Validating Repeatability Performance of the Axcend Focus LC®: Single- and Multi-Day Study

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Abstract

Innovative tools that purport to improve workflows through saving time, space, or cost must also provide analytical results that are nominally the same or better than incumbent technologies. In liquid chromatography, repeatability is a key performance indicator of any modern system. This application note demonstrates the performance of the Axcend Focus LC® in both single- and multi-day repeatability studies. For single-day analysis, a 30-injection sequence of alkylphenones was performed. The highest relative standard deviations (RSDs) for peak area and retention time were 1.49% and 0.194%, respectively. In addition, peak areas and retention times were monitored for a series of 5 injections made over three days. The highest peak area and retention time fluctuations were 0.851% and 0.706%, respectively. All fluctuations fell within the traditionally accepted limits of <2% RSD for peak area and <1% RSD for retention time. This clearly demonstrates the ability of the Axcend Focus LC to produce repeatable chromatographic data.

Introduction

High performance liquid chromatography (HPLC) is one of the most widely used analytical techniques in the world. To operate in regulated environments, HPLC instruments must be capable of producing repeatable separations across a single day, and between days. Although repeatability tolerances can vary between methods and regulatory environments, a relative standard deviation (RSD) of <2% for peak area and <1% for retention time is typically considered acceptable [1,2]. The Axcend Focus LC is a capillary scale HPLC usually referred to as capillary LC which utilizes columns with an inner diameter (i.d.) of 0.1-0.3 mm and operated at mobile flow rates between 0.5 and 10 µL/min. The decrease in solvent consumption results in solvent savings of over 99% when compared to analytical scale LC systems [3]. The goal of this work was to demonstrate the repeatability of the Focus LC in single- and multi-day studies to validate its use in regulated environments. To accomplish this, a single-day sequence of 30 separations was performed as well as a three-day comparison of the same separation.

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Materials and Methods

HPLC grade water and acetonitrile (ACN) were used for the mobile phase as well as the sample solvent. The sample consisted of thiourea, acetophenone, propiophenone, and butyrophenone at a concentration of 50 parts per million (ppm) each in a 70:30 mixture of Water/ACN.

LC System	Axcend Focus LC with Heated Column Cartridge
Column	Agilent Eclipse Plus C18 0.3 x 100 mm, 1.8 µm particles
Mobile Phase	A) 85:15 Water/ACN with 0.2% formic acid B) 100% ACN
Temperature	35˚C
Detection Wavelength	255 nm
Flow Rate	4 µL/min
Elution Mode	Isocratic at 30% B
Run Time	8 minutes
Injection Method	250 nL external injection loop toggled at 0.01 min for a 40 nL injection.

Results and Discussion

A series of 30 replicate injections was performed within a single day to demonstrate intraday repeatability. The results are shown in Figure 1. Thiourea was primarily used as a dead-time marker and, therefore, was not included in the quantitative results. The three alkylphenones were analyzed for retention time and peak area, with the results listed in Table 1. The highest observed RSD for peak area was 1.497% for butyrophenone and the highest RSD for retention time was 0.194% for acetophenone. Both RSDs fall below the desired 2% and 1% limits for area and retention time, respectively. This demonstrates that the Axcend Focus LC produces daily repeatable data.

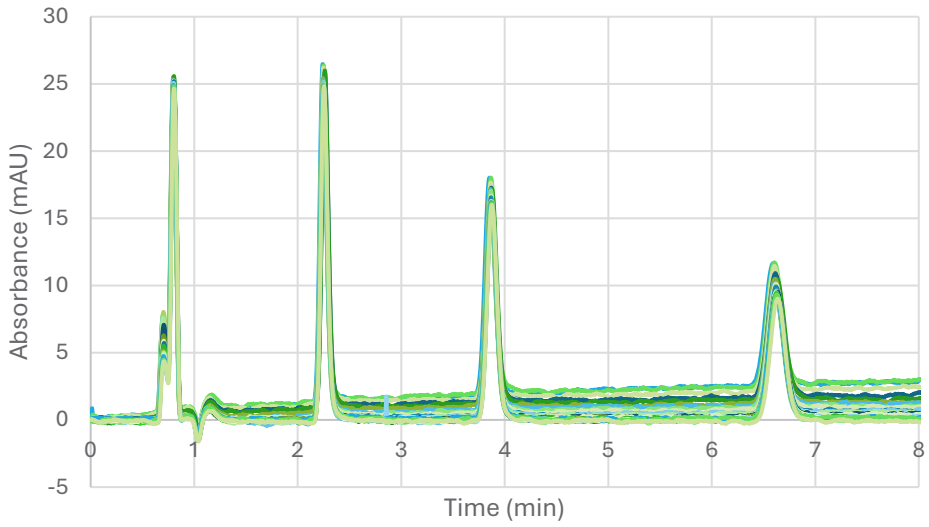

Figure 1. Overlaid chromatograms of 30 repetitive injections of a mixture of alkylphenones obtained in a single day.

