APPLICATION BRIEF

Liquid Chromatography

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HPLC Analysis of Amlodipine Besylate in Accordance with the United States Pharmacopeia

Introduction

Amlodipine besylate belongs to a class of drugs known as dihydropyridine calcium channel blockers. It is primarily used to treat hypertension and angina as well as other conditions which arise as a result of coronary artery disease. As a calcium channel blocker, it prevents diffusion of calcium into cardiac muscle and the smooth muscle of the blood vessels. This causes vasodilation, lower heart contractility and therefore lower blood pressure. In addition, production of nitric oxide (a known vasodilator) increases.[1] Amlodipine is listed on the World Health Organization's (WHO) list of essential medicines as an antihypertensive medicine, meaning that it considered among the safest and most effective compounds needed to meet the requirements of a basic healthcare system.[2]

This application brief describes the use of a PerkinElmer Epic™ C18 column to analyze amlodipine besylate (Figure 1) in accordance with the official USP monograph.[3]

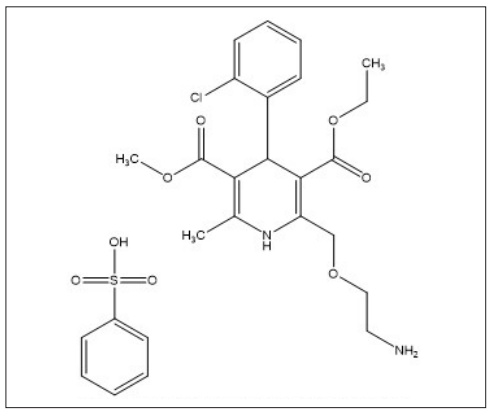

Figure 1: Chemical structure of amlodipine besylate.

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Experimental conditions

Method Parameters

All HPLC method parameters are shown in Table 1.

Table 1: HPLC Method Parameters.

Instrument	PerkinElmer LC 300 HPLC System with LC 300 Multi-wavelength UV/Vis (MWD) Detector
Column	Epic C18 150 x 4.6 mm, 5 µm (P/N: 135291-EC18)
Mobile Phase	**A:** Buffer (Triethylamine, Water, Phosphoric acid, pH 3.0) **B:** ACN **C:** MeOH 50% **A** 15% **B** 35% **C**
Flow Rate	1.0 mL/min
Temp	25°C
Wavelength	237 nm
Injection Volume	10 µL
Analyte	Amlodipine besylate

Solvents and Samples

All solvents were HPLC grade and samples were filtered using a 13 mm nylon filter, P/N: 02542880.

A standard solution of USP amlodipine besylate (0.05 mg/mL) was prepared using pre-mixed mobile phase as diluent.

The mobile phase buffer solution was prepared by dissolving 7.0 mL triethylamine in 800 mL of water. The pH was then adjusted to pH 3.0 by adding concentrated phosphoric acid. The solution was then diluted with water to a volume of 1,000 mL.

Results and Discussion

The USP monograph specifies that a column with L1 packing (150 x 3.9 mm) be used with a 1 mL/min flow rate. In this application, a 150 x 4.6 mm L1 column was used due to pressure limitations associated with the system. The USP <621> allowed changes allows the inner diameter to be changed so long as linear velocity remains constant. A flow rate of 1.4 mL/min is required to ensure this compliance. The USP <621> allows flow rate to be adjusted by ± 50% and so to prevent a further increase in backpressure the flowrate was reduced from 1.4 mL/min to 1.0 mL/min, a change well within USP allowances.

The use of ion pair reagents, such as triethylamine, can significantly increase the time required for equilibration. In this application, the column was equilibrated for approximately 80 column volumes to ensure proper equilibration and stable retention.

The analysis of amlodipine besylate has been carried out using an Epic C18 (150 x 4.6 mm, 5 µm) column (P/N: 135291-EC18), Figure 2. The USP monograph requires that the standard deviation of peak area and retention time for five replicate injections be no more than 2%. The Epic C18 column gave an efficient and repeatable separation with an efficiency of 57,747 plates per meter (N/m, calculated using the tangential method) and RSD values of 0.67% and 0.10% for peak area and retention time, respectively. The superior base deactivation and high-density bonding technology of the Epic C18 phase allows for excellent peak shape and the impressive performance displayed for this application.

