Mass-based fraction collection at high flow rates for the purification of compounds in the lower gram scale

Application Note

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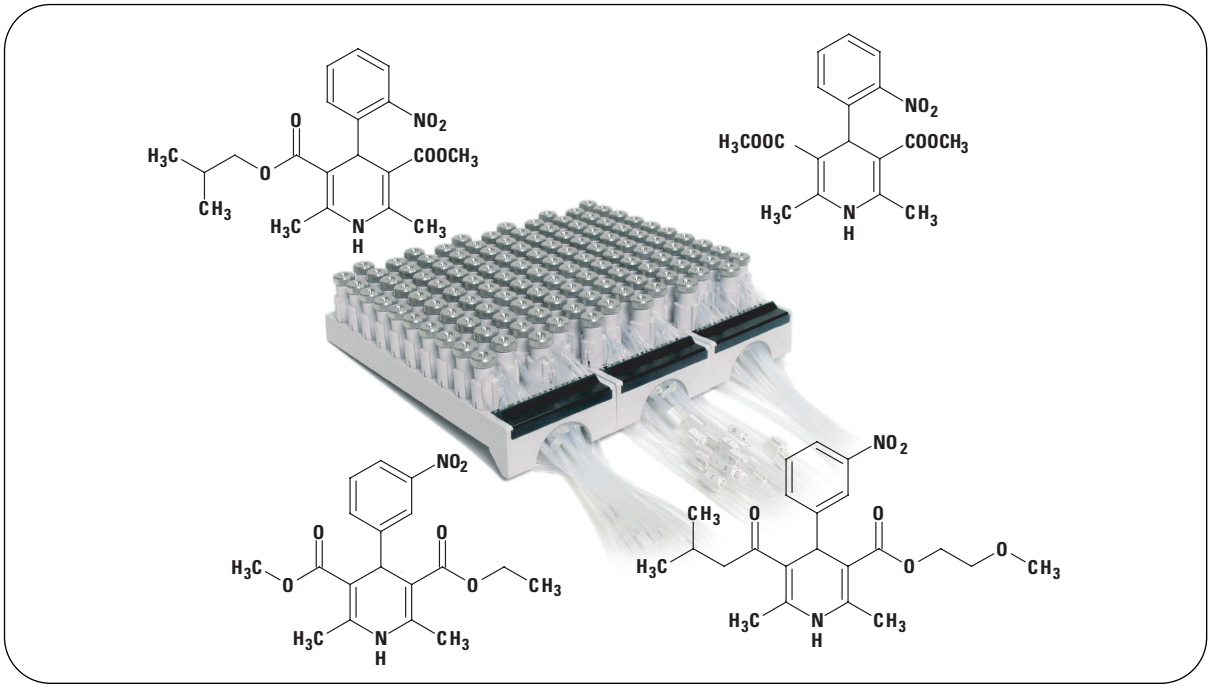

Abstract

In this Application Note the mass-based purification of low gram amounts of compounds is demonstrated using the Agilent 1100 Series purification platform. Starting the experiments on 21.2-m i.d. columns two application examples were scaled up to a 50-mm i.d. column operated at 100 mL/min. Fractions with purities of 85 and 95 % were obtained by mass-based fraction collection even though the column overload did not allow the UV-trace to show separated peaks.

Agilent Technologies

Introduction

Combinatorial compound libraries for drug discovery are routinely purified by automated preparative LCMS-systems. Samples of 30 to 50 mg compound mixture are separated on 20 mm diameter columns at flow rates of 20 to 35 mL/min using mass-based fraction collection. Until now purification of several grams of starting material needed for the synthesis of compound libraries had to rely on classical purification methods like crystallization or manual flash chromatography on silica gel. In this Application Note we demonstrate the operation of the Agilent 1100 Series purification system[1,2] in mass-based fraction collection mode at flow rates of 100 mL/min for the purification of up to 4 grams of a crude product in a single run on 50 mm diameter columns.

