APPLICATION NOTE 73916

Overcoming strong solvent effects during method transfer of a compendial method for the analysis of hesperidin

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Goal

To demonstrate the use of the Thermo Scientific™ Vanquish™ Core HPLC System equipped with a strong solvent loop to mitigate strong sample solvent effects occurring in a compendial method.

Application benefits

- A compendial method for the analysis of hesperidin was transferred to the Vanquish Core HPLC system.
- Different approaches to handling strong solvent effects are demonstrated to be successful.
- A simple and compliant way to mitigate strong sample solvent effects is demonstrated.

Introduction

It is considered good chromatographic practice to dissolve a sample in a solvent or solvent mixture that is close to, or identical to, the starting conditions of the chromatographic method. Following this practice will avoid a mismatch in elution strength between the injected sample plug and the mobile phase. In case this approach cannot be followed, a weaker eluting solvent is typically not an issue, but the injection of a sample dissolved in a stronger solvent may be problematic and result in peak broadening, peak symmetry distortions, and in severe cases, as peak splitting in the chromatogram. The strong solvent effect typically negatively impacts resolution and signal-to-noise ratios in a chromatographic analysis.

However, in some instances, it is not feasible to match the elution strength of the sample solvent and initial mobile phase. Most often this is due to limits of solubility of one or more analytes or the result of the sample preparation procedure. In these cases, it is often easier and more straightforward to mitigate the strong solvent effect while conserving the chromatographic method. To find a suitable mitigation strategy, it is necessary to recognize the factors that influence the severity of the strong solvent effect. The most relevant are:

i) The magnitude of mismatch in elution strength between injected sample solvent and initial mobile phase

ii) The ratio of injected volume to column diameter: The larger the diameter, the more sample dilution takes place at the column head, helping to lower the elution strength

iii) The degree of pre-column mixing of sample and mobile phase, which depends strongly on the volume between point of injection and column head. Similar to ii), mixing of the sample plug with mobile phase effectively reduces the solvent strength

Mitigation strategies can be derived from these root causes. Accommodating i) is the least preferred solution as this requires modification of the sample solvent, the avoidance of which is the main reason why strong solvent effects occur. Addressing ii) is straightforward. The simplest way is to reduce the injection volume if the method performance is not compromised by this modification. By transferring a method to a larger diameter column, e.g., changing from 2.1 to 4.6 mm inner diameter, the ratio can also be tuned to be less prone to strong solvent effects, however, this usually also decreases the sensitivity of the method. To address the limited mixing, as described in iii), two approaches are possible. One consists of the use of custom injection programs (CIPs), as implemented on the Vanquish Core HPLC system, another consists of physically adjusting the volume, i.e., introducing a large volume capillary, a mixer, or similar item into the flow path between injector and column. On the Vanquish Core HPLC system, this can be done by utilizing the strong solvent loop, which is shown in Figure 1.

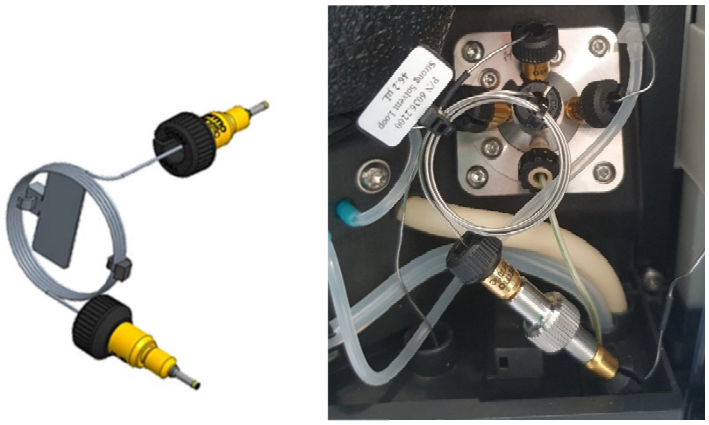

Figure 1. Strong solvent loop schematic (left) and installed in the Vanquish Core Autosampler (right)

Consideration of ii) and iii) also highlights the importance of strong solvent effects during the transfer of methods from legacy instruments to modern HPLC systems. The dispersion in modern HPLC instruments is inherently less because of better manufactured tubing (e.g., more consistent bore) and more precise connections, resulting in less disturbance of flow. This means that issues resulting from insufficient mixing of sample solvent and initial mobile phase—peak fronting or splitting—are prevented.

