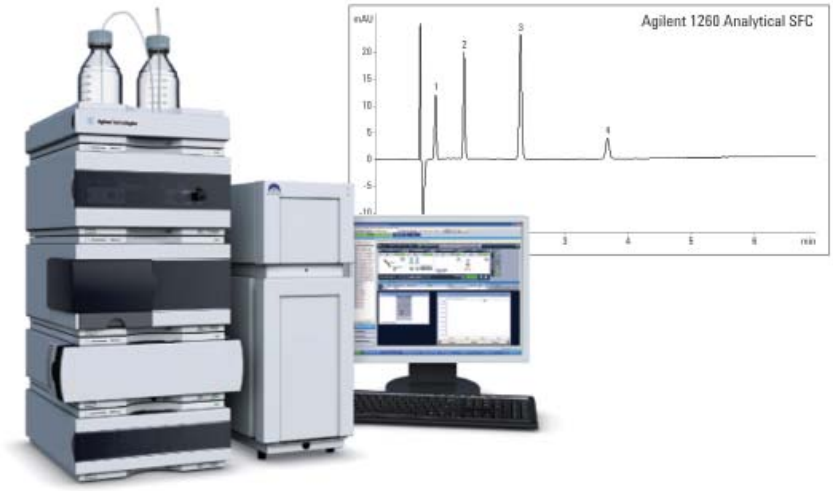
# Comparison of UV detection limits between the Agilent 1260 Infinity Analytical SFC System and an Agilent 1200 Series LC System

## Application Note

Drug Development





## Authors

Melissa Dunkle, Gerd Vanhoenacker, Frank David, and Pat Sandra
Research Institute for Chromatography
President Kennedypark 26,
8500 Kortrijk, Belgium

Udo Huber
Agilent Technologies, Inc.
Waldbronn, Germany



## Abstract

In this Application Note, the UV sensitivity and detection limits obtained on an Agilent 1260 Infinity Analytical SFC System and on an Agilent 1200 Series LC System were compared. Two applications were selected and similar separation was obtained using the same column, the same detector, and typical separation conditions for both supercritical fluid chromatography (SFC), a normal phase-like separation technique, and reversed phase liquid chromatography (RPLC). First, a standard mixture of polycyclic aromatic hydrocarbons (PAH) was analyzed. In a second example, 2,6-dichloroaniline, a typical potential genotoxic impurity (PGI), in a drug substance (diclofenac) was determined. Although the Agilent 1200 Series LC System was slightly more sensitive, the Agilent 1260 Infinity Analytical SFC System obtained near- HPLC sensitivity.


Agilent Technologies

## Introduction

UV sensitivity between RPLC and SFC was compared using an Agilent 1200 Series LC System and an Agilent 1260 Infinity Analytical SFC System. In order for this to be a fair comparison, the same column, same injection volume, same samples, and same UV detector and flow cell were used on both configurations. Typical SFC modifier (MeOH) and LC mobile phases (Water/ACN) were used. Because RPLC and SFC are orthogonal separation techniques, finding samples that would separate in a similar time frame was a limiting factor. Finally, the separation of a mixture of four polycyclic aromatic hydrocarbons (PAHs) and the determination of 2,6-dichloroaniline, a potential genotoxic impurity (PGI) in an active pharmaceutical ingredient (API), dichlorfenac, were selected as applications, since the obtained chromatographic profiles in SFC were similar to those in RPLC, facilitating a good comparison of sensitivity and limits of detection.

## Experimental

### Solutions

Stock solutions of the PAHs were made up at 5,000 ppm in dichloromethane. A dilution series was prepared between 0.1 and 100 ppm in methanol; the same dilution series was injected in both the SFC and LC systems.

Stock solutions were prepared at 5,000 ppm in methanol for 2,6-dichloroaniline and in 50/50 water/methanol with 0.05 % formic acid for diclofenac. The PGI (2,6-dichloroaniline) was spiked at concentrations between 0.01% and 1% in the diclofenac solution; the same solutions were injected in both the SFC and LC systems.

