A practical HPLC approach to save acetonitrile in regulated environments

Case study on Agilent ZORBAX column parameter optimization shows drastic reduction of acetonitrile consumption while maintaining EP/USP requirements.

Application Note

Drug Development

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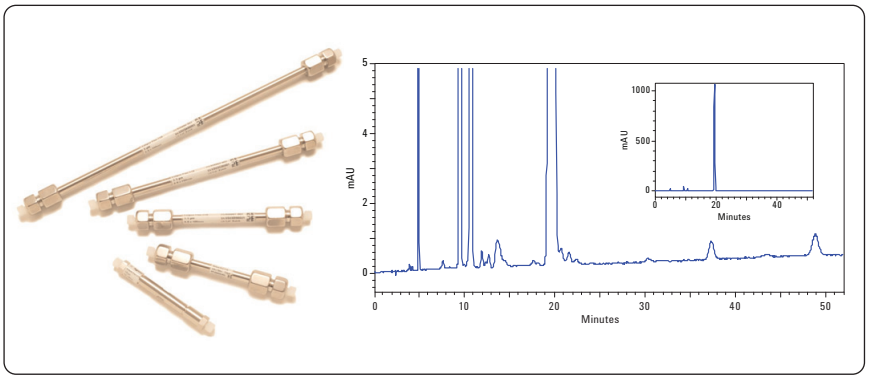

Abstract

This Application Note demonstrates efficient methods for conserving acetonitrile using Agilent's ZORBAX columns as a solution to the global acetonitrile shortage. Varying the liquid chromatography column dimensions and particle size according to the allowed method adjustment criteria from European (EP) and US pharmacopeia (USP) provides a significant reduction in acetonitrile consumption without compromising results. Since the modifications are within the flexible limit provided by authorities, adopting these changes is unproblematic for regulated environments. Methylprednisolone sodium succinate was used as the analytical target for this study. The selection of shorter columns with a smaller internal diameter and decreased particle size, effectively reduced run time from approximately 52 minutes to 20 minutes. This resulted in an 83% savings in total solvent volume.

Agilent 1260 Infinity LC Verified

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Introduction

The current shortage of acetonitrile is a major global concern. A large percentage of all pharmacopeia HPLC (high pressure liquid chromatography) methods as well as the majority of users in the pharmaceutical and chemical industry, rely on acetonitrile as the preferred organic solvent. The shortage of acetonitrile has resulted in tremendous pressure to reduce consumption or replace acetonitrile with a suitable solvent. Reducing internal diameter, length or particle size of the HPLC columns within the EP and USP limits provide a reduction solvent consumption without deviating from regulation. We used methylprednisolone sodium succinate, a synthetic glucocorticoid drug, as a model compound for this study.

Experimental

Materials

Methylprednisolone sodium succinate (for injection) was from Pfizer (Solumedrol, Batch No: R05169). All solvents were of HPLC grade; acetonitrile was purchased from Labsacan, acetic acid from Fluka, and Millipore deionized water was used.

Instrumentation and chromatographic conditions

The suggested column dimension for methylprednisolone as per EP is 250 mm × 4 mm using octadecylsilyl silica gel with 5-µm particle size. The mobile phase was a premixed solution of acetonitrile and 3.0% glacial acetic acid in the ratio 33:67. The LC system was operated at 1.0 mL/min. The column was maintained at room temperature (25 °C). In this study all analyses were performed using the Agilent 1200 Series RRLC system. The system components included a binary pump, mobile phase vacuum degassing unit, autosampler, temperature controlled column compartment and a diode array detector. An Agilent ZORBAX Eclipse Plus (5 µm particles, 4.6 mm id × 250 mm length) column was used for performing chromatographic separations.

Results

The chemical structure of methylprednisolone sodium succinate is shown in Figure 1. A chromatographic representation of methylprednisolone sodium succinate related substance analysis, per EP method, is as shown in Figure 2. The run time for the analysis is approximately 50 min when the method parameters are as described in section 2.2. This means about 50 mL of mobile phase is required for one acquisition for related substance analysis. The amount of mobile phase required for equilibrating the 250 mm long column is additional.

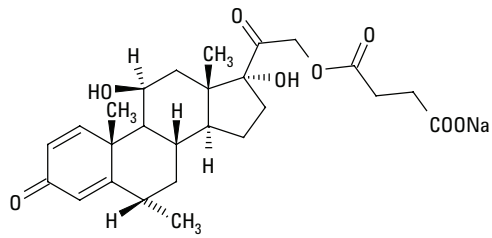

Figure 1
Structure of methylprednisolone sodium succinate.