Equipment

- The experiments were performed on an Agilent 1100 Series purification system containing the following modules:
- 2 Agilent 1100 Series preparative pumps
- Agilent 1100 Series dual-loop autosampler PS (10000-µL loop)
- Agilent 1100 Series column organizer
- Agilent 1100 Series multiwavelength detector (flow cell: 0.06-mm path length)
- Agilent 1100 Series fraction collector AS (with 0.8-mm i.d. capillaries and semi-prep needle, funnel tray)
- Agilent 1100 Series MSD
- Agilent 1100 Series isocratic pump (make-up pump)
- Active splitter
- Agilent 1100 Series UIB

The system was controlled using the Agilent ChemStation (rev. B.01.01).

Results and discussion

Columns and compounds

An Agilent Prep-C18 column (21.2 x 50 mm, 5 µm) was used for testing the column overload conditions of the large-scale experiments. The experiments were then performed on a 50 x 200 mm column packed with Agilent Prep-C18 material with 10 µm particle size[3]. Two test mixtures were used as samples, test mix A containing nifedipin, nimodipin and nisoldipin (figure 1a), test mix B containing nitrendipin and nimodipin (figure 1b). As a performance evaluation measure nimodipin was collected from test mix A and nitrendipin

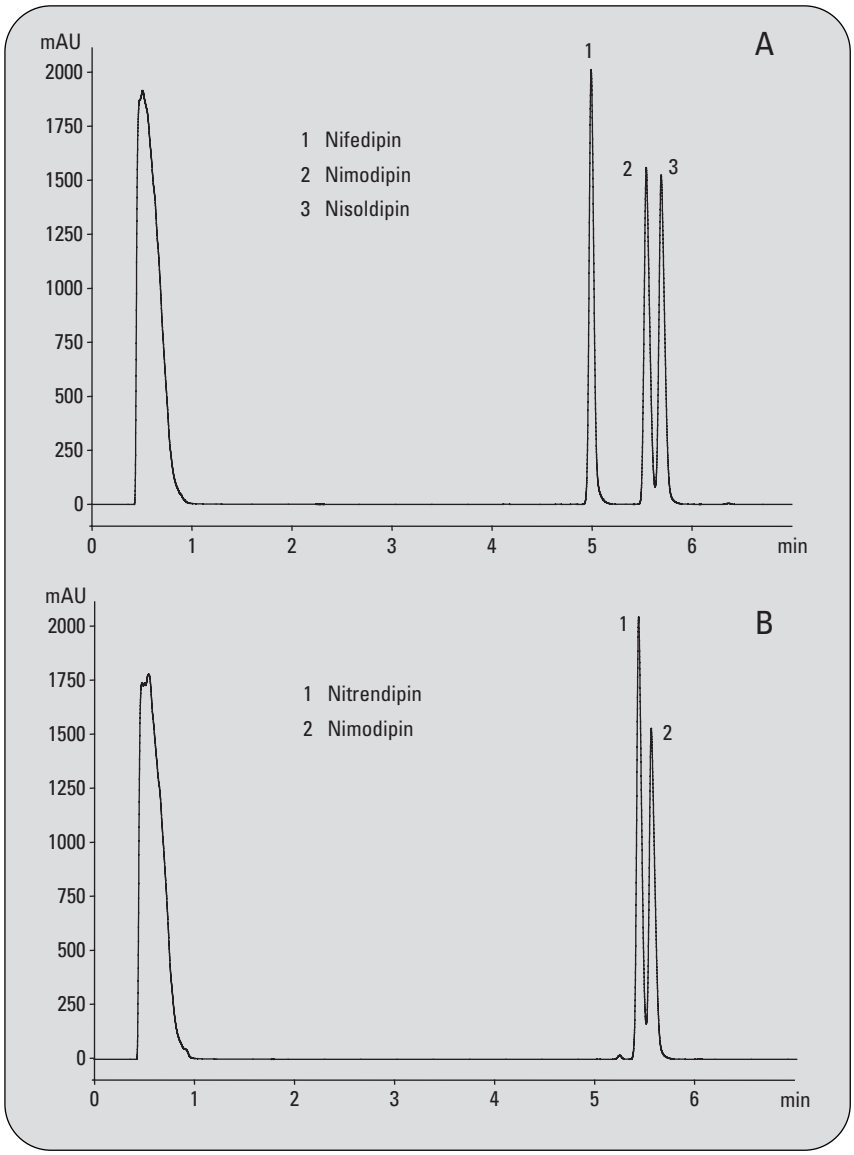

Figure 1
A) Test mix A (10 mg each in 500 µL DMSO).
B) Test mix B (10 mg each in 500 µL DMSO).

