High Efficiency, High Throughput LC and LC/MS Applications Using ZORBAX Rapid Resolution HT Columns

Application

Drug Manufacturing/QA/QC

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Introduction

Short chromatographic run times increase productivity because more samples can be run in less time when the analysis time is reduced. Short run times are achieved by using shorter HPLC columns. To truly maintain productivity, resolution and efficiency need to be maintained with short columns. This is done effectively by using short columns with smaller particle sizes. For the greatest efficiency in the shortest column lengths (50 mm and shorter), new 1.8-µm particles can be used for a wide range of high throughput liquid chromatography (LC) and liquid chromatography/mass spectrometry (LC/MS) applications.

Experimental

To demonstrate the potential of the 1.8-µm particle size columns, we converted a standard USP assay method into a high throughput analysis by using a Rapid Resolution High Throughput (RRHT) column. In this first example, we converted the USP analysis of triamcinolone into a high throughput method without changing the resolution required. The traditional USP method calls for a 3.9 × 300 mm, 10-µm L1 column and suggests a retention time of 10 minutes while requiring Rs >3 for triamcinolone and the internal standard hydrocortisone. This is easily achieved, as shown in Figure 1A.

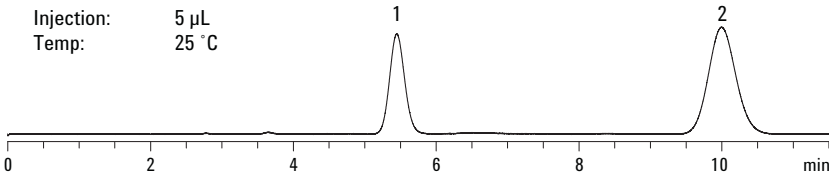
To convert this to a high throughput application, a 4.6 × 30 mm, 1.8-µm Eclipse XDB-C18 was substituted using the same analysis conditions. The result (Figure 1B) is a 2-minute retention time, increasing sample throughput 5×. The resolution and efficiency of this 2-minute analysis exceed that of the original method using a column with 1/10 the length. The original USP method also suggested starting with a mobile phase composition of 60% methanol: 40% water. When this mobile phase was used a run time of 0.7 minutes was achieved (Figure 1C) while the resolution was more than double what is required. The end result is a method with 10× the sample throughput of the original method with no compromise in resolution.

Highlights

- Standard USP assay methods for triamcinolone and guaifenesin were converted into high throughput methods using Agilent RRHT columns with 1.8-µm particles, while still exceeding minimum resolution requirements.
- Throughput increases of 8 to 10× were demonstrated.

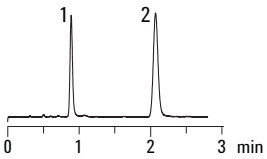
1A

USP L1 Column: 3.9 × 300 mm, 10 µm
Eluent: 47% Methanol: 53% Water
Flow: 1.5 mL/min
Injection: 5 µL
Temp: 25 °C



Peak	RT	N	Rs
1	5.45	3199	0
2	9.99	3212	8.1

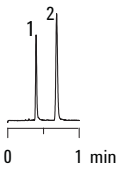
1B



RRHT Column (L1)
ZORBAX Eclipse XDB-C18: 4.6 × 30 mm, 1.8 µm
Part number 923975-902
Eluent: 47% Methanol: 53% Water
Flow: 1.5 mL/min
Injection: 1 µL
Temp: 25 °C

Peak	RT	N	Rs
1	0.89	3256	0
2	2.07	4851	11.8

1C



RRHT Column (L1)
ZORBAX Eclipse XDB-C18: 4.6 × 30 mm, 1.8 µm
Part number 923975-902
Eluent: 60% Methanol: 40% Water
Flow: 1.5 mL/min
Injection: 1 µL
Temp: 25 °C

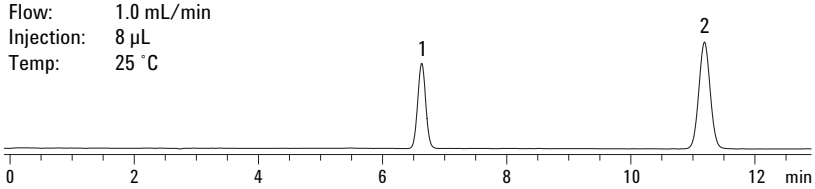
Peak	RT	N	Rs
1	0.40	2991	0
2	0.69	4025	6.9

Figure 1. Throughput comparisons for triamcinolone using different column and gradient conditions. Peak 1: triamcinolone, 0.2 µg/µL, Peak 2: hydrocortisone, 0.3 µg/µL. Minimum required resolution: 3.0.

Figure 2 shows a dramatic increase in throughput for the USP assay of guaifenesin. This calls for a 4.6 × 250-mm L1 column and a Rs >3 for the guaifenesin and benzoic acid internal standard (Figure 2A). Both 4.6 × 50 mm and 4.6 × 30-mm RRHT columns were substituted to evaluate different choices in column lengths for high throughput applications. The 50-mm column length (Figure 2B) improves throughput 5× while maintaining 70% of efficiency and 78% of the resolution of the 250 mm, 5-µm column. The 30 mm, 1.8 µm provides 8× the sample throughput and Rs of 8.6, nearly 3× the method requirements (Figure 2C).

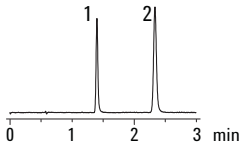
2A

USP L1 Column
ZORBAX Eclipse XDB-C18: 4.6 × 250 mm, 5 µm
Eluent: 40% Methanol: 60% Water: 1.5% Glacial Acetic Acid
Flow: 1.0 mL/min
Injection: 8 µL
Temp: 25 °C



Peak	RT	N	Rs
1	6.63	12,737	0
2	11.19	18,552	15.8

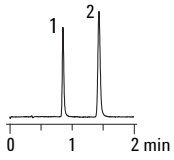
2B



RRHT Column (L1)
ZORBAX Eclipse XDB-C18: 4.6 × 50 mm, 1.8 µm
Eluent: 40% Methanol: 60% Water: 1.5% Glacial Acetic Acid
Flow: 1.0 mL/min
Injection: 2 µL
Temp: 25 °C

Peak	RT	N	Rs
1	1.40	11,421	0
2	2.33	12,909	12.3

2C



RRHT Column (L1)
ZORBAX Eclipse XDB-C18: 4.6 × 30 mm, 1.8 µm
Eluent: 40% Methanol: 60% Water: 1.5% Glacial Acetic Acid
Flow: 1.0 mL/min
Injection: 2 µL
Temp: 25 °C

Peak	RT	N	Rs
1	0.85	5,855	0
2	1.43	7,300	8.6

Figure 2. Throughput comparisons for guaifenesin using different column and gradient conditions. Peak 1: guaifenesin, 0.04 µg/µL, Peak 2: benzoic acid, 0.10 µg/µL. Minimum required resolution: 3.0.

Conclusion

These two examples show that RRHT columns with 1.8-µm particles can be used to increase productivity and sample throughput for LC analyses. These columns are also available with a 2.1-mm internal diameter to achieve the same throughput increases for LC/MS analyses. Time savings with these columns can be dramatic while still achieving high resolution.

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