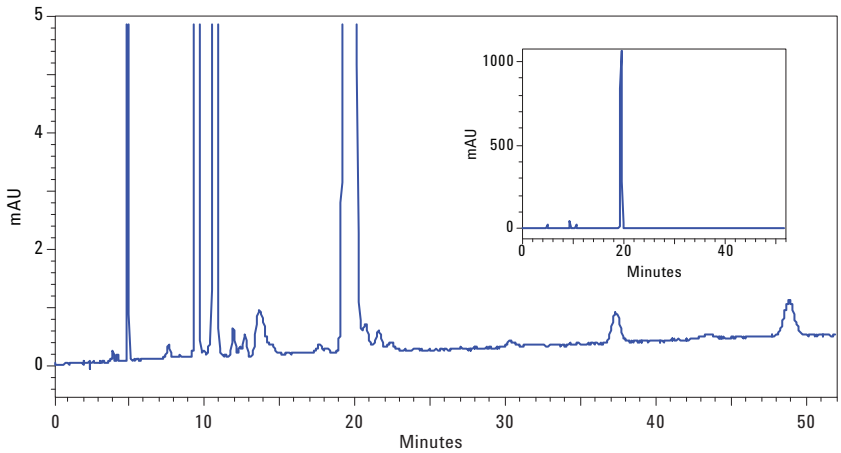

Figure 2
Chromatogram obtained for methylprednisolone sodium succinate as per EP method (with Full view inset) using Agilent ZORBAX Eclipse Plus C 18 (5 µm particles, 4.6 mm id × 250 mm length)

The amount of mobile phase can be effectively reduced by changing column length, internal diameter and particle size. However, in a regulated environment, these parameters can be changed only within certain boundaries prescribed by the USP and EP. The adjustment limits are shown in Table 1.

Parameter	Method adjustment limit (USP and EP) (%)
Column length	± 70
Internal diameter	± 25
Particle size	–50

Table 1
Method adjustment parameters for column dimensions as per EP and USP (3).

Reducing column length shortens run time and saves solvent but compromises resolution

The first step towards reducing solvent consumption is to shorten the separation time. Chromatograms obtained with columns of different lengths and a flow rate of 1 mL/min is shown in Figure 3. Because there is a direct correlation between run time and solvent consumption, reducing the column length from 250 mm to 100 mm (deviation: -60%, still within the accepted limit) shortens the run time to 21 minutes and decreases solvent consumption by 61%, compared to the original method. The use of a 50-mm column would further reduce solvent consumption and run time but is outside of the acceptable limit. However, as the column length is reduced resolution is compromised.