Xingsu Zhike Koufuye is a modern pharmaceutical version of a TCM. It is an oral liquid extract prepared from six different herbs and is used to treat cough and asthma caused by the cold virus. Hesperidin, derived from one of the herbs (*Citrus reticulata* Blanco—tangerine), is used for quality control of the medicine. Using a compendial method for the determination of hesperidin as described in the 2020 Chinese Pharmacopeia[1], we demonstrate the occurrence of strong solvent effects when the method is run unaltered on a Vanquish Core instrument. We show the use of custom injection programs as a software-based mitigation strategy, as well as the use of a strong solvent loop as a hardware-based solution to mitigate the negative affect of strong solvent injections and improve the peak shape.

Experimental

Chemicals

Chemical name	Part number
Deionized water, 18.2 MΩ·cm resistivity or higher	N/A
Fisher Scientific™ Methanol, Optima™ LC/MS grade	A456-212

Chemical name	Part number
Fisher Scientific o-Phosphoric acid	A260-500
Hesperidin, standard substance	For this study hesperidin was obtained from the National Institutes for Food and Drug Control China (P/N X5UW-6RTM) but can also be obtained from reputable vendors.

Sample handling

Item name	Part number
Vials (amber, 2 mL), Fisher Scientific	03-391-6
Cap with Septum (Silicone/PTFE), Fisher Scientific	13-622-292
Fisher Scientific™ Titan3™ Regenerated Cellulose Syringe Filters	52213-RC

The Fisher Scientific codes can be unique to different countries; the codes given above should be compatible across the EU and USA.

Instrumentation

Thermo Scientific™ Vanquish™ Core HPLC system consisting of:

Module	Part number
System Base Vanquish Core	VC-S01-A-02
Vanquish Quaternary Pump C	VC-P20-A
Vanquish Split Sampler CT	VC-A12-A
Vanquish Thermostatted Column Compartment C	VC-C10-A-03
Vanquish Diode Array Detector C	VC-D11-A
Flow cell, standard, path length 10 mm (13 µL, SST)	6083.0510
Strong solvent loop, V = 46.2 µL	6036.2200

Sample preparation

A stock solution of hesperidin was prepared by accurately weighing 30.0 mg and dissolving in 100 mL of pure methanol. Working standards were prepared by dilution with pure methanol to a final concentration of 30 µg/mL.

A commercial sample of Xingsu Zhike Koufuye was prepared for analysis by tenfold dilution with methanol, followed by filtration. The filtered solution was directly transferred to vials and subjected to chromatographic analysis.

For the chromatographic runs using a CIP, an additional vial filled with pure aqueous 0.1% phosphoric acid was prepared.

Chromatographic conditions

Table 1. Chromatographic conditions

Parameter	Setting
Column	Thermo Scientific™ Acclaim™ C18, 4.6 × 250 mm, 5 µm, P/N 059149
Mobile phase	A: Methanol/0.1% aqueous phosphate buffer (33/67 v/v)
Flow rate	1 mL/min
Column temperature	25 °C
Autosampler temperature	8 °C
Injection volume	10 µL
Detector settings	Wavelength: 283 nm; Data rate: 10 Hz; Response time: 0.12 s

Custom Injection Program

A Custom Injection Program was used in some of the experiments, following the guidelines outlined in *Application Note 73186—Improving peak results using a custom injection program to reduce solvent strength prior to sample injection*[2]. Briefly, the CIP initiates a sandwich injection, where the sample plug is located between two plugs of a weak eluting solvent to perform an online dilution of the sample. This is done by the following commands using the mode “Replace normal injection”:

