Seamless Transfer of Methods to the New Agilent 1200 Series Diode Array Detector

Technical Note

Abstract

This Technical Note compares the Agilent 1100 Series diode array detector (DAD) “B” model) to its successor the Agilent 1200 Series DAD “D model”. For this comparison both detectors were interchangeably connected to an Agilent 1100 Series LC system. To exclude undesired instrument bias the same Agilent 1100 Series LC system was used for both series of experiments. The evaluated performance criteria included retention time, peak width, resolution, peak area, peak height, signal-to-noise, limit of detection, limit of quantitation and linearity. Overall, the Agilent 1200 Series DAD compared favorably to the Agilent 1100 Series DAD, delivering the same or even noticeably better results. Based on these results it was concluded that an Agilent ChemStation method that was developed and validated on an 1100 Series DAD is fully compatible with the 1200 Series DAD. This means that an 1100 Series DAD can be replaced by a 1200 Series DAD with no or minimal revalidation effort.

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Introduction

In pharmaceutical quality control UV-visible detectors are used for the quantification of main compounds and impurities. The Agilent 1100 Series DAD in combination with the Agilent 1100 Series LC system has been the ideal instrument for accurate and reliable quantification results over the past years. This Technical Note compares the Agilent 1100 Series DAD "B" model to its successor, the Agilent 1200 Series DAD "D model"[1]. The performance of the 1200 Series DAD was determined to be not only as good as but even noticeably better than the 1100 Series DAD. This warrants the replacement of an 1100 Series DAD, even in a regulated environment, with no or minimal revalidation effort.

Equipment

The Agilent 1100 Series LC system comprised the following modules:

- Agilent 1100 Series binary pump
- Agilent 1100 Series well-plate autosampler
- Agilent 1100 Series thermostatted column compartment
- Agilent 1100 Series diode array detector "B" model (standard flow cell: 10-mm path length, 13-µL volume, Agilent part number 61315-60012), or Agilent 1200 Series diode array detector "D" model (standard flow cell: 13-µL volume, 10-mm path length, Agilent part number 61315-60022)

The system was controlled using the Agilent ChemStation software. The required ChemStation revision to control the new Agilent 1200 Series DAD is version B.01.03 SR 2 or higher, or B.2.01 SR 2 or higher. The instrument modules were equipped with the firmware version A.06.02 or higher. Other vendor's control software was not included in the evaluation. However, some distinct conclusions can be drawn provided the vendor in question has an official control code exchange agreement with Agilent Technologies.

Results and discussion

A sample consisting of tramadol and its four main impurities (A,B,C,D) was used as described in a previous Application Note[2] to compare the chromatographic performance (figure 1). The sample contained 1 mg/mL tramadol and 2.5 µg/mL of each impurity and a volume of 10 µL was injected. The injected amount on column was 10 µg tramadol and 25 ng of each impurity. Ten consecutive runs were carried out on an 1100 Series system using the 1100 Series DAD and the 1200 Series DAD, respectively, and the results were averaged. The relative standard deviation (in %) was calculated for all results, however results are only displayed if there was a relevant difference between the two

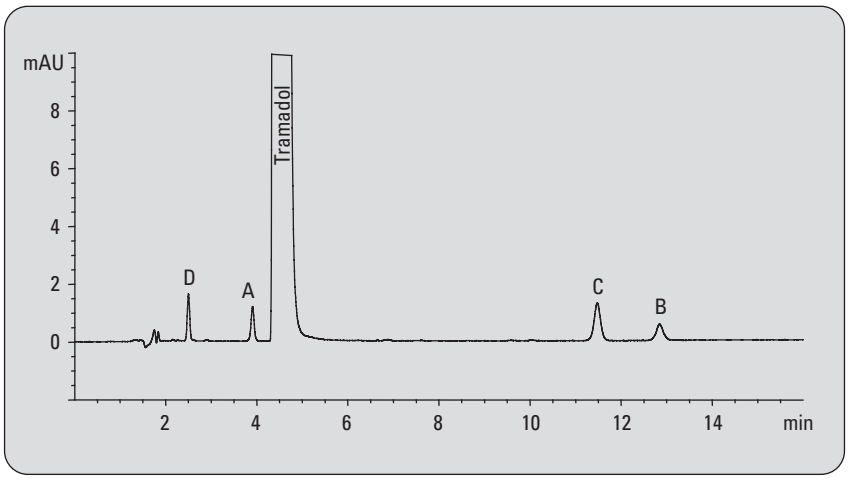

Figure 1
Chromatogram of tramadol and the four main impurities.

