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Application Data Sheet

No. AD-0064

Nexera X2- UHPLC

Enhancement of Sensitivity of Photodiode Array Detector SPD-M30A by New HS Capillary Flow Cell

❑ Introduction

The performance of Shimadzu new generation photodiode array detector SPD-M30A designed for Nexera X2 UHPLC was described previously [1]. The high sensitivity and high resolution of SPD-M30A was greatly due to the new capillary flow cell which features total internal light reflection and extremely small diameter and volume (1uL). Shimadzu introduced recently another high sensitivity (HS) capillary flow cell with an extra long optical path of 85 mm compared to 10 mm of a standard flow cell. The sensitivity is further enhanced to about five times on average. The HS cell offers a new option for users in analysis of trace levels of organic compounds like impurities, residues and pollutants in pharmaceuticals, food and environmental samples by HPLC with UV detection methods.

❑ System and Conditions

The system used in this study was a Nexera SR UHPLC with a SPD-M30A detector. Both standard cell and high sensitivity (HS) cell were used to compare their sensitivity and other performance. The samples used in the study were anthracene and caffeine, which show very strong UV absorption at 250 nm and 272 nm, respectively. The samples were prepared from high purity chemicals using mobile phase as solvent. The UHPLC analysis was carried out in isocratic mode. This was to evaluate and compare the sensitivity and resolution of different flow cells under exactly same conditions without gradient effect.

Table 1A: System and experimental conditions

System	Nexera SR with SPD-M30A
Mobile Phase	ACN/Water (70/30)
Column	XR-ODSIII 50x2.0 mm, 1.6µm
Flow Rate	0.4 mL/min
Detection wavelength	250nm (Anthracene) 272nm (Caffeine)
Slit width	8nm
Cell temperature	40ºC
Injection Volume	5 µL

Table 1B: SPD-M30A detector and flow cells

Feature	Standard cell	HS Cell
Optical Path length	10mm	85mm
Cell volume	1µL	9µL
Noise level (x10^5 AU)	0.4	0.6

❑ Chromatograms and Calibration Curves of Caffeine and Anthracene

Figure 1 shows the overlay chromatograms of mixed standard samples. The lowest concentrations of caffeine and anthracene tested were 100ppb and 1ppb respectively. The calibration curves of the sample set are shown in Figure 2.

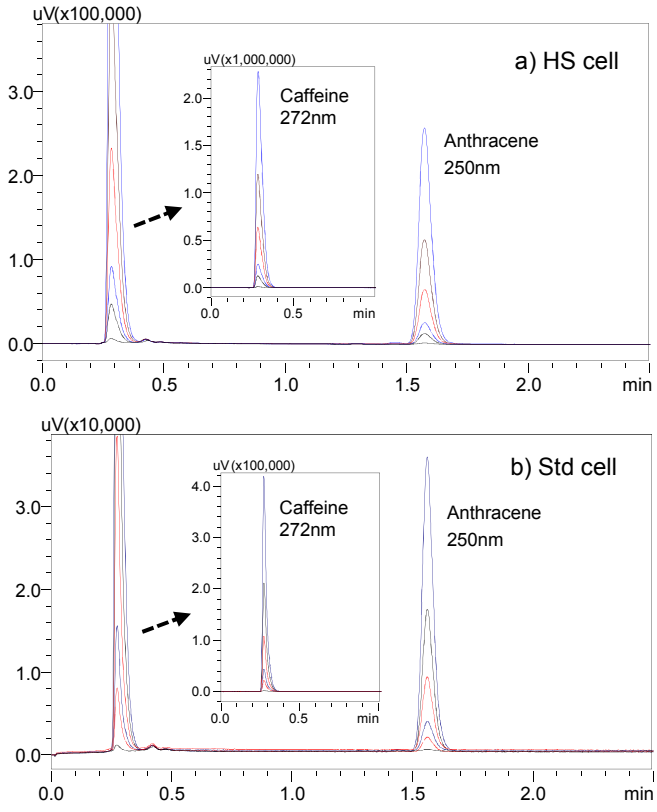

Fig 1: Overlay chromatograms of mixed samples of anthracene and caffeine obtained with HS cell (a) and standard cell (b). Caffeine conc.: 0.1, 1, 2, 5, 10, 20 **ppm (ug/mL)**; Anthracene conc.: 1, 10, 20, 50, 100 and 200 **ppb (ng/mL)**.

The peak intensity and peak area of both caffeine and anthracene using the HS cell are 6.8 ~ 9.3 times greater than that of standard cell. This increase of UV absorption was simply due to increase of optical path length of the HS cell. A small discrepancy from the proportional relation between intensity and light path length was due to peak dispersion of a bigger volume of the HS cell.