Table 2. Custom Injection Program

No.	Command	Parameters
1	UDP_PrepareLiquidHandling	Volume = 50 µL
2	UDP_Draw	Volume = 20 µL Position = *aq. phosphoric acid*
3	UDP_Draw	*Parameter taken from injection table*
4	UDP_Draw	Volume = 20 µL Position = *aq. phosphoric acid*
5	UDP_Wait	10 s
6	UDP_NeedleWash	–
7	UDP_PrepareInject	–

Chromatography data system

The Thermo Scientific™ Chromeleon™ 7.3 Chromatography data system (CDS) was used for data acquisition and processing.

Results and discussion

The initial injection of the hesperidin standard according to the compendial method did show a severe peak distortion, as shown in Figure 2A. To check that this effect can be indeed attributed to a strong solvent effect, the injection volume was reduced from 10 µL to 5 µL; the result is shown in Figure 2B. It is evident that with the reduction in injection volume the peak symmetry is greatly improved, although this comes at the cost of reduced sensitivity. The use of the custom injection program is shown in Figure 2C. The shape and symmetry of the hesperidin peak are vastly improved. The injection of the hesperidin standard after installation of the strong solvent loop and subsequent requalification of the system is shown in Figure 2D.

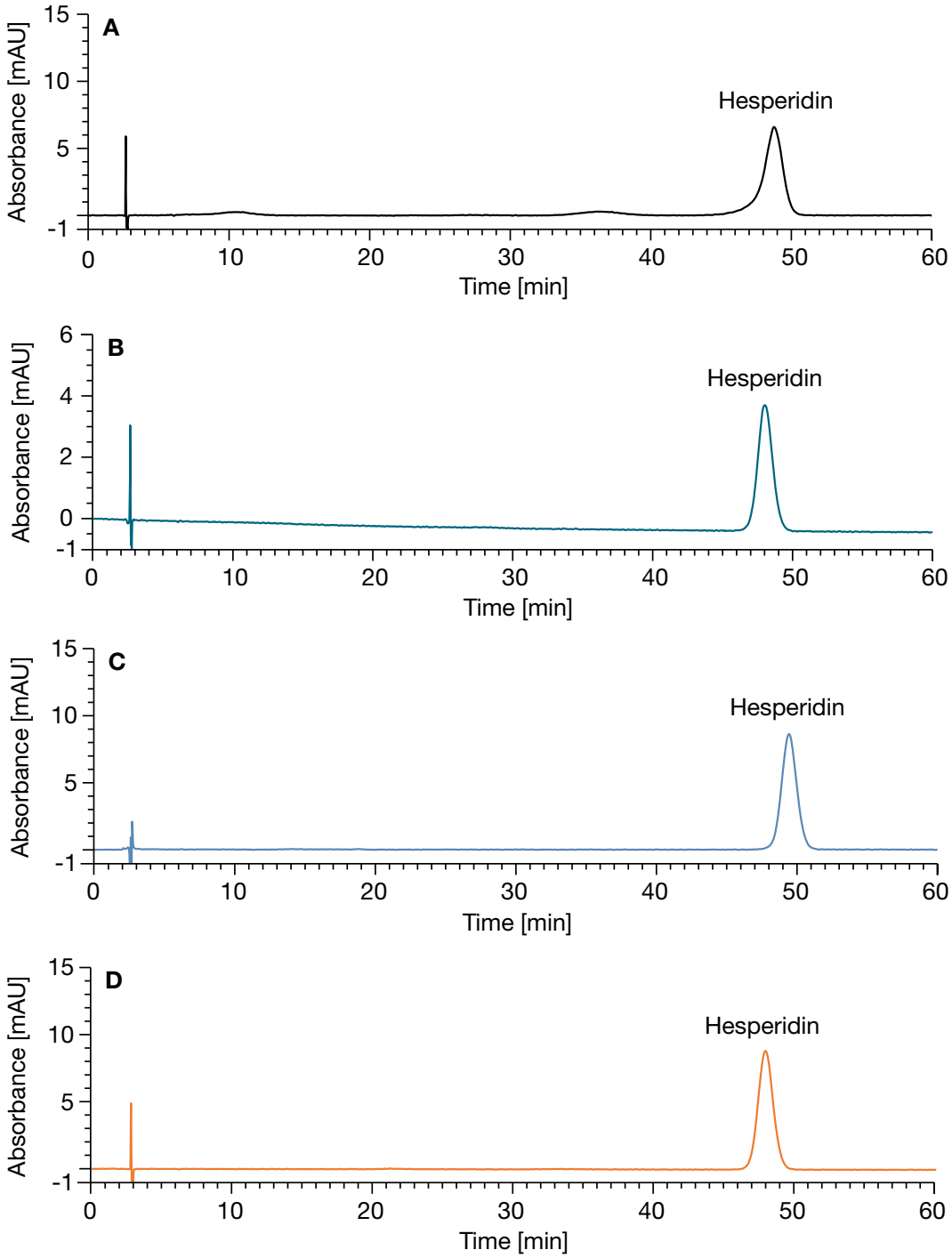

Figure 2. Comparison of default injection (A, Inj. vol. = 10 µL), reduced volume injection (B, Inj. vol. = 5 µL), CIP (C), and Strong solvent loop (D) for the injection of the 30 µg/mL hesperidin standard

The direct comparison of the different injections for the hesperidin standard is summarized in Table 3. While all three approaches mitigate the strong solvent effect, it is notable that, as expected, a reduction of injection volume is also accompanied by a reduced signal-to-noise ratio, while the other two strategies effectively increase the signal-to-noise ratio, due to the sharper peaks.

The injection of the sample following the compendial method is shown in Figure 3. Both the strong solvent loop and the custom injection program help to reduce the strong solvent effect to an extend that enables to run this assay.

Table 3. Comparison of hesperidin peak properties using different strong solvent effect mitigation strategies

Mode	Peak width (10%) [min]	Peak symmetry (EP)	Signal-to-noise
Default	3.11	0.79	320.7
Reduced inj. vol.	2.21	1.06	232.0
CIP	2.23	1.02	380.6
Strong solvent loop	2.22	1.04	428.9

