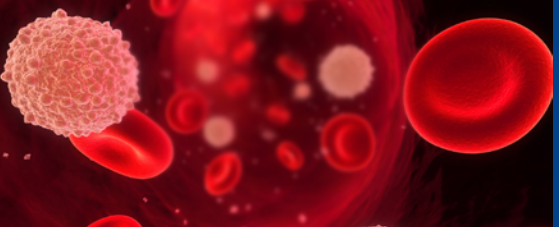

Amlodipine Besylate and Related Substances – USP

MACHEREY-NAGEL application department · Dr. H. R. Wollseifen, T. Kretschmer, R. Nuessgen

Application benefits

- HPLC method with faster separations within allowable adjustments.
- Shorter runtimes
- Lower solvent consumption
- Optimized system suitability

MN products

REF 720120.46
EC HPLC column (analytical), NUCLEOSIL® 100-5 C18, 5 µm, 150x4.6 mm

REF 763156.46
EC HPLC column (analytical), NUCLEOSHELL® RP 18, 5 µm, 150x4.6 mm

REF 702107
Screw closure, N 9, PP, yellow, center hole, Silicone white/PTFE red, 1.0 mm

REF 702079
Screw neck vial, N 9, 11.6x32.0 mm, 1.5 mL, label, flat bottom, amber, silanized

MN application numbers

HPLC: 129500
HPLC: 129510

Keywords

Amlodipine Besylate, USP, NUCLEOSHELL® RP18, L1, United States Pharmacopeia

Introduction

The USP monograph describes the separation of Amlodipine Besylate from impurities. This work demonstrates the use of superficially porous HPLC phases and shows their benefits.The method optimization was performed to achieve shorter run times and system suitability results within allowable adjustments.

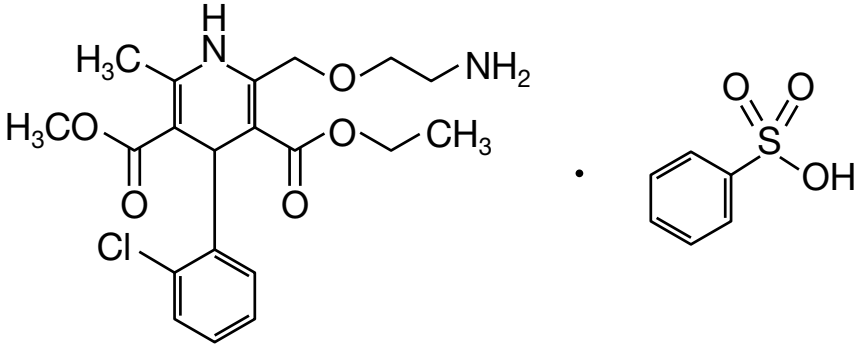

Figure 1: Structure of Amlodipine Besylate.

USP Monograph: Amlodipine Besylate Method Details

Method Parameter	Description
System suitability solution	Dissolve about 5 mg of Amlodipine Besylate in 5 mL of hydrogen peroxide, and heat at 70 °C for 45 minutes.
Standard preparation	Dissolve USP Amlodipine Besylate RS in mobile phase to obtain a concentration of 0.003 mg/mL
Test solution	Dissolve 50 mg of Amlodipine Besylate in a 50 mL volumetric flask and dilute to volume with mobile phase.
Column size	150 x 3.9 mm
Stationary phase	Base-deactivated packing L1, 5 µm
Mobile phase	pH 3.0 Buffer: Dissolve 7.0 of triethylamine in 800 mL of water. Adjust with phosphoric acid to a pH of 3.0 ± 0.1, and dilute with water to 1 L. pH 3.0 Buffer, methanol and acetonitrile (50:35:15).
Flow rate	1.0 mL/min
Detection	237 nm
Injection	10 µL
Elution order	1. Impurity A 2. Amlodipine Besylate
Suitability requirements	
Resolution:	NLT 4.5 between Amlodipine and Impurity A.
Tailing factor:	NMT 2.0 for Impurity A and Amlodipine Besylate.
Realtive standard deviation:	NMT 5.0% for Impurity A and NMT 2.0% for Amlodipine Besylate.

* Amlodipine Besylate (USP -1029501) and Amlodipine Related Compound A (USP-1029512) were purchased from Labmix24 GmbH; Postal address: Industriestrasse 18A – 46499 Hamminkeln (Germany).