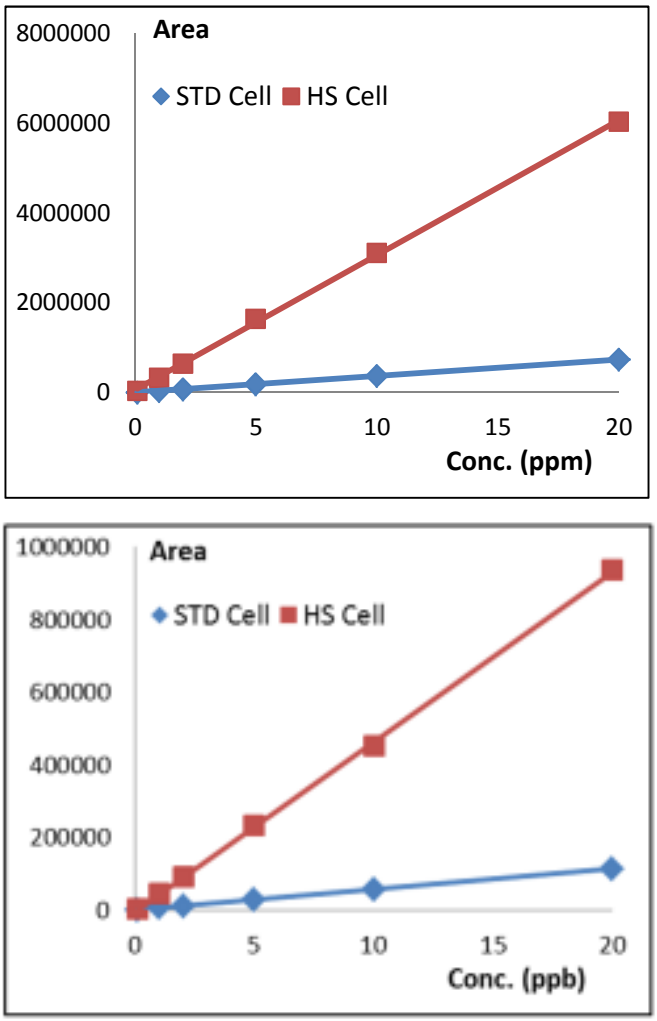

Fig 2: Peak area calibration curves of caffeine (top) and anthracene (bottom) with different flow cells of SPD-M30A. The conc. are same as in Fig 1.

❑ Enhancement of Sensitivity by HS Cell

The increase of sensitivity and overall performance of SPD-M30A due to use of HS cell are illustrated with the results of the lowest concentration sample. As shown in Table 2, the peak area, intensity and S/N ratio obtained with HS cell were 4.8 to 9.3 times higher than the standard cell. Peak width of HS cell was 20% broader than the standard cell for anthracene. This was due to dispersion effect of the greater volume of HS cell. The repeatability of HS cell was better than the standard cell. These data indicate that the HS cell enables SPD-M30A detector to gain about five times enhancement in sensitivity (S/N ratio).

Table 2: Comparisons of flow cells with mixed sample of 100ppb caffeine and 1ppb anthracene

Peak	HS Cell		Standard Cell		Numerical ratio (HS/Std)	
	Caff	Anthr	Caff	Anthr	Caff	Anthr
Area	39931	4368	4291	583	9.3	7.5
Intensity (mAU)	13771	1214	2017	179	6.8	6.8
S/N	126.5	19.7	26.3	3.5	4.8	5.6
Width at 50% (sec)	0.039	0.056	0.027	0.045	1.4	1.2
*RSD% (n=6)	0.16	0.10	0.37	0.38	0.43	0.26

** Repeatability test: 1000ppb caffeine and 10ppb anthracene.*

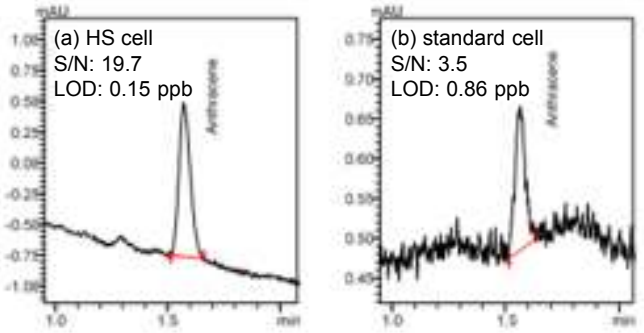

Fig 3: Anthracene peak of 1ppb with HS cell (a) and standard cell (b) by SPD-M30A Detector

Anthrancene is one of PAHs, toxic industrial pollutants found in air, water and smoked food etc. The EPA methods for quantitative analysis of 16 concerned PAHs were based on GC or HPLC (RF and UV) depending on the type of samples [2]. The detection limits required are 1ppb for anthrancene and other 11 PAHs using HPLC with fluorescence detection and 10 ppb for 4 PAHs with UV detection. The very high sensitivity (LOD: 0.15ppb or 0.75pg) of anthrancene as shown in Figure 3 reveals a possibility to use new generation SPD-M30A PDA detector with HS capillary cell for analysis of more PAHs.

❑ Summary

The new HS capillary cell enhances the sensitivity of SPD-M30A detector 4.8 and 5.7 times for caffeine and anthracene respectively. The LOD of anthracene, one of the concerned PAHs, was 0.15ppb or 0.75pg.

❑ References

1. Application Data Sheet AD-0058, Shimadzu Asia Pacific
2. US PEA Method 610: polynuclear aromatic hydrocarbons

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