Chromatographic conditions:

Column:	ZORBAX SB-C18 4.6 x 150 mm, 5 µm
Mobile phases:	Water + 0.1 % TFA = A
	Acetonitrile + 0.1 % TFA = B
Gradient:	at 0 min 25 % B
	at 14 min 32 % B
	at 16 min 50 % B
Stop time:	16 min
Post time:	5 min
Flow:	1 mL/min
Injection:	10 µL
Column temperature.:	30 °C
UV detector:	DAD: 270 nm/30 (ref. 360 nm/50)
	Flow cell (10 mm path length)

detectors. The results for retention time, peak area and peak height, peak width and resolution, as well as signal-to-noise, limit of detection (LOD) and limit of quantitation (LOQ) were compared as shown in section *Signal-to-noise, LOD and LOQ*. Further, the linearity for both detectors was measured from the detection limit to the upper end of the linear range. In this study the latest "D" model of the 1200 Series DAD and the "B" model of the 1100 Series DAD were used. Technically earlier "B" models of the 1200 Series DAD resemble the 1100 Series DAD, hence the results shown in the note are also applicable to earlier "B" models of the 1200 Series DAD.

Retention time

Figure 2A shows the overlay of two chromatograms acquired with the 1100 Series DAD and 1200 Series DAD, respectively. The retention times (figure 2B) were identical for both detectors. The repeatability of the 1100 Series system was generally below the specified value of 0.3 % RSD.

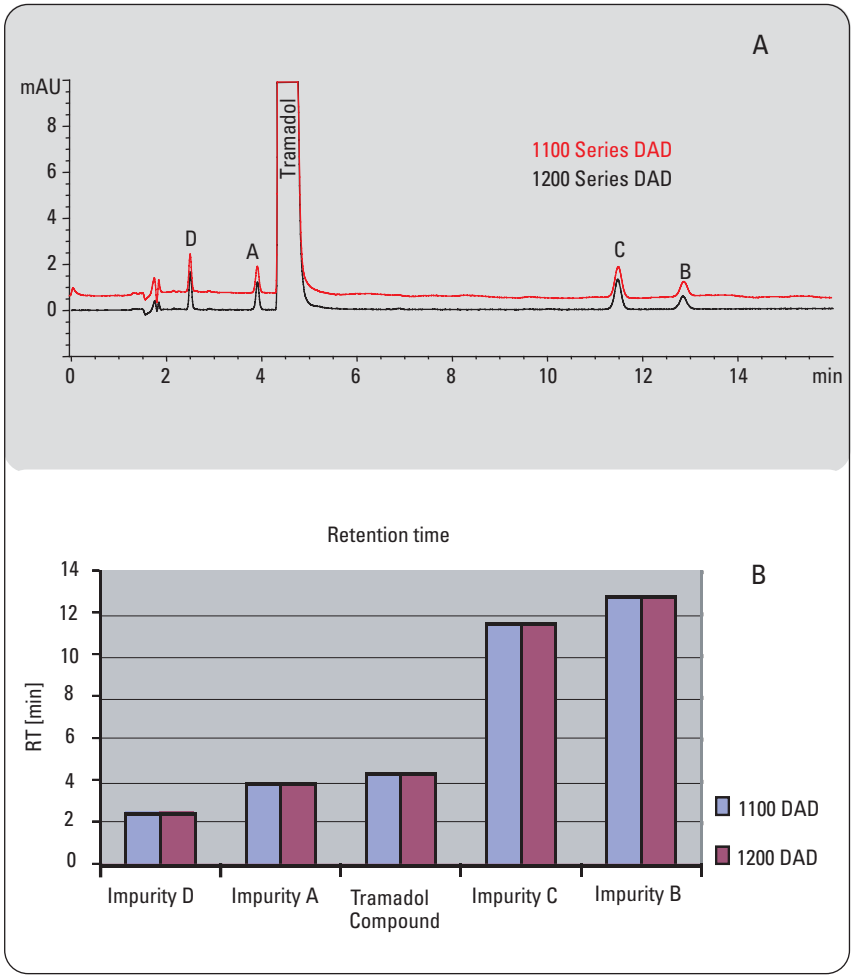

Figure 2
Overlay of two chromatograms acquired with the 1100 Series DAD and 1200 Series DAD (A). Comparison of retention times (B) for tramadol and the four main impurities determined with the 1100 and 1200 Series DAD.