Column:	Agilent Prep-C18
	21.2 x 50 mm, 5 µm
Mobile phases:	Water = A
	Acetonitrile = B
Gradient:	at 0 min 10 % B
	At 2 min 10 % B
	At 6 min 90 % B
	At 7 min 90 % B
Stop time:	7 min
Post time:	3 min
Flow:	25 mL/min
Injection:	450 µL
Column temp.:	ambient
UV detector:	DAD 220 nm/4 (ref. off)
Polarity:	Positive
Mode:	Scan (200 – 700)
Fragmentor:	70 V
Drying gas flow:	13 L/min
Nebulizer pr.:	55 psig
Drying gas temp:	350 °C
Make-up solv.:	Methanol + 0.1 % HCOOH
Split ratio:	1:9500
FC threshold:	500000 counts

was collected from test mix B and the resulting fractions were analyzed for purity. For method see figure 2.

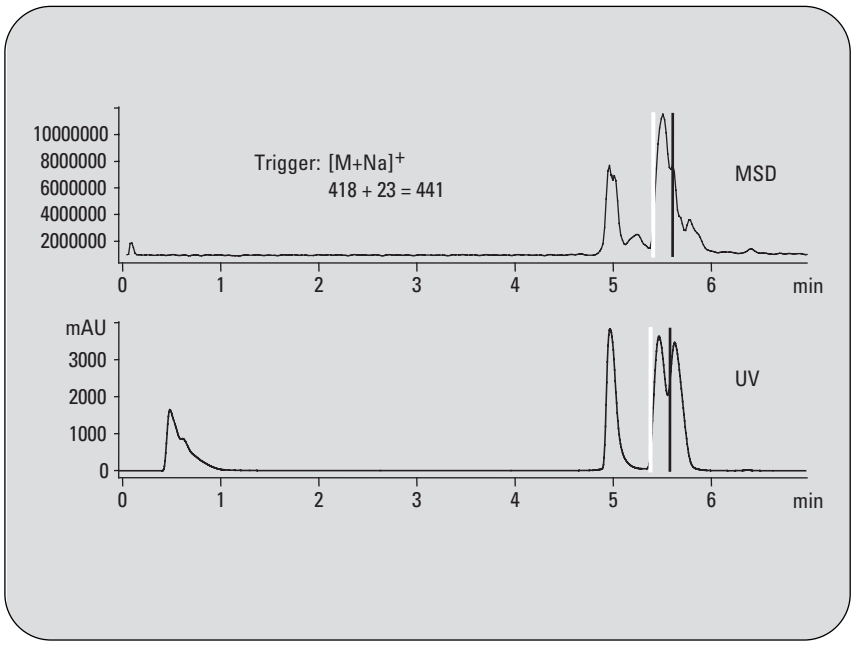

Figure 2
Result of mass-based fraction collection.

Testing the overload conditions on 20 mm diameter columns

Test mix A (50 mg of each compound in 450 µL of DMSO) was injected on the 21.2 x 50 mm Agilent Prep-C18 column. Nimodipin was collected based on the trigger mass of 441 because the compound showed a strong sodium adduct (418 plus the expected adduct $[M+Na]^+$ results in a trigger mass of 441) as shown in figure 2.

Re-analysis of the collected fraction presented a purity (based on area percent in the UV signal) of 91.3 % for nimodipin as shown in figure 3.