Table 1: USP Monograph: Amlodipine Besylate Method Details

Chromatographic methodology improvements

Figure 2: a

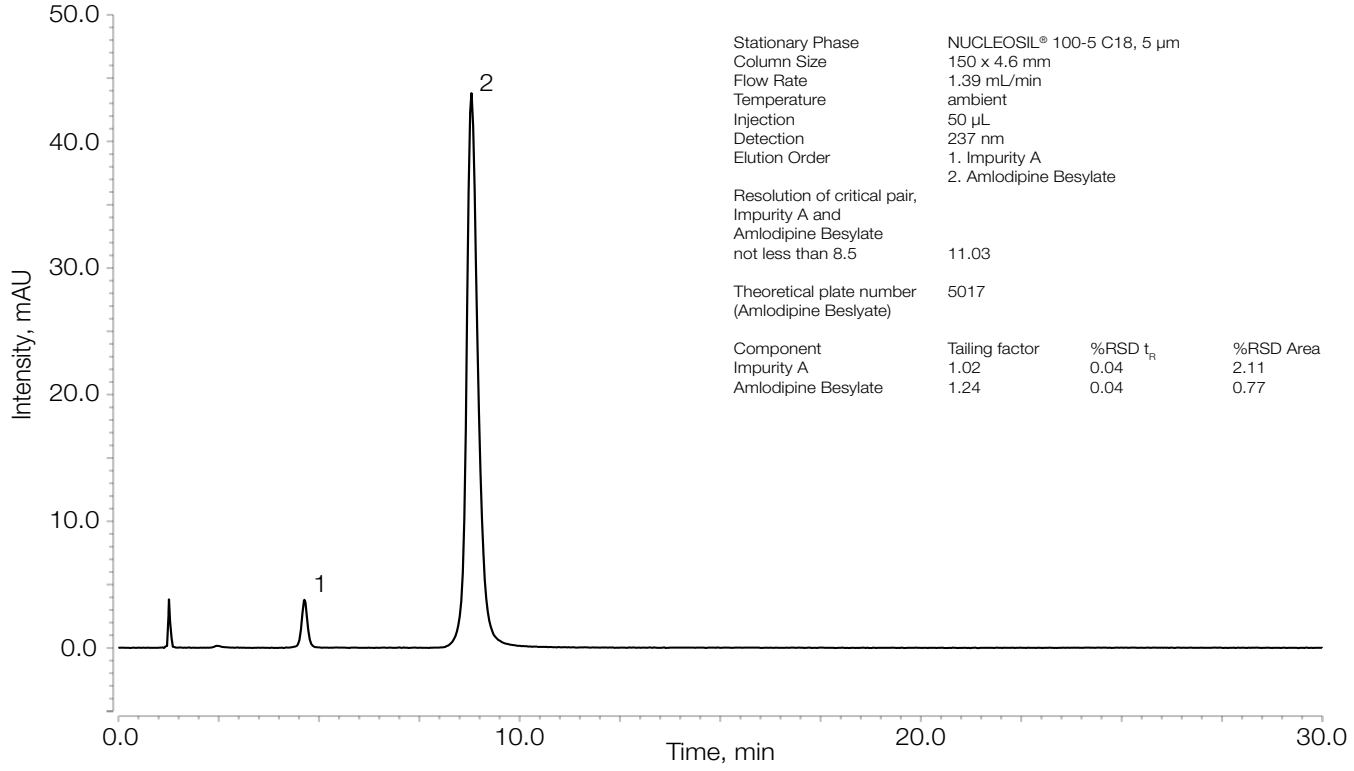

Figure 2: b

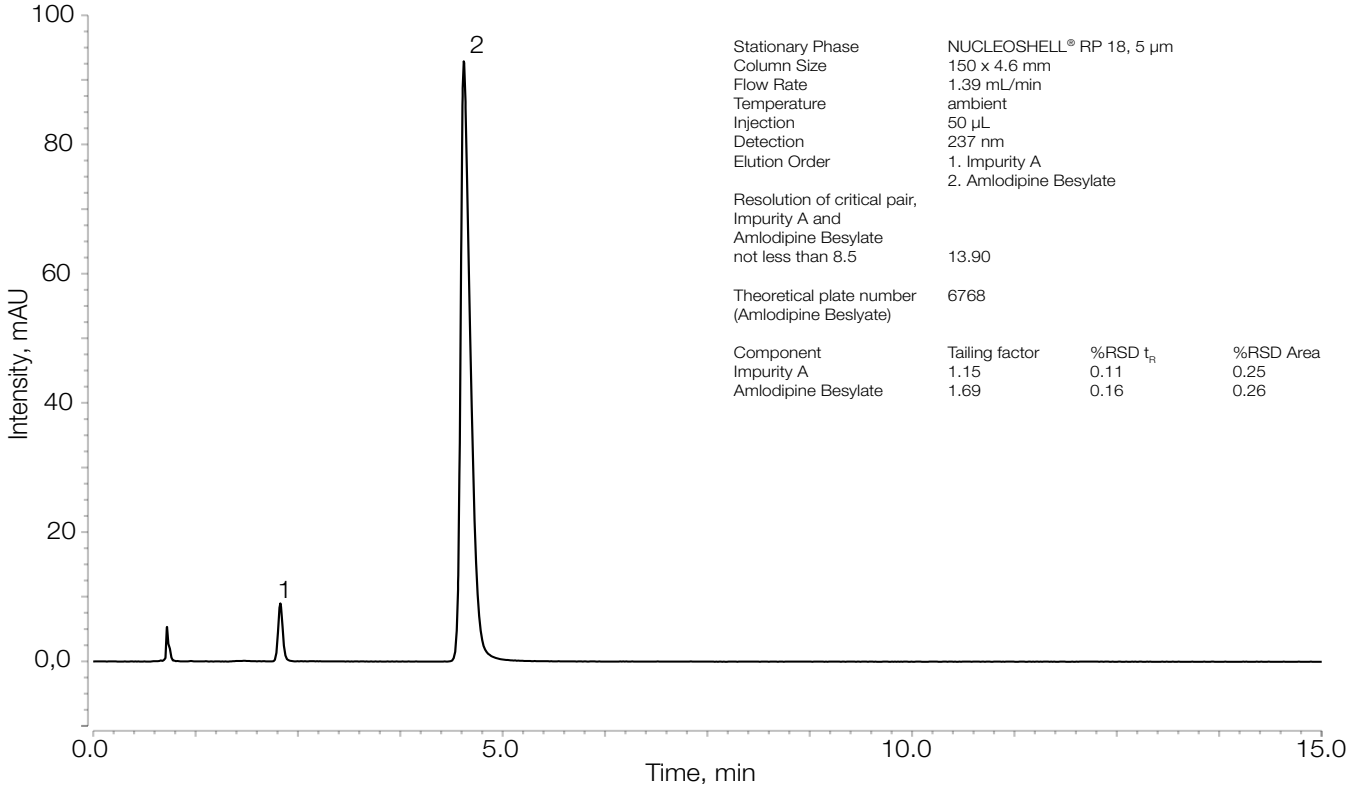

Figure 2: a: EC HPLC column (analytical), NUCLEOSIL® 100-5 C18, 5 µm, 150x4.6 mm, b: EC HPLC column (analytical), NUCLEOSHELL® RP 18, 5 µm, 150x4.6 mm.

Results

Method Parameter	Allowed Adjustments (isocratic elution)	Method 1 (figure 2: a)	Method 2 (figure 2: b)
Mobile phase pH	± 0.2 units	As specified	As specified
Concentration of salts in buffer	± 10%	As specified	As specified
Composition of the mobile phase	± 30% relative; cannot exceed ± 10% absolute change; cannot be reduced to zero	As specified	As specified
Stationary phase	No change of C18 allowed	NUCLEOSIL® 100-5 C18	NUCLEOSHELL® RP 18
Ratio column length/particle size	Column length to particle size diameter ratio can be adjusted between – 25% and + 50%	150 mm / 3 µm as specified	150 mm / 3 µm as specified
Column internal diameter	Can be adjusted so long as linear velocity is maintained	3.9 mm	3.9 mm
Flow rate	± 50% after adjustment due to a change in column dimensions	1.39 ml/min (± 0% after adjustments)	1.39 ml/min (± 0% after adjustments)
Column temperature	± 10 °C	ambient as specified	ambient as specified
Injection volume	Can be adjusted as much as needed; must be consistent with linearity, precision, and detection requirements	50 µL as specified	50 µL as specified
Detection [nm]	No change permitted	237 nm as specified	237 nm as specified
Retention time Amlodipine Besylate [min]		8.793 min	4.520 min (– 50.5%**)