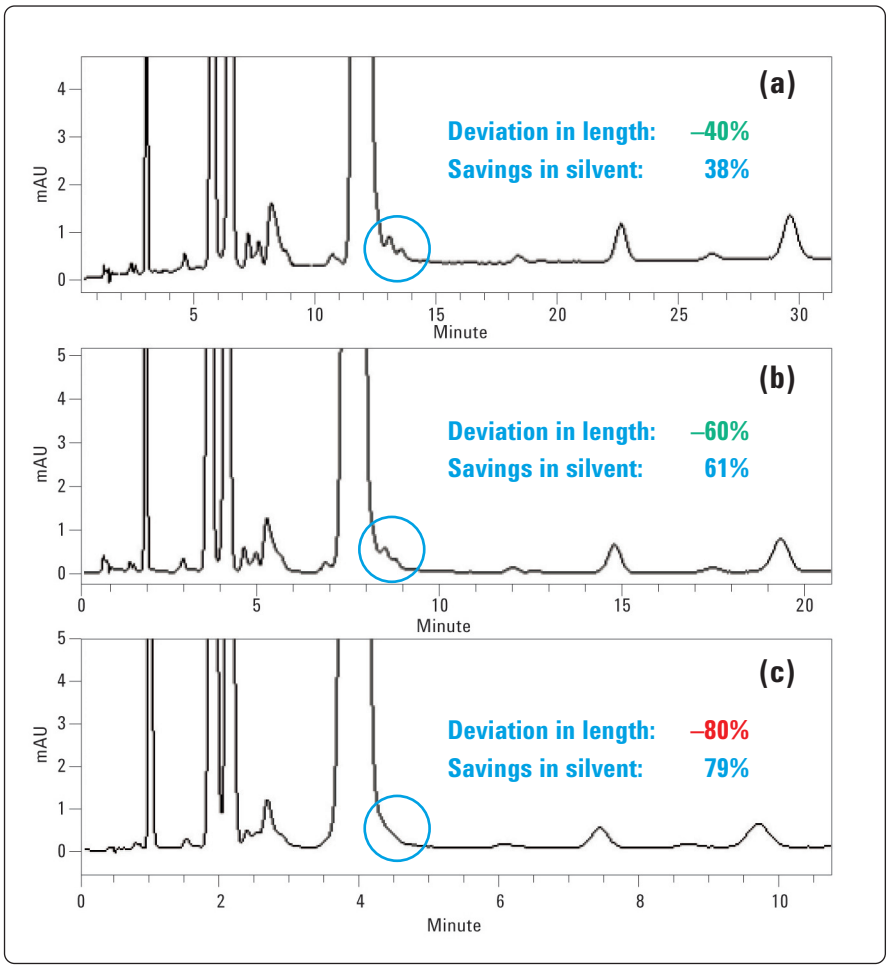

Figure 3
HPLC trials with reduction in column length with effect on run time, resolution and percentage of solvent saved compared to original EP method.
(a): ZORBAX Eclipse Plus C18 Analytical, 150 mm × 4.6 mm, 5 µm
(b): ZORBAX Eclipse Plus C18 Analytical, 100 mm × 4.6 mm, 5 µm
(c): ZORBAX Eclipse Plus C18 Analytical, 50 mm × 4.6 mm, 5 µm

Reducing particle size allows use of shorter columns while maintaining resolution

Reducing a column's particle size will increase peak resolution. Figure 4 shows separations of methylprednisolone sodium succinate with columns of decreasing particle size. Runs (a), (b) and (c) were performed with 50 mm length × 4.6 mm id columns with particle sizes of 5, 3.5, and 1.8 µm. Analysis with sub 2 µm columns (1.8 µm particle size) gives the best resolution compared to the other two columns. The increased resolution provided by smaller particles allows the use of shorter columns without a loss of resolution compared to the original method while saving solvent. In this case, the use of a short 50 mm × 4.6 mm column with 1.8 µm particles reduces solvent consumption by 79% compared to the original method, without compromising in resolution.

HPLC trial runs with reduction in column internal diameter

Decreasing the internal diameters (id) from 4.6 to 3.0 to 2.1 mm while keeping the particle size constant at 1.8 µm allows further reduction in solvent consumption. However, changing the internal diameter to 2.1 mm will require method revalidation as this change is outside the pharmacopeia method adjustment limits (54% reduction of id). In addition, when changing the column id, revalidation of instrumentation may be required as dead volumes have to be adapted to the new smaller column volume.

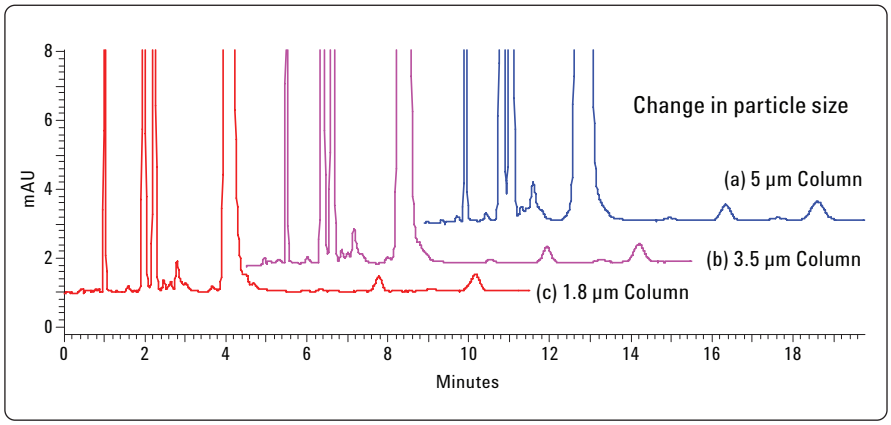

Figure 4
Separations of Methylprednisolone sodium succinate with columns of varying particle size.
Trace-(a): ZORBAX Eclipse Plus C18 Analytical, 50 mm × 4.6 mm, 5 µm; Flow: 1 mL
Trace-(b): ZORBAX Eclipse Plus C18 Rapid Resolution, 50 mm × 4.6 mm, 3.5 µm;Flow: 1 mL
Trace-(c): ZORBAX Eclipse Plus C18 Rapid Resolution HT, 50 mm × 4.6 mm, 1.8 µm; Flow: 1 mL