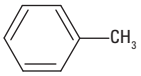
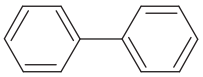
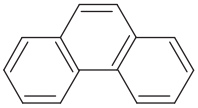
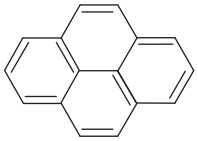
| Compound name | Peak ID | Structure |
|---|---|---|
| Toluene | 1 |  |
| Biphenyl | 2 |  |
| Phenanthrene | 3 |  |
| Pyrene | 4 |  |

**Table 1**
**List of PAHs.**

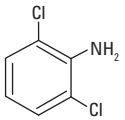
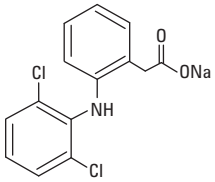
| Compound name | Peak ID | Structure |
|---|---|---|
| 2,6-dichloroaniline | PGI |  |
| Diclofenac | API |  |

**Table 2**
**List of API and PGI.**

## Experimental

The same reversed phase column (Agilent ZORBAX Eclipse XDB C18), injection volume, and detector, flow cell, and detector settings were used for both SFC and LC experiments.

The experimental conditions are summarized in Table 4. These conditions can be considered as generic for SFC and RPLC.

| | Agilent 1260 Infinity Analytical SFC (G4309A) | Agilent 1200 Series LC |
|---|---|---|
| Degasser | G1322A | G1322A |
| Agilent 1260 SFC Binary Pump | G4302A | N/A |
| SFC A5 Fusion | G4301A | N/A |
| Quaternary Pump | N/A | G1311A |
| Autosampler | G4303A | G1329A |
| Thermostatted Column Compartment | G1316B | G1316A |
| Diode Array Detector | G1315C | G1315C |

**Table 3**
**System modules.**

| Conditions | |
|---|---|
| Column: | Agilent ZORBAX Eclipse XDB C18 (4.6 × 150 mm, 5 µm) |
| Supercritical fluid: | $CO_2$ |
| Modifier (SFC): | MeOH w. 2% water |
| Mobile phase (LC): | A) Water (PAHs)<br>A) Water with 0.05% formic acid (PGI)<br>B) ACN (PAHs and PGI) |
| Outlet pressure (SFC): | 120 bar |
| Flow rate: | 2.0 mL/min (LC and SFC) |
| Modifier/MP gradient: | 0-20 min: 5-40% (SFC PAHs and PGI)<br>0-8 min: 60-100%B (LC PAHs)<br>0-8 min: 50-80%B (LC PGI) |
| Temperature: | 40 °C (SFC)<br>25 °C (LC) |
| Injection volume: | 5 µL (SFC and LC) |
| Detection: | 254(4) Ref. 360 (100) (PAHs)<br>296(5) Ref. 450 (50) (PGI) |

**Table 4**
**Experimental conditions.**

## Results and Discussion

### PAHs

Using the same column and a typical SFC modifier and LC mobile phases, similar separations were achieved for the PAH mixture in SFC and LC. Figure 1 shows the SFC and LC separation of the PAH mixture at 5 ppm, and, as can be seen, the elution order remained the same in both separation modes. Using the dilution series, calibration curves were constructed, and it was determined that excellent linearity was obtained in both SFC and LC. Using the calibration curves, the limits of detection (LOD) with a signal-to-noise ratio (S/N) greater than 3 for each system were calculated (Table 5). Also seen in Table 5 are the S/N at 0.5 ppm and the RSD values on the S/N at 0.5 ppm, as well as the linearity and the slope (m) from the calibration curves.

