Amlodipine Besylate and Related Substances

From Particulate to Monolithic Column

The current impurity profiling method in USP36-NF31 for amlodipine besylate is based on TLC (test 1) and HPLC (test 2) where the liquid chromatograph is equipped with a 237 nm detector and a 150x3.9 mm column that contains packing L1. The flow rate is about 1.0 mL per minute.

Performance criteria to be met:
-For the purpose of identification, the relative retention times are about 0.2 for benzene sulfonate, 0.5 for amlodipine impurity A, and 1.0 for amlodipine. Amlodipine impurity A is 3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methylpyridine-3,5-dicarboxylate

The monograph was followed using a 150x4.6 mm Purospher® STAR RP-18 endcapped with 5 µm particle size, but not adjusting the flow-rate for the slightly larger inner diameter, page 10.
The monograph method was thereafter transferred using another column, see page 11.
No specific particle size is mentioned wherefore any type of column backbone can be used, and thus to comply with pharmacopoeial changes and perform only partial revalidation, the method can be changed by:

- Reduction of particle size to maximum 2.5 µm (50% since the first method uses a 5 µm particle in the written standard operating procedure - SOP) or use a monolithic column
- Shortening the column to a length of 45 mm (70%)
- Reduction of inner diameter if linear velocity is kept constant
- Reduction of injection volume as long as limit of detection (LOD) and linearity is OK.

A Chromolith® HighResolution RP-18 endcapped 100x4.6 mm column was chosen as the alternative for amlodipine besylate and its related impurities, page 11.

The alternative column met the performance criteria so why change to this alternative?

1. **The method will run faster** (Time-saving: 10 minutes per sample)
   (the column length is 33% shorter but total chromatographic analysis time is shortened by 50%).
2. **Higher chromatographic resolution**
   (Chromolith® HighResolution provide performance corresponding to sub-3 µm particle packed columns)
3. **Sensitivity enhancement**
   (Eluting peaks will have narrower width on a more efficient and shorter column and thus higher peak amplitude is attained)

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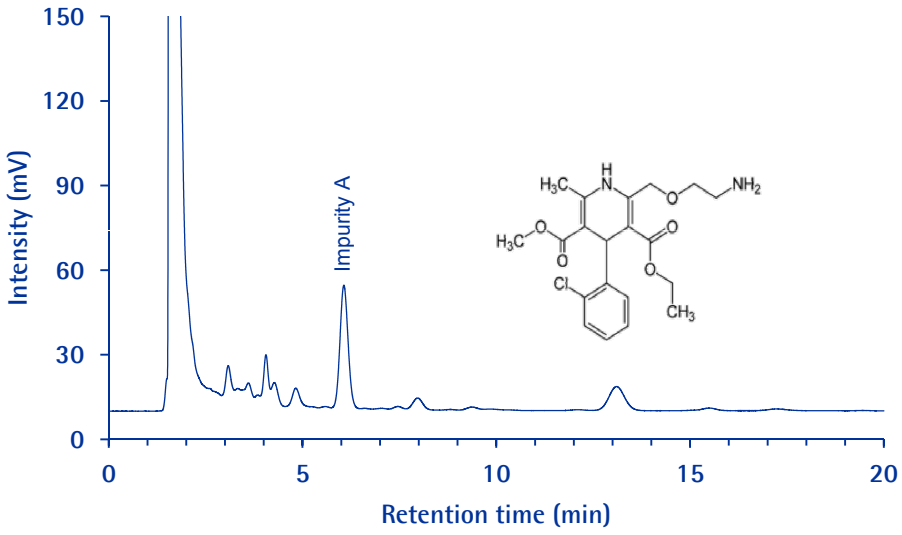
Purospher® STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher® STAR RP-18 endcapped (5µm) Hibar® RT 150x4.6 mm	1.51455.0001
Injection:	10 µL	
Detection:	UV 237 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Acetonitrile:Methanol:Buffer 15:35:50 (v/v)	
Buffer:	Add 7.0 ml of triethylamine in 1000 mL Milli-Q water, mix and adjust pH to 3.0 with orthophosphoric acid. Sonicate.	
Temperature:	25 °C	
Diluent	Mobile phase	
Standard:	Weigh 10 mg of Amlodipine Besylate in 20 mL volumetric flask. Add about 5.0 mL diluent and sonicate it. Dilute it up to the mark with the same. Further dilute 1.0 ml to 100 ml with diluent.	
Resolution Solution:	Weigh 5.0 mg of Amlodipine Besylate in 5.0 mL volumetric flask. Add 5.0 ml hydrogen peroxide. Heat it at 70°C for 45 min in water bath.	
Sample:	Crush 20 tablets. Weigh 50mg equivalent powder in 50 ml volumetric flask. Add about 30 ml diluent and sonicate it for 20 min. Dilute it up to the mark with the same.	