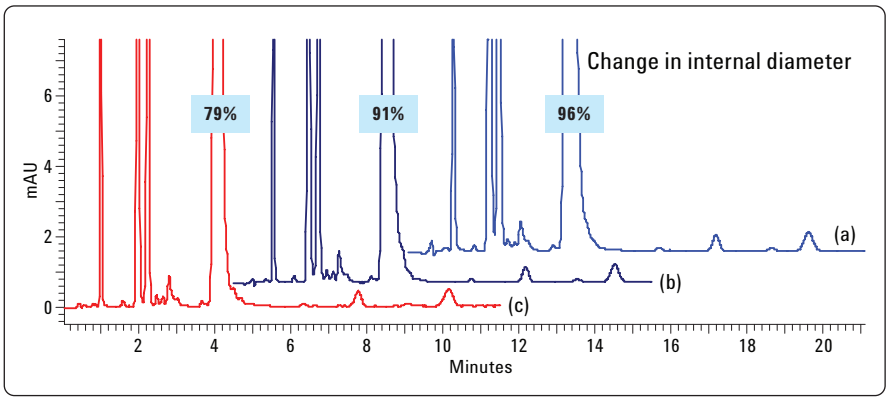

Figure 5
Trials with columns of varying internal diameter labeled with respective amount of solvent saved compared to EP method.
Trace-(a): ZORBAX Eclipse Plus C18 Narrow bore RRHT, 50 mm × 2.1 mm, 1.8 µm, Flow: 0.21 mL
Trace-(b): ZORBAX Eclipse Plus C18 Solvent saver HT, 50 mm × 3.0 mm, 1.8 µm, Flow: 0.43 mL
Trace-(c): ZORBAX Eclipse Plus C18 Rapid resolution HT, 50 mm × 4.6 mm, 1.8 µm, Flow: 1 mL

HPLC trial run with optimum deviation in column dimensions

Optimizing column length, particle size and internal diameter while staying within the accepted method adjustment parameters as per EP and USP provides solvent savings while maintaining separation performance. In this case, column length is reduced to 100 mm (deviation: –60%), particle size to 3.5 µm (deviation: –30%) and internal diameter to 3.0 (deviation: –25%). These adjustments allow an 83% solvent savings. The chromatogram in Figure 6 demonstrates uncompromised separation performance, compared to the original method.

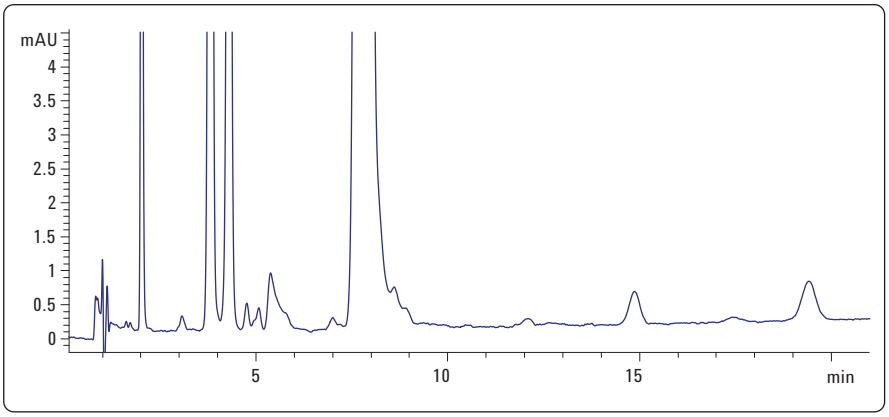

Figure 6
LC trial with adjusted column parameters within the limits of EP and USP method adjustment criteria. Column used ZORBAX Eclipse Plus Solvent saver plus, 100 mm × 3.0 mm, 3.5 µm; Flow: 0.43 mL..

Conclusion

Solvent consumption in liquid chromatography can be efficiently reduced by changing the column dimensions and particle size. Labs working in a regulatory environment can change the column dimensions and particle size according to method adjustment parameters from the European and US Pharmacopeia. Results show that solvent consumption can be drastically reduced (in this case by 83%) after incorporating the allowed column dimension variations without compromising separation performance.

Using solvent saver columns with sub 2-µm particles are the best option for developing new methods as they enable the highest reduction of column length without loss of resolution.

References

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