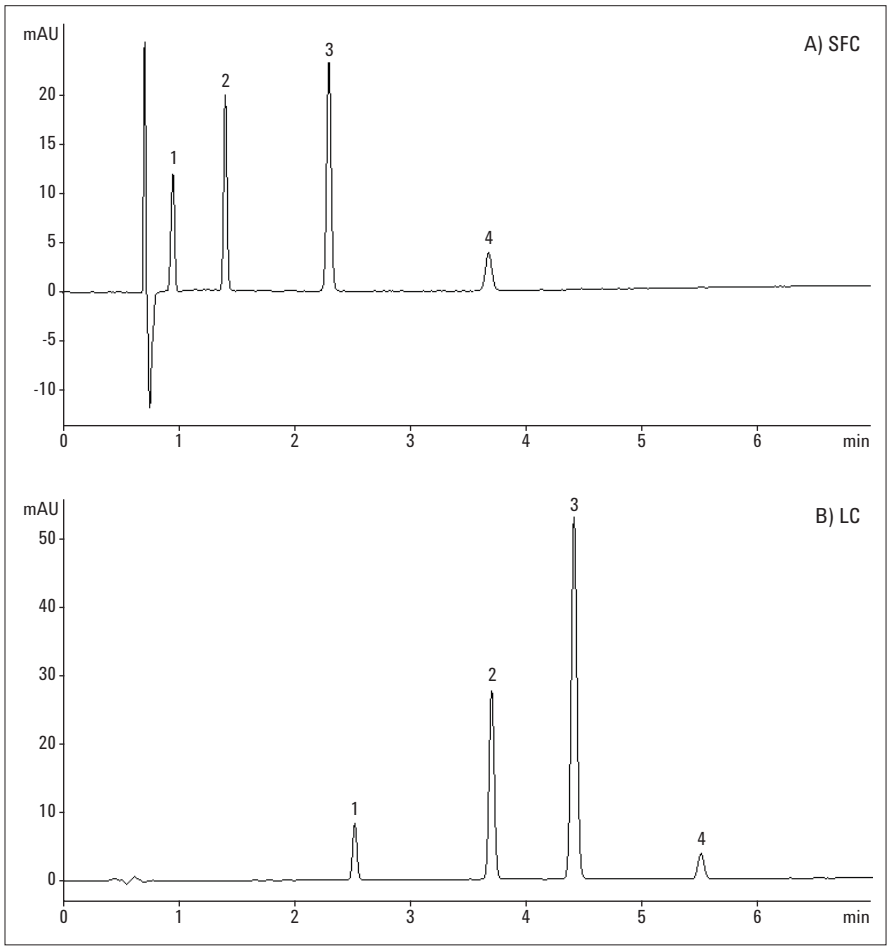



**Figure 1**
**Separation of the PAH mixture using A) SFC and B) LC. The separation conditions are stated in Table 4.**

| | SFC mode | | | | | LC mode | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | S/N (0.5 ppm) | %RSD (S/N) | LOD (ppm) | $R^2$ | m (slope) | S/N (0.5 ppm) | %RSD (S/N) | LOD (ppm) | $R^2$ | m (slope) |
| Toluene | 8.5 | 10.5 | 0.2 | 0.998 | 5.9 | 16.1 | 9.4 | 0.1 | 0.990 | 6.6 |
| Biphenyl | 13.1 | 11.3 | 0.1 | 0.999 | 8.4 | 44.8 | 8.8 | 0.04 | 0.999 | 18.5 |
| Phenanthrene | 14.7 | 8.1 | 0.07 | 0.999 | 13.1 | 86.3 | 9.0 | 0.02 | 0.999 | 38.3 |
| Pyrene | 3.0 | 7.3 | 0.5 | 0.999 | 3.1 | 6.2 | 10.9 | 0.25 | 0.999 | 3.0 |

**Table 5**
**Limits of detection (S/N = 3), RSD (%) on S/N (n = 3), LOD (at S/N = 3), linearity ($R^2$) and slope (m) of calibration curves for PAHs.**

The baseline noise for the two systems at 5 ppm are given in Table 6; the time windows for both systems was the same, 6 – 6.5 min. Overall, the LODs were in the same order of magnitude between the two techniques; typically, the LODs in LC mode are between two and four times lower than in SFC mode. Interestingly, the slope of the calibration curves (that are also a measure of sensitivity) are similar for toluene and pyrene, and somewhat steeper (more sensitive) for LC analysis of biphenyl and phenanthrene.

| | SFC mode | | LC mode | |
|---|---|---|---|---|
| | 6 × SD | P to P | 6 × SD | P to P |
| Noise | 0.0872 | 0.0779 | 0.0361 | 0.0337 |

**Table 6**
**Noise levels (expressed as 6 × SD or peak-to-peak noise) for the two systems at 5 ppm.**

## Determination of 2,6-dichloroaniline (PGI) in dichlofenac (API)

Separations of diclofenac (API) and 2,6-dichloroaniline (PGI) were achieved in both SFC and LC modes. In both cases, the PGI eluted before the API. Figure 2 shows the SFC and LC chromatograms for the separation of 2,6-dichloroaniline spiked at 0.05% (w/w) in dichlofenac. Adequate resolution between the PGI and API were obtained in both cases: R = 3.54 (SFC) and R = 3.46 (LC). Both modes of separation were sensitive enough so that the limit of quantification (LOQ) was below the 0.01% impurity level (S/N > 13). Using a series of dilutions of PGI in API, a calibration curve was constructed, and the LOQs (S/N = 10) for both methods were determined (Table 7). Also seen in Table 7 are the S/N at 0.05 % (w/w) spiking level and the RSD values on the S/N at the 0.05% (w/w) spiking level, as well as the linearity ($R^2$) and the slope (m) from the calibration plots. The values obtained by both techniques are very similar.