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Analyte	RSD Peak Area	RSD Retention Time
Acetophenone	0.838%	0.194%
Propiophenone	0.992%	0.134%
Butyrophenone	1.497%	0.139%

Table 1. Single-day Repeatability of Alkylphenones Peak Areas and Retention Times.

An interday repeatability study was also conducted over the course of three days. For this, a series of 5 consecutive injections of the alkylphenone mixture were made each day. A comparison of the first run of each day is shown in Figure 2. The peak area and retention time RSDs were recorded across the three days, with the results listed in Table 2. Also given in Table 2 is the overall RSD calculated from the average retention time or peak area of each day. The highest peak area RSD was 0.851% for butyrophenone and the highest retention time RSD was 0.706% also for butyrophenone. Again the repeatability of the system fell within the acceptance threshold, demonstrating that the Axcend Focus LC maintain good repeatability across multiple days.

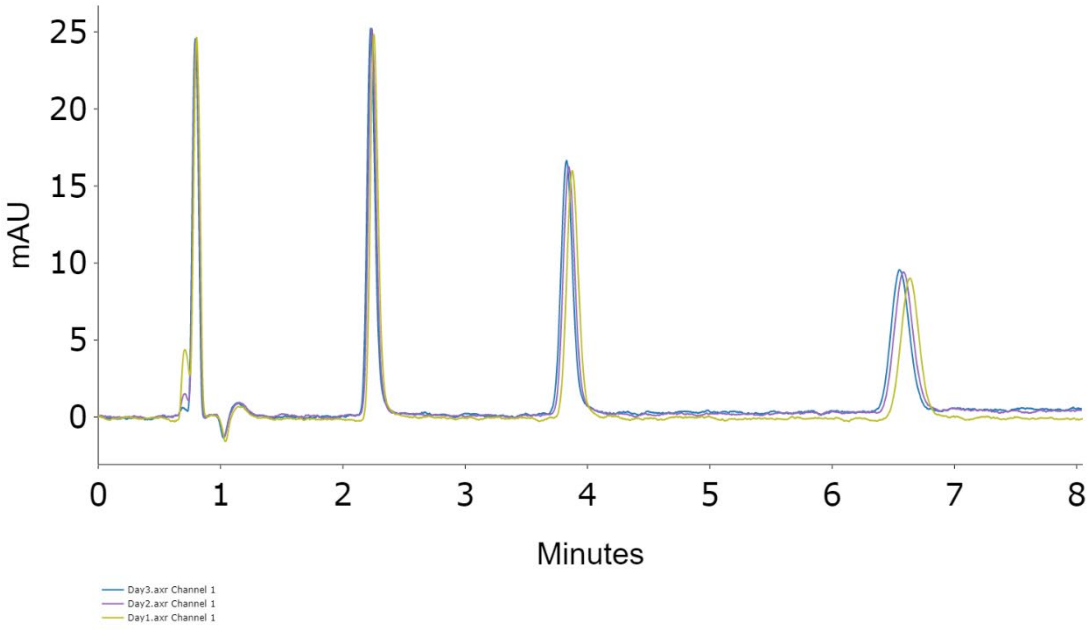

Figure 2. Overlaid chromatograms of the first of 5 repetitive injections of a mixture of alkylphenones obtained on each of 3 different days.

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Analyte	Day 1 Area RSD	Day 1 Ret Time RSD	Day 2 Area RSD	Day 2 Ret Time RSD	Day 3 Area RSD	Day 3 Ret Time RSD	Overall Area RSD	Overall Ret Time RSD
Acetophenone	0.544	0.376	0.419	0.289	0.525	0.371	0.362	0.635
Propiophenone	1.23	0.205	0.812	0.353	1.58	0.504	0.181	0.638
Butyrophenone	0.966	0.183	1.30	0.956	0.655	0.656	0.851	0.706

Table 2. Multi-Day Repeatability of Alkylphenone Peak Areas and Retention Times.

Conclusions

The results of both intraday and interday repeatability studies of chromatographic results obtained using the Axcend Focus LC demonstrate that it can deliver consistent and reliable capillary-scale chromatographic performance. Over the course of 30 injections in a single day, all analytes exhibited RSD values for peak area and retention time well within the commonly accepted thresholds of <2% and <1%, respectively. Similarly, interday studies conducted over 3 days also showed excellent repeatability with the highest observed RSDs for peak area and retention time being 0.851% and 0.706%, respectively. These findings confirm that the Axcend Focus LC is suitable for routine analytical use in regulated environments where repeatability is critical. Furthermore, the ability of the Axcend Focus LC to achieve this performance with significantly lower solvent and sample volumes highlights its potential as a greener and more economical alternative to traditional HPLC systems.

References

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