Peak width and resolution

The peak width at half height of the peaks measured with the 1200 Series DAD is slightly smaller than with the 1100 Series DAD. As a result the resolution is about 5 % higher, as shown in figures 3A and 3B. The impact is mainly seen at low concentrations, as in the example for impurities A, B and C.

Peak area and peak height

Figure 4B and tables 1a and 1b show that the peak height for all peaks are identical for both detectors (tramadol is not shown), but the peak area using the 1200 Series DAD is about 5 % lower than with the 1100 Series DAD. However, the overall sensitivity of the 1200 Series DAD is higher, because of the much lower noise as illustrated in section *Signal-to-noise, LOD and LOQ*. The relative standard deviation for the peak areas of the small impurity peaks was between 1 and 5 % on both detectors.

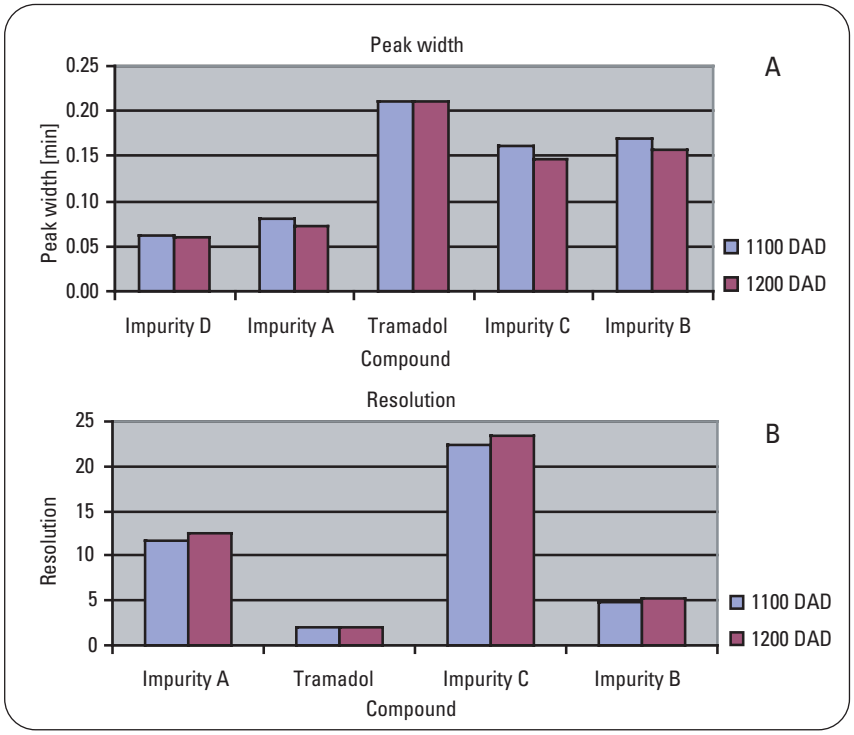

Figure 3
Comparison of peak width at half height (A) and resolution (B) for tramadol and the four main impurities determined with the 1100 and 1200 Series DAD.