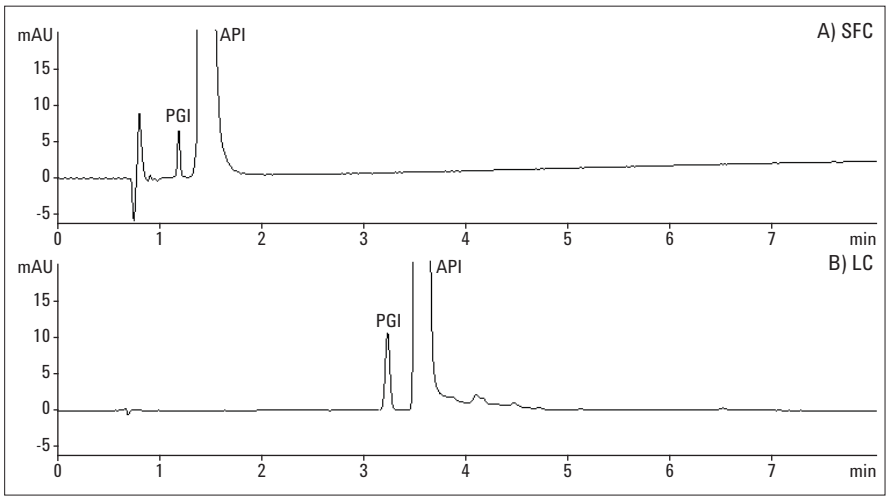



**Figure 2**
**Separation of diclofenac and the impurity 2,6-dichloroaniline in A) SFC and B) LC. The separation conditions are stated in Table 4.**

| | SFC mode | | | | | LC mode | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | S/N (0.01% w/w) | %RSD (S/N) | LOQ (%) (w/w) | $R^2$ | m | S/N (0.01% w/w) | %RSD (S/N) | LOQ (%) (w/w) | $R^2$ | m |
| 2,6-Dichloroaniline | 12.6 | 11.0 | 0.01% | 0.999 | 217.6 | 17.0 | 19.1 | 0.007% | 0.999 | 692.8 |

**Table 7**
**Signal-to-noise, RSD on S/N (n = 3), Limits of quantification (S/N > 10),Linearity ($R^2$) for 2,6-Dichloroaniline in dichlofenac and slope of calibration curve (m) for SFC and HPLC analysis.**

The noise for the two systems at the 0.05% spiking level are given in Table 8; the time windows were 2 – 2.5 min for the LC system and 2.5 – 3 min for the SFC system. Overall, it was determined that the sensitivities were nearly identical, with the LC method being only slightly more sensitive than the SFC method.

| | SFC mode | | LC mode | |
|---|---|---|---|---|
| | 6 × SD | P to P | 6 × SD | P to P |
| Noise | 0.1625 | 0.1204 | 0.1118 | 0.0807 |

**Table 8**
**Noise levels (expressed as 6 × SD or peak-to-peak noise) for the two systems at the 0.05% (w/w) spiking level.**

## Conclusion

The sensitivity of an Agilent 1200 Series LC System and the Agilent 1260 Infinity Analytical SFC System were compared. The same column, injection volume, detector, flow cell, and detector settings were used in both separation modes. Two samples were chosen based on their ability to be separated in a similar way, under both SFC and LC conditions. For both samples, excellent linearity and detection limits were obtained using both separation modes. It was determined that in both cases, the LC separation mode was slightly more sensitive than the SFC mode; however, SFC does give near-HPLC sensitivity and results in a significantly reduced analysis time at comparable resolution.

**www.agilent.com/chem/sfc**

© Agilent Technologies, Inc., 2011
Published in USA, November 1, 2011
Publication Number 5990-9195EN

Agilent Technologies