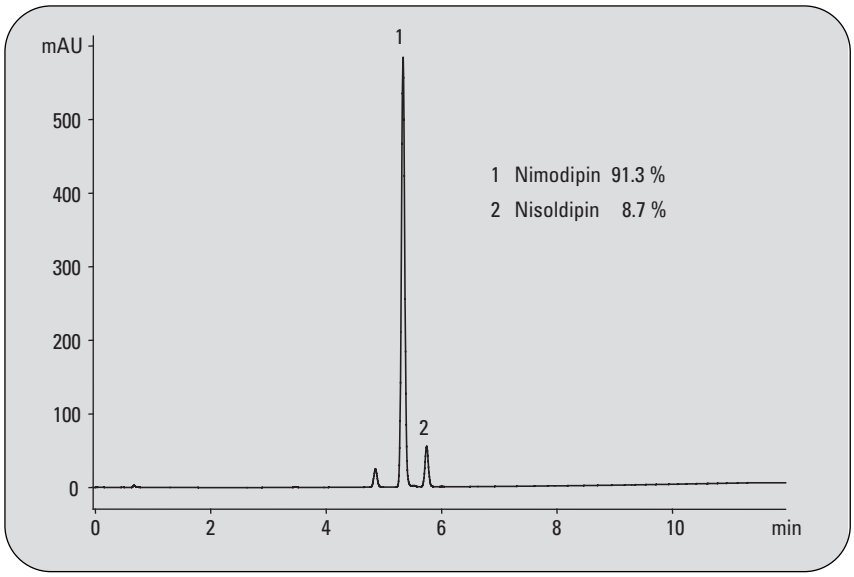

Figure 3
Re-analysis of the collected fraction.

The purification experiment was repeated with test mix B. Figure 4a shows the result of the purification of nitrendipin and nimodipin (90 mg each in 750 µL DMSO) based on a trigger mass of 383 (360 plus the expected adduct $[M+Na]^+$ results in a trigger mass of 383) and the re-analysis of the collected fraction (figure 4b). The purity of nitrendipin was 85.9 % (based on area percent in the UV signal). See figure 2 for purification method.

System configuration for mass-based fraction collection at 100 mL/min

To achieve the high flow rate of 100 mL/min 0.7-mm i.d. capillaries were used to connect the preparative pumps to the dual-loop autosampler and the autosampler to the column. The 0.8-mm i.d. capillary shipped with the 0.06 mm pathlength preparative flow cell was used to connect the column to the UV detector. A 10-mL capillary was installed between the active splitter and the fraction collector because a time delay of about five seconds between MSD and fraction collector is required. A 10-mL sample loop was installed in the dual-loop autosampler for the injection of larger sample volumes. The sample was injected in three portions because the injection of more than 10 mL of DMSO leads to significant peak broadening. After transferring the first portion of the sample into the sample loop the injection valve was switched to mainpass to transfer the sample portion onto the column and wash away the DMSO. After the last portion was transferred to the column a *start pulse* was transmitted to trigger the run. This was achieved using the injector program shown in table 1.