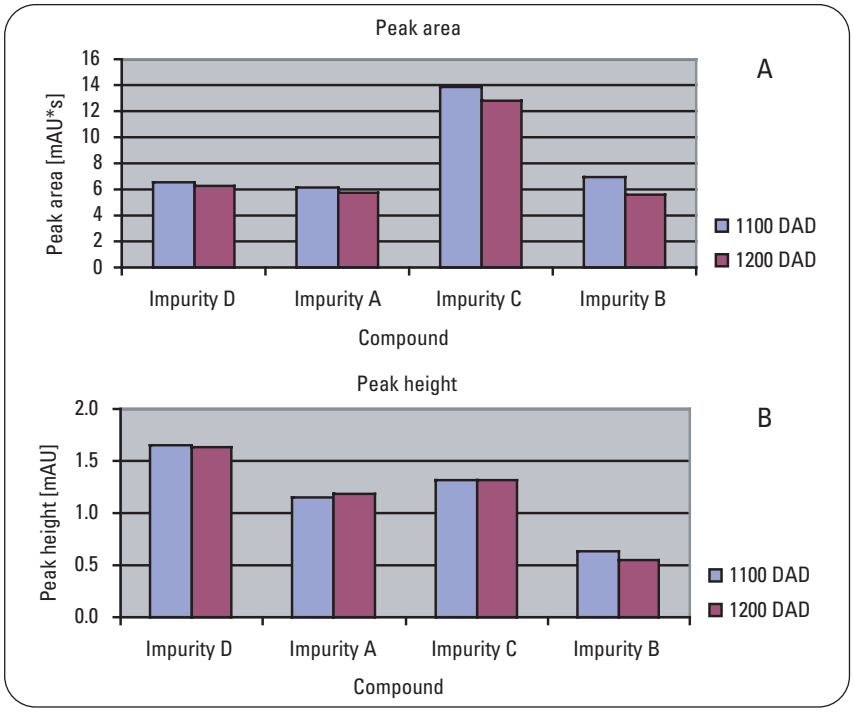

Figure 4
Comparison of peak area (A) and peak height (B) for the four main impurities determined with the 1100 and 1200 Series DAD.

Signal-to-noise, LOD and LOQ

With the new electronics in the Agilent 1200 Series DAD "D" model, the noise and drift specifications have been improved. Depending on the application this results in an increase of the signal to noise (typically by a factor 2-3), as shown in figure 5A.

Limit of detection and limit of quantitation were determined based on the signal-to-noise ratio using the Agilent ChemStation "Performance and Noise" report. For details on the calculation formula please refer to the Agilent ChemStation manual[3]. As a result, the LOD (signal-to-noise = 2) and the LOQ (signal-to-noise = 10) is also about 2-3 times lower for the new 1200 Series DAD, as shown in figures 5B and 5C.

Peak area	Impurity D		Impurity A		Tramadol		Impurity C		Impurity B	
DAD model	"B"	"D"	"B"	"D"	"B"	"D"	"B"	"D"	"B"	"D"
Mean	6,59	6,26	6,08	5,72	2426,66	2354,73	13,82	12,68	6,91	5,54
Std. Dev.	0,10	0,05	0,15	0,11	2,61	3,65	0,30	0,15	0,30	0,23
RSD [%]	1,58	0,86	2,47	1,97	0,11	0,16	2,14	1,16	4,33	4,14
Confidence (95 %)	0,02	0,01	0,03	0,02	0,56	0,78	0,06	0,03	0,06	0,06

Table 1a
Mean, standard deviation, relative standard deviation and confidence (95 %) of peak area for tramadol and the four main impurities.

Peak height	Impurity D		Impurity A		Tramadol		Impurity C		Impurity B	
DAD model	"B"	"D"	"B"	"D"	"B"	"D"	"B"	"D"	"B"	"D"
Mean	1,64	1,62	1,15	1,19	181,18	175,58	1,32	1,30	0,64	0,55
Std. Dev.	0,01	0,01	0,01	0,02	0,47	0,16	0,01	0,02	0,02	0,02
RSD [%]	0,50	0,41	0,97	1,63	0,26	0,09	1,05	1,79	2,95	4,38
Confidence (95 %)	0,00	0,00	0,00	0,00	0,10	0,03	0,00	0,00	0,00	0,01

Table 1b
Mean, standard deviation, relative standard deviation and confidence (95 %) of peak height for tramadol and the four main impurities.