'does not need full revalidation'

Chromatographic Data :

No.	Compound	Retention Time (min)	RRT	Resolution	Theoretical plates
1	Amlodipine Impurity A	6.0	0.5	-	3966
2	Amlodipine Besylate	13.1	1.0	12.5	5026

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
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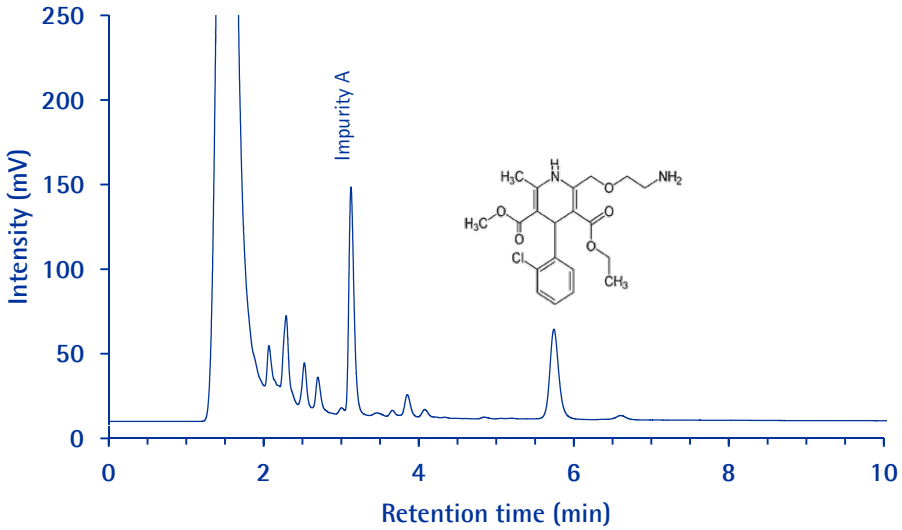
Amlodipine Besylate and Related Substances

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® High Resolution RP-18 endcapped 100x4.6mm	1.52022.0001
Injection:	10 µL	
Detection:	UV 237 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Acetonitrile:Methanol:Buffer (15:35:50) (v/v)	
Buffer:	Add 7.0 mL of triethylamine in 1000 mL Milli-Q water. Mix and adjust pH to 3.0 with orthophosphoric acid. Sonicate.	
Temperature:	25 °C	
Diluent	Mobile phase	
Standard:	Weigh 10 mg of Amlodipine Besylate in 20 mL volumetric flask. Add about 5.0 mL diluent and sonicate it. Dilute it up to the mark with the same. Further dilute 1.0 ml to 100 ml with diluent.	
Resolution Solution:	Weigh 5.0 mg of Amlodipine Besylate in 5.0 mL volumetric flask. Add 5.0 mL hydrogen peroxide. Heat it at 70°C for 45 min in water bath.	
Sample:	Crush 20 tablets. Weigh 50mg equivalent powder in 50 ml volumetric flask. Add about 30 mL diluent and sonicate it for 20 min. Dilute up to the mark with diluent.	
Pressure Drop:	65-68 Bar (943-986 psi)	



'does not need full revalidation'

Chromatographic Data :

No.	Compound	Retention Time (min)	RRT	Resolution	Theoretical plates
1	Amlodipine Impurity A	3.1	0.5	-	11163
2	Amlodipine Besylate	5.7	1.0	16.1	12438

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