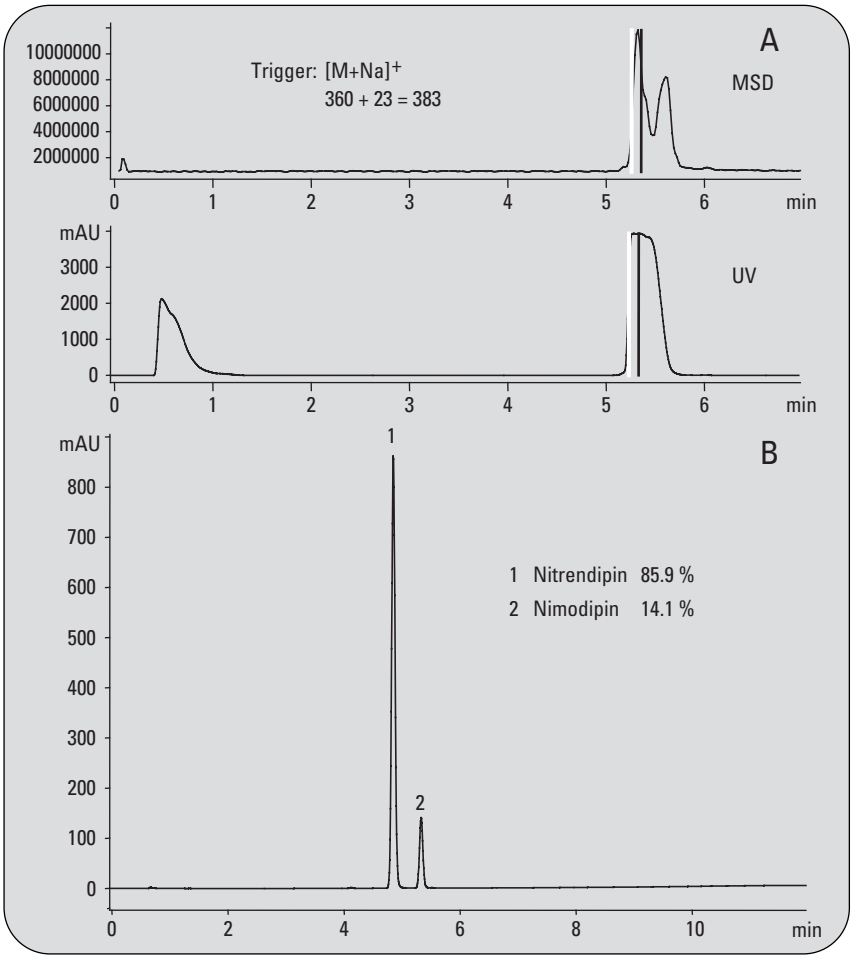

Figure 4
A) Result of mass-based fraction collection. B) Re-analysis of collected fraction.

#	Command
1	VALVE bypass
2	DRAW 50.0 µL from air, def. speed
3	DRAW 4000.0 µL from P1-C-01, def. speed, def. offset
4	NEEDLE wash in flush port, 10.0 sec
5	EJECT max. amount into seat, def. speed
6	VALVE mainpass
7	WAIT 0.10 min
8	VALVE bypass
9	DRAW 50.0 µL from air, def. speed
10	DRAW 4000.0 µL from P1-C-02, def. speed, def. offset
11	NEEDLE wash in flush port, 10.0 sec
12	EJECT max. amount into seat, def. speed
13	VALVE mainpass
14	WAIT 0.10 min
15	VALVE bypass
16	DRAW 50.0 µL from air, def. speed
17	DRAW 4000.0 µL from P1-C-03, def. speed, def. offset
18	NEEDLE wash in flush port, 10.0 sec
19	EJECT max. amount into seat, def. speed
20	VALVE mainpass + start pulse
21	DRAW 5000.0 µL from flush, def. speed
22	EJECT max. amount into seat, def. speed

Table 1
Injection of 12000 µL of sample in 3 portions of 4000 µL.

Column:	50 x 200 mm, Agilent Prep-C18, 10 µm
Mobile phases:	Water = A Acetonitrile = B
Gradient:	at 0 min 10 % B At 5.5 min 10 % B At 25 min 90 % B At 30.5 min 90 % B
Stop time:	30.5 min
Post time:	3 min
Flow:	100 mL/min
Injection:	12000 µL
Column temp.:	ambient
UV detector:	DAD 220 nm/4 (ref. off)
Polarity:	Positive
Mode:	Scan (200 – 700)
Fragmentor:	70 V
Drying gas flow:	13 L/min
Nebulizer pr.:	55 psig
Drying gas temp:	350 °C
Make-up solv.:	Methanol + 0.1 % HCOOH
Split ratio:	1:38000
FC threshold:	500000 counts