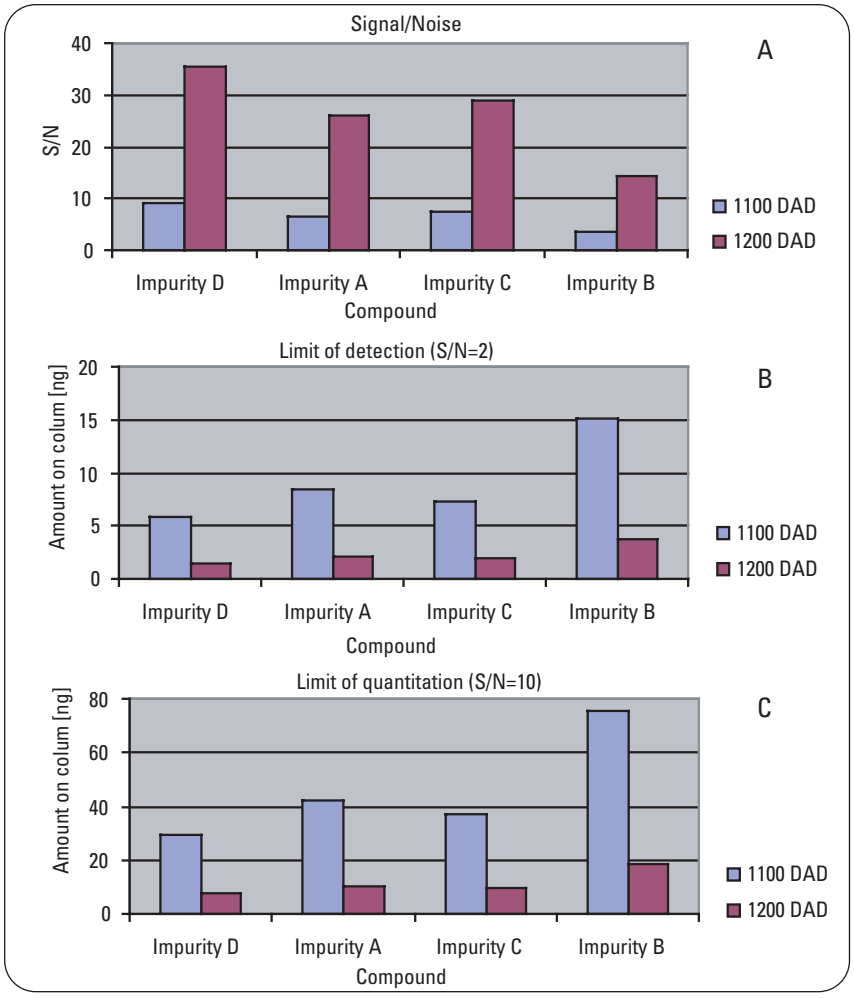

Figure 5
Comparison of Signal-to-Noise (A), LOD (B) and LOQ (C) for the four main impurities determined with the 1100 and 1200 Series DAD.

Linearity

A sample consisting of nifedipin, nimodipin and nisoldipin[4] (3 mg each in 1 mL DMSO) was used to measure detector linearity.
A chromatogram of the sample is shown in figure 6. Six dilutions were made from this stock solution by diluting each sample with DMSO in a ratio of 1:5. As a result, seven injections were carried out with the injected sample amount on column shown in table 2.

Level	Amount on column [µg]
1	15.00000
2	3.00000
3	0.60000
4	0.12000
5	0.00480
6	0.00096

Table 2
Amount injected on column from stock solution (level 1) and dilutions.

The linearity results of the two detectors are shown in figures 7A and 7B. In tables 3a and 3b the linearities, intercepts and slopes for the calibration curves for both detectors are shown. The linearity results determined for the 1200 Series DAD are better than for the 1100 Series DAD (a deviation from linearity does not occur within the first 5 digits). Also the intercept results are about 50 % closer to the origin for the 1200 Series DAD.

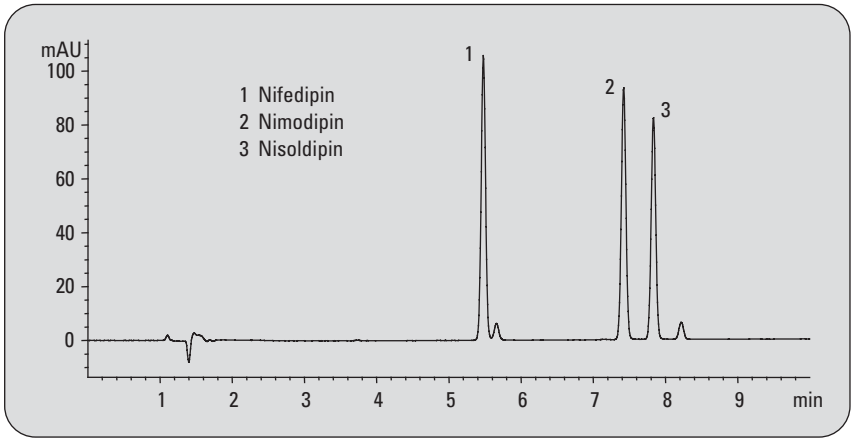

Figure 6
Chromatogram of nifedipin, nimodipin and nisoldipin.

Chromatographic conditions:

Column:	ZORBAX SB-C18 4.6 x 150 mm, 5 µm
Mobile phases:	Water = A Acetonitrile = B
Gradient:	at 0 min 40 % B at 10 min 95 % B
Stop time:	10 min
Post time:	5 min
Flow:	1 mL/min
Injection:	5 µL
Column temperature:	30 °C
UV detector:	DAD: 335 nm/16 (ref. 500 nm/80) Flow cell (10 mm path length)

