 4
Calibration run results.

5.3 Control sample

The control sample is used to check the method performance with respect to resolution and limit of detection.

5.3.1 Resolution for all peaks must be > 2.

5.3.2 Limit of detection must be <0.01 % level for all impurities.

Compound	Amount	Resolution	S/N	% Level of LOD
A	4.9 µg/mL	17.5	22	0.008 %
B	4.5 µg/mL	3.81	23.6	0.008 %
C	5.1 µg/mL	17.88	31.5	0.0078 %
D	4.4 µg/mL	-	22	0.008 %
Main compound	1 mg/mL	2.6	-	-

Table 5
Control sample results.

Table 6 shows the area and retention time precision results of the main compound and the impurities of the analyzed samples.

In table 7 the amounts found are summarized. The amounts of impurities do not exceed the limit of 0.5 % for all 3 samples.

5.4 Sample run precision and determination of amounts

Samples must be injected 3 times and precision for areas and retention times must be determined for the main compound and the impurities.

5.4.1 Area precision of the main compound must be < 1 % rsd.

5.4.2 Retention time precision must be < 0.5 % rsd.

5.4.3 Precision for areas of impurities in the 0.05 up to the 0.4 % level must be < 10 % rsd, below the 0.05 % level down to the 0.02% level area precision should be < 20 % rsd

5.4.4 Retention time precision for impurities must be < 0.5 % rsd

5.4.5 Determination of the amount of the main compound in ng/mL.

5.4.6 Determination of the impurity level in %.

5.4.7 Percentage of allowed total impurity amount must be < 0.5 %.

	Sample 1	Sample 2	Sample 3
Main compound			
RSD area	0.145	0.233	0.093
RSD retention time	0.104	0.045	0.037
Impurities			
RSD area	1.33-10.54	0.563-3.98	5.50-8.49
RSD retention time	0.039-0.106	0.058-0.24	0.013-0.050

Table 6
Results obtained from the main compound of the 3 analyzed samples, 3 injections each.

	Sample 1, total 0.071 % impurities (passed)	Sample 2, total 0.257 % impurities (passed)	Sample 3, total 0.11 % impurities (passed)
Amount of main compound	999.58 ng/mL	1001.51 ng/mL	998.93 ng/mL
Impurity A, % level	0.02 %	0.066 %	0.028 %
Impurity B, % level	0.019 %	0.066 %	0.03 %
Impurity C, % level	0.017 %	0.07 %	0.03 %
Impurity D, % level	0.015 %	0.055 %	0.022 %

Table 7
Amounts of main compound and impurities found in the analyzed samples.

Conclusion

Analytical QA/QC departments are faced with increasing amounts of samples and increasing demands regarding data quality and reliability. Typically, for one sample run, 10 to 15 ancillary runs have to be performed to ensure correctness of qualitative and quantitative data. Fast LC methods, which have been thoroughly validated, can help to increase sample throughput significantly without compromising data quality. The sequence used included 47 runs, which took about 3.9 hours including 30 minutes for system suitability testing. A sequence using a conventional method of about a 20-min cycle time would take approximately 15.7 hours. This represents a significant increase in sample throughput for the fast LC method. Solvent savings are also significant. With a 20-min cycle time, 47 runs and a flow rate of 2.2 mL/min, 2068 mL of solvent are required. Using the fast method described here a 5-min cycle time requires only 517 mL solvent for 47 runs. The cost per analysis will therefore drop significantly by approximately a factor of 3 to 4. See table 8 for an example. Revalidation of a method can take up to 2 weeks and cost about $4,000. In our example, after the analysis of about 500 runs updating a method to a fast analysis is cost effective.

Cycle time	20 min cycle time	5 min cycle time
Runs/year*	26208	104832
Approx. costs/analysis	$11.35	$2.85
Approx. cost/1000 runs**	$11,350	$2,850
Cost savings /1000 runs	-	$8,500
Increase in throughput	-	4 times

Table 8
Increase in sample throughput and cost savings using fast LC methods
***24 hour/day, 7 days/week, 52 weeks/year**
****Solvents and disposal = $31/l, labor/h = $30.**

References

1.
Michael Frank “Impurity Profiling with the Agilent 1200 Series LC System- Part 3: Rapid Condition Scouting for Method Development”, *Agilent Application Note*, *publication number 5989-5619EN*, **2006.**

2.
Angelika Gratzfeld-Huesgen “Impurity Profiling with the Agilent 1200 Series LC system- Part 4: Method Validation of a Fast LC Method”, *Agilent Application Note*, *publication number 5989-5620EN*, **2006.**

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