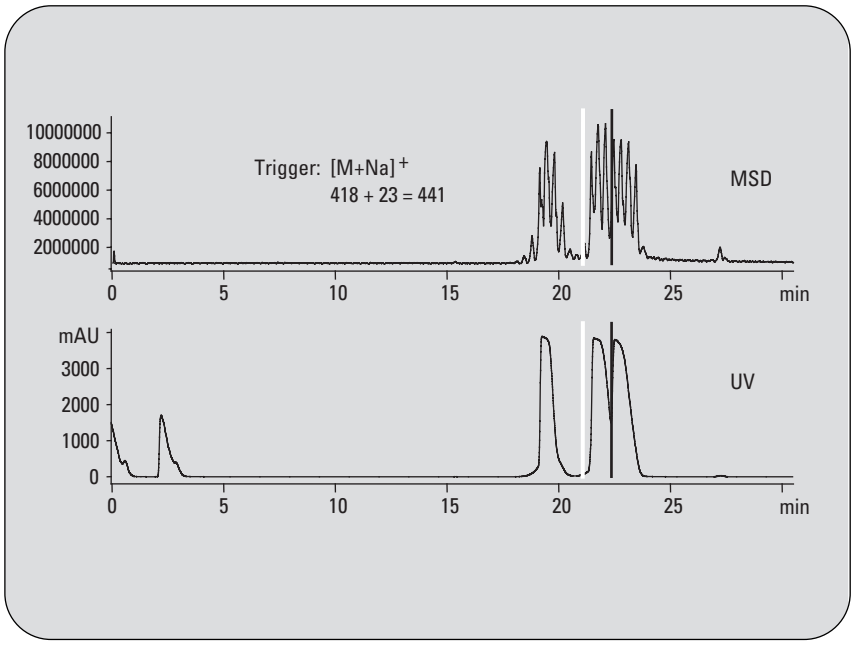

Figure 5
Result of mass-based fraction collection.

The analytical scale fraction collector was modified with the 0.8-mm i.d. preparative tubing kit (G1364-68711) and with the high flow needle (G1364-87202) and was equipped with the 40-funnel tray (G1364-84512) for the collection of high volumes.

Purification of gram amounts

Test mix A (1333 mg of each compound in 12000 µL of DMSO) was injected onto the 50 x 200 mm column packed with Agilent Prep-C18 material for the purification of approximately 4 grams. Nimodipin was collected based on the trigger mass of 441 because the compound showed a strong sodium adduct (418 plus the expected adduct $[M+Na]^+$ results in a trigger mass of 441) as shown in figure 5.

Re-analysis of the collected fraction presented a purity (based on area percent in the UV signal) of 96.2 % for nimodipin as shown in figure 6.

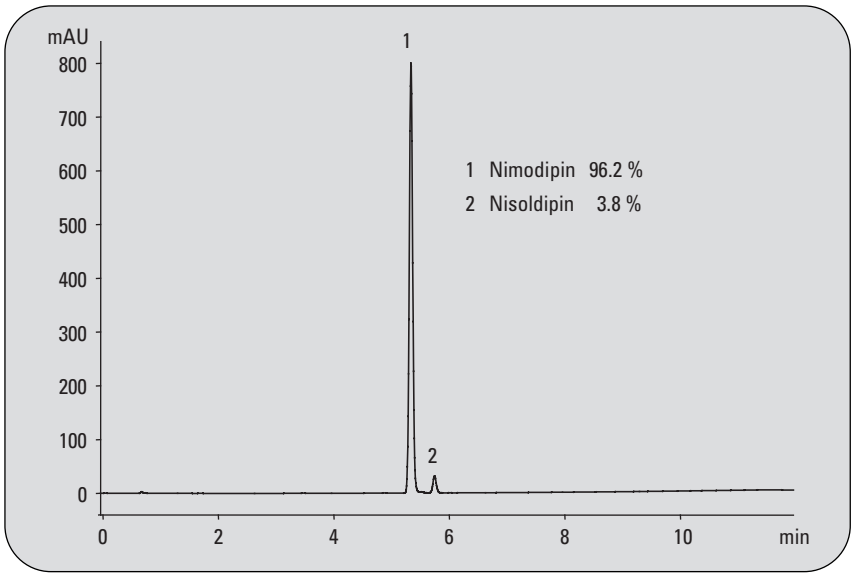

Figure 6
Re-analysis of the collected fraction.

The purification experiment was repeated with test mix B. Figure 7a shows the result of the purification of nitrendipin and nimodipin (1800 mg each in 14700 µL DMSO) based on a trigger mass of 383 (360 plus the expected adduct $[M+Na]^+$ results in a trigger mass of 383) and the re-analysis of the collected fraction (figure 7b). The purity of nitrendipin was 91.4 % (based on area percent in the UV signal). See figure 5 for purification method.

Conclusion

In this Application Note the mass-based purification of low gram amounts of compounds on a 50-mm i.d. column operated at 100 mL/min was demonstrated. Compounds could be isolated in high purity even for non-baseline separated peaks with some minor system modifications. The UV signals could no longer be separated even using a preparative flow cell with a very short pathlength. The fraction collection and re-analysis results are summarized in table 2.