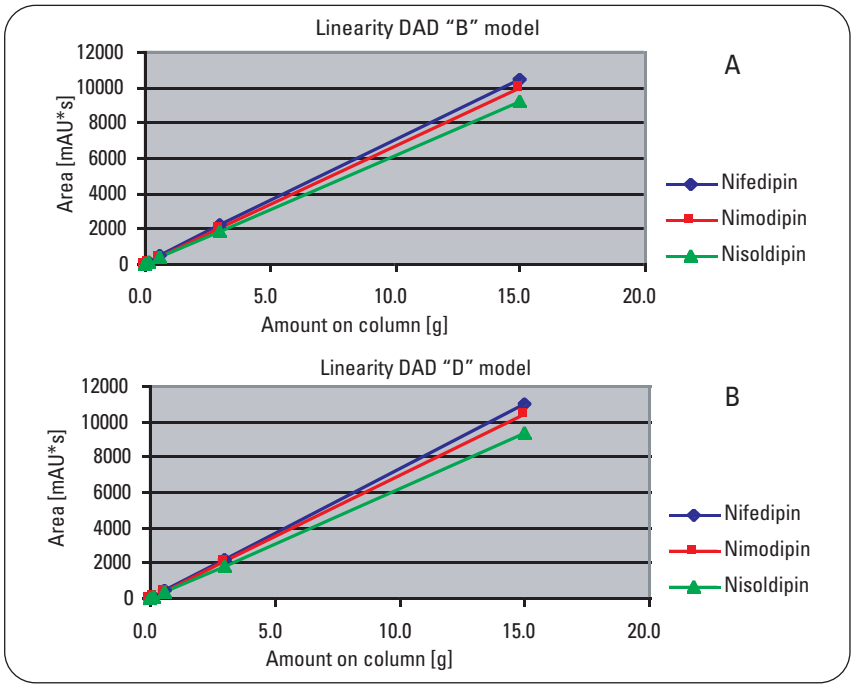

Figure 7
Comparison of the linearity results for the 1100 Series DAD (A) and 1200 Series DAD (B).

Conclusion

The 1200 Series DAD shows comparable and most of the time noticeably better results than the 1100 Series DAD if:

- it is used in the same Agilent 1100 or 1200 Series system configuration with identical columns, capillary connections, etc.
- the original standard flow cell for each detector is used, and
- the detector settings such as wavelength, bandwidth, reference wavelength and peak width are identical.

It has to be considered that the original standard flow cells for the detectors are different in design.

All experiments in this Application Note were entirely done with the corresponding original flow cell that is shipped with each of the detectors. The performance of the 1200 Series DAD measured with the original flow cell cannot be guaranteed if the flow cell from the 1100 Series DAD is used instead.

In this study it was proven that no changes to the analysis methods are necessary to obtain similar to significantly better performance results. As a result, it can be concluded that methods can be transferred easily from the 1100 Series DAD to the new 1200 Series DAD with no or minimal revalidation effort. Similar results can be expected when making the same evaluation with other vendor's control software. This statement holds true only as long as the vendor has entered an official control code exchange agreement with Agilent. Such an agreement ensures that vendors are informed ahead of time of new firmware versions and planned changes. Technically earlier "B" models of the 1200 Series DAD resemble the 1100 Series DAD, hence the results shown in the note are also applicable to earlier "B" models of the 1200 Series DAD.

If you require more detailed information, please contact an Agilent Technologies sales representative.

	Nifedipin	Nimodipin	Nisoldipin
Linearity	0.99989	0.99996	1.00000
Intercept	26.623	15.836	4.0535
Slope	696.70	662.16	609.93

Table 3a
Linearity, slope and intercept for the 1100 Series DAD.

	Nifedipin	Nimodipin	Nisoldipin
Linearity	0.99999	1.00000	1.00000
Intercept	9.8036	4.2852	-1.6876
Slope	737.21	693.80	625.28

Table 3b
Linearity, slope and intercept for the 1200 Series DAD.

References

1.
"Agilent 1200 Series UV-visible Detectors", *Agilent Technologies Data Sheet*, *publication number 5989-5574EN*, **2006.**

2.
"Impurity Profiling with the Agilent 1200 Series LC/MS System – Part 2: Isolation of Impurities with Preparative HPLC", *Agilent Technologies Application Note*, *publication number 5989-5618EN*, **2006.**

3.
"Understanding your Agilent ChemStation", *Agilent Technologies manual*, *part number G2070-91123*, **2006**.

4.
"Mass-based Fraction Collection at high flow rates for the purification of compounds in the lower gram scale", *Agilent Technologies Application Note*, *publication number 5989-2469EN*, **2005.**

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