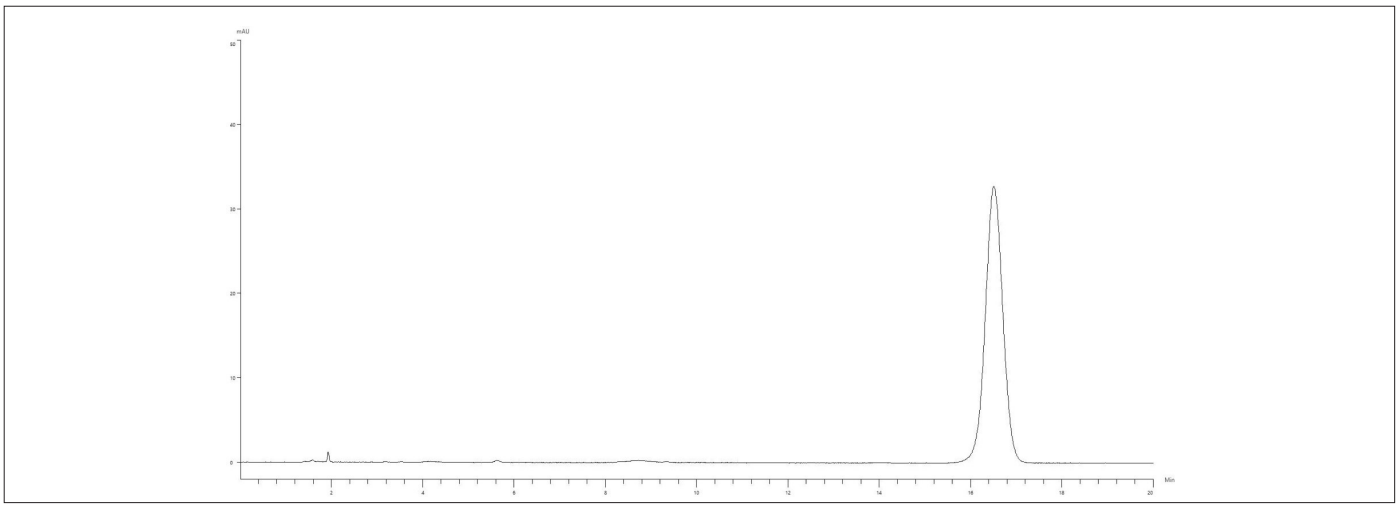

Figure 2: Analysis of amlodipine besylate using an Epic C18 column (150 x 4.6 mm, 5 µm).

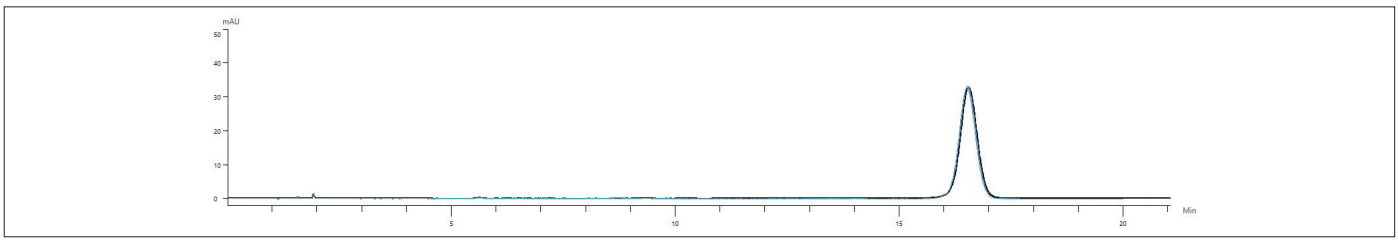

Figure 3: Overlay of 5 successive reps of amlodipine besylate.

Table 2: Results summary. RSD calculated from 5 successive injections.

Column	Peak Area RSD (%)		Retention Time RSD (%)		Column Efficiency (N/m)	Tailing Factor
	Epic	USP Requirement	Epic	USP Requirement		
Epic C18	0.67	≤ 2.0	0.10	≤ 2.0	57747	1.00

Conclusion

- The Epic C18 column (150 x 4.6 mm, 5 µm) provides repeatable and efficient separations for amlodipine besylate, giving an RSD well within USP requirements.
- The superior base deactivation and high-density bonding technology of Epic C18 results in excellent peak shape.
- If the method needn't be USP compliant, it could be altered to avoid the use of triethylamine which drastically increases equilibration times.

References

1. Drugbank database, https://www.drugbank.ca/drugs/DB00381, (accessed 15/07/20)
2. WHO list of essential medicines, https://apps.who.int/iris/bitstream/handle/10665/325771/WHO-MVP-EMP-IAU-2019.06-eng.pdf?ua=1, (accessed 15/07/20)
3. USP monograph amlodipine, https://online.uspnf.com/uspnf/document/1_GUID-9F661B2E-005A-4B58-B939-ACD8F118AA67_4_en-US?source=Quick%20Search&highlight=amlodipine, (accessed 15/07/20)

Consumables

Component	Description	Part Number
Column	Epic C18 150 x 4.6 mm, 5 µm	135291-EC18
HPLC Vials	2 mL Amber 9 mm Screw Top Vial with Write-on Patch and Fill Lines (100/pack)	N9307802
HPLC Vial Caps	9 mm Screw Top Blue (polypropylene) Cap with PTFE/Silicone pre-slit Septa (100/pack)	N9306203
Syringes	Syringe 1 mL BD Luer-Lok Disposable, Pack of 100	02542890
Syringe Filters	0.45 µm particle size syringe filter (100/pack)	02542880
PEEK Fittings	Finger-tight for 1/16" OD PEEK tubing	09920513
Stainless Steel Fittings	OptiTech Reusable Nut/Ferrule for UHPLC	N9306301

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