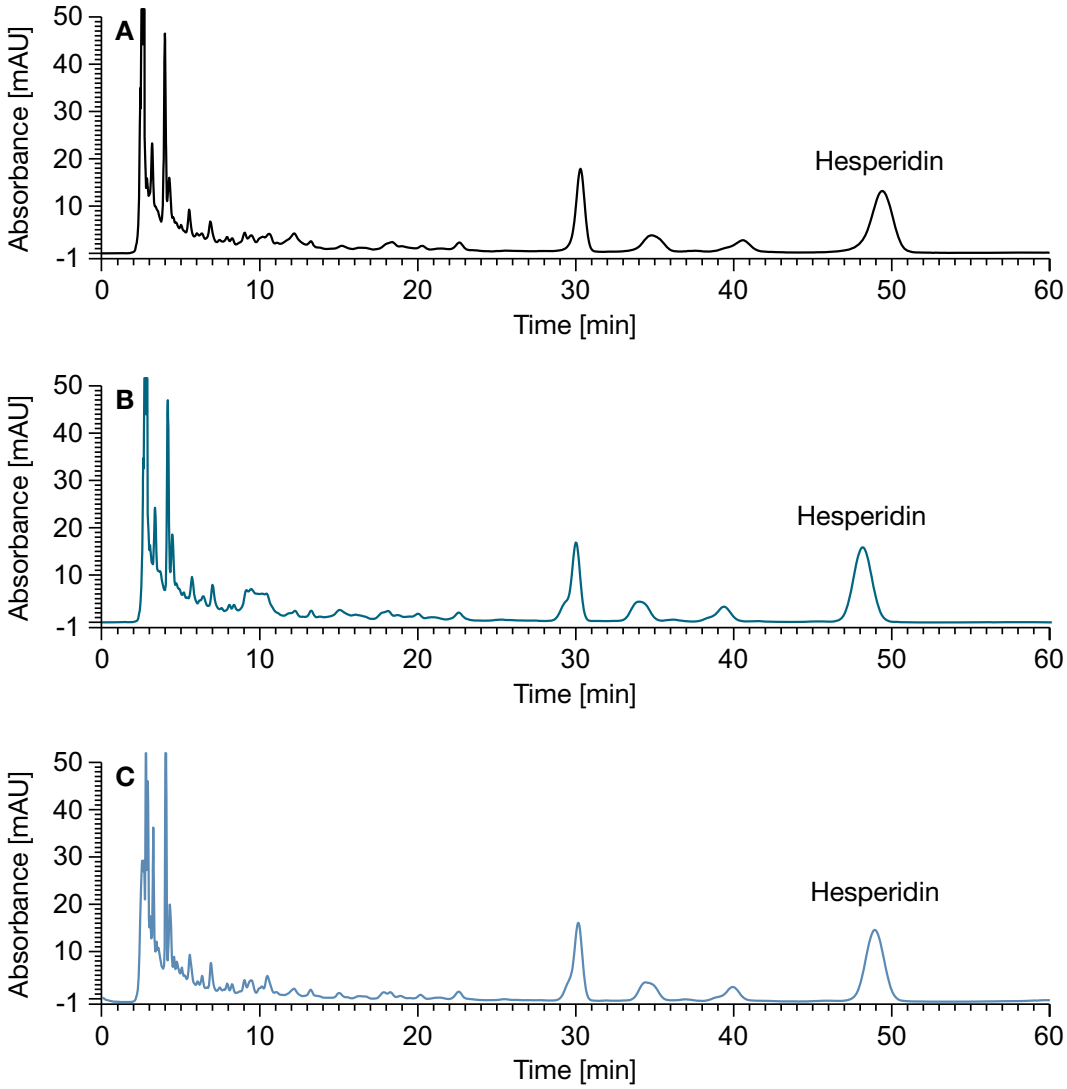

Figure 3. Analysis of hesperidin containing sample (Xingsu Zhike Koufuye) using default configuration/injection (A), the strong solvent loop (B), and the CIP (C)

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For practical purposes, reducing the injection volume is often the easiest approach, which is also considered an acceptable modification in a regulated environment, as long as the system suitability test is still passed and not impaired by the reduced signal-to-noise ratio. The use of a strong solvent loop is straightforward and does not rely on any additional steps during method setup or modifications of the method. The increase of extra-column dispersion, which accompanies the installation of the strong solvent loop, prohibits the flexible use of this system for UHPLC methods. The strong solvent loop as a system modification is considered acceptable in a regulated environment, although the installation/deinstallation of a strong solvent loop may mandate requalification of the system to maintain compliance. CIP do not require this requalification and preserve the full system performance. However, familiarity with CIPs is required for the correct setup of the method, and although not explicitly mentioned in pharmacopeia compendia, the use of CIPs in injection methods may be regulated by local SOPs.

A preference for one method over the other is therefore dependent on the laboratory regulations, user preference, and system utilization.

Conclusion

- Strong solvent effects are sometimes observed when legacy methods are transferred to modern HPLC instruments.
- The strong solvent effect impacts the resolution, peak symmetry, and signal-to-noise ratio.
- These effects can be mitigated by either implementing a chromatographic solution (reducing injection volume), a hardware solution (strong solvent loop), or a software-based approach (custom injection program).
- With an assay method for the analysis of hesperidin, a strong solvent loop and custom injection programs were demonstrated to successfully eliminate the strong solvent effect.

References

1. Xingsu Zhike Koufuye (Hesperdin)—ChP.2020.1000-1001
2. Improving peak results using a custom injection program to reduce solvent strength prior to sample injection. *Thermo Scientific Application Note 73186*, **2019**. https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/an-73186-lc-results-custom-injector-reduce-solvent-strength-an73186-en.pdf

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