Run time (3 x Amlodipine Besylate [min])		26.379 min (3 x 8.793 min)	13.56 min (- 50.5%**)
Theoretical plate number (Amlodipine Besylate)	Within –25% to 50%, relative to the prescribed column***	5017	6768 (+ 34.9%**)
Suitability requirements			
Resolution:	NLT 8.5 between Impurity A and Amlodipine Besylate	11.03	13.90
Tailing factor:	NMT 2.0 for Impurity A and Amlodipine Besylate	1.02 – 1.24 (see Figure 2a)	1.15 – 1.69 (see Figure 2b)
%RSD tR (Impurity A)	NMT 5.0% for Impurity A	0.04	0.11
%RSD Area (Impurity A)	NMT 5.0% for Impurity A	2.11	0.16
%RSD t_R (Amlodipine Besylate)	NMT 2.0% for Amlodipine Besylate	0.04	0.25
%RSD Area (Amlodipine Besylate)	NMT 2.0% for Amlodipine Besylate	0.77	0.26

* change in comparison to USP method ** change in comparison to method 1 *** column used in method 1.

Conclusion

The fully porous NUCLEOSIL® 100-5 C18, 5 µm, 150x4.6 mm HPLC column from MACHEREY NAGEL fulfills all requirements of the USP monograph (Amlodipine Besylate). By using superficially porous NUCLEOSHELL® analytical columns the runtime of the method can be reduced by up to 50.5% (with NUCLEOSHELL® RP 18, 5 µm, 150x4.6 mm) compared to fully porous NUCLEOSIL® silica gel, while keeping all method parameters well within the allowed adjustment range of the United States Pharmacopeia. The reduction in runtime leads to a lower solvent consumption optimizing the analysis of Amlodipine Besylate with regard to the guidelines of green chemistry. We were also able to improve the resolution (from 11.03 to 13.90) as well as the peak intensity with NUCLEOSHELL® columns compared to the original method with fully porous silica gel.

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