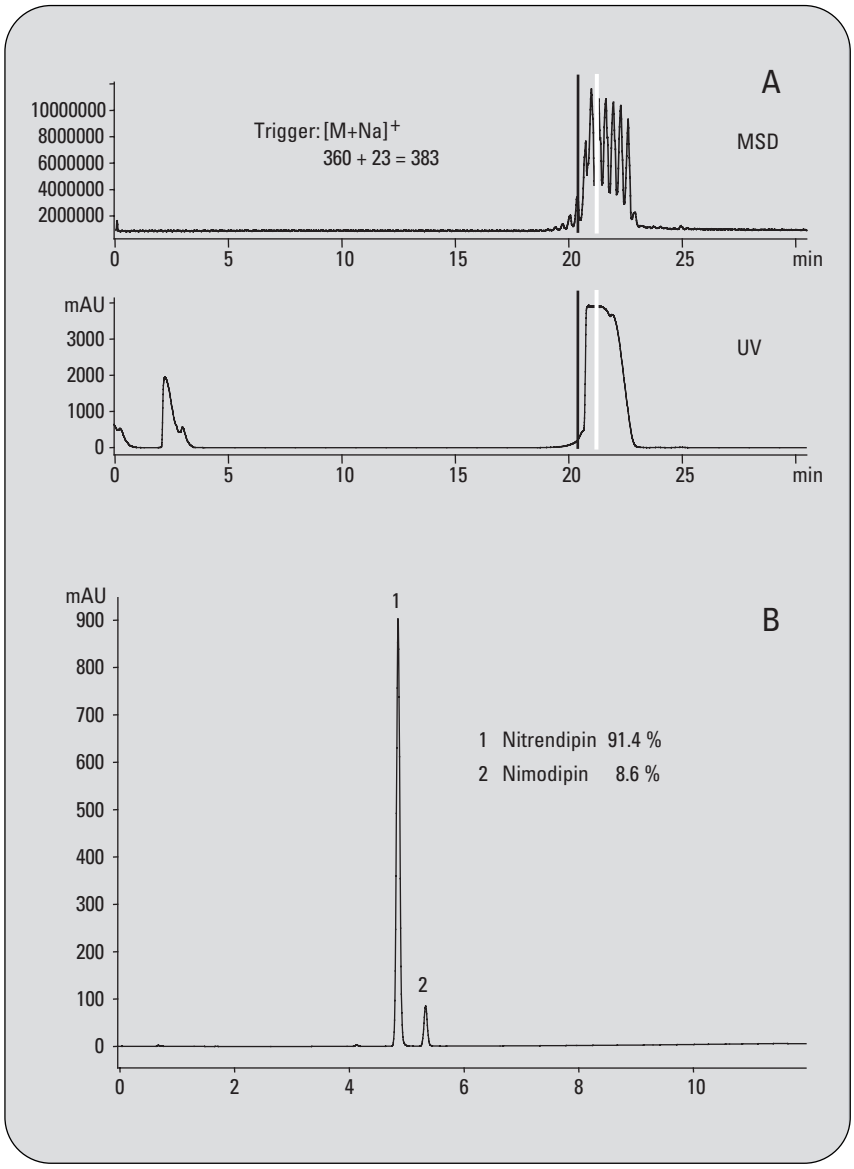

Figure 7
A) Result of mass-based fraction collection.
B) Re-analysis of collected fraction.

Column	Flow rate [mL/min]	Target compound	Amount per compound [mg]	Injection volume [µL]	Split ratio	MS threshold [counts]	Fraction purity [area%]
21.2 x 50 mm	25	Nimodipin	3 x 50	450	1:9500	500000	91.3
21.2 x 50 mm	25	Nitrendipin	2 x 90	750	1:9500	500000	85.9
50 x 200 mm	100	Nimodipin	3 x 1333	12000	1:38000	500000	96.2
50 x 200 mm	100	Nitrendipin	2 x 1800	14700	1:38000	500000	91.4

Table 2
Summary of purification results.

References

1.
"Principles in preparative HPLC" *Agilent Technologies primer publication number 5989-0652EN*, **2004.**

2.
"Agilent 1100 Series purification system" *Agilent Technologies Brochure, publication number 5989-1255EN*, **2004**.

3.
Column hardware: Merck packing stand NW50, by courtesy of U.Rosentreter, BayerHealthcare AG.

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www.agilent.com/chem/purification

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