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DEVELOPMENT AND VALIDATION OF SENSITIVE METHOD TO SIMULTANEOUSLY DETERMINE AMLODIPINE AND BESYLATE ION BY HPLC

Annotation: *In the present analytical study, a rapid, simple, sensitive and accurate HPLC method has been developed and validated for simultaneous determination of amlodipine and besylate in pharmaceutical preparations.*

Keywords: *High Performance liquid chromatography, Method validation, Simultaneous analysis, Amlodipine, Besylate.*

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РАЗРАБОТКА И ПРОВЕРКА ЧУВСТВИТЕЛЬНОГО МЕТОДА ОДНОВРЕМЕННОГО ОПРЕДЕЛЕНИЯ АМЛОДИПИНА И ИОНА БЕЗИЛИТА С ПОМОЩЬЮ ВЭЖХ

Аннотация: *В настоящем аналитическом исследовании был разработан и утвержден быстрый, простой, чувствительный и точный метод ВЭЖХ для одновременного определения амлодипина и безилата в фармацевтических препаратах.*

Ключевые слова: *высокоэффективная жидкостная хроматография, валидация метода, одновременный анализ, амлодипин, безилат.*

1. Introduction:

Amlodipine besylate is a calcium channel blocker belonging to the second generation of dihydropyridine [1, c.1022]. It inhibits calcium ion influx across cell membranes selectively, with a greater effect on vascular smooth muscle cells than on cardiac muscle cells [2, c.129]. It used in the treatment of hypertension and chronic stable angina with a dose of 5 mg according to WHO [3, c.1].

Amlodipine besylate is white to off-white, crystalline powder with a molecular weight of 567.06. It is slightly soluble in water and freely soluble in methanol. It has absolute bioavailability (60-65%), half-life (35-50 h) and classified as BCS class I drug [4, c.143][5, c.11].

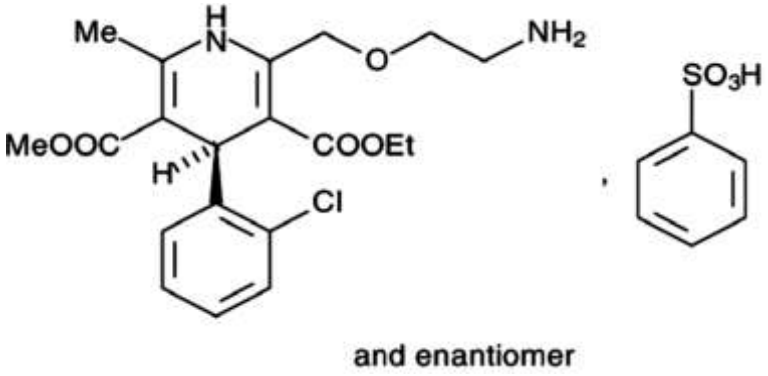

Figure.1. Structure of Amlodipine besylate

2. Materials and methods:

2.1. Materials and solvents: Standard amlodipine besylate powder (RAKSHIT/INDIA) was obtained from Human Pharma for pharmaceutical industry, amlodipine besylate 5 mg tablets were purchased from the local pharmacies, acetonitrile HPLC grade (Segma/Germany), ammonium acetate (Scharlau/Germany), phosphoric acid (Segma/Germany), methanol HPLC grade (Honey well/Germany), water for HPLC and distilled water.

2.2. Chromatographic conditions for HPLC: The analytical column was C 18, 150 x 4.6 mm I.D, 4μm particle size. Temperature of the column was maintained at 30 Ů and the data were analyzed by Agilent Technologies software. The mobile phase was a mixture of 30% acetonitrile and 70% 100 mM ammonium acetate pH 5. The mobile solvent was filtered under vacuum. The flow rate was 1 ml/minute with 5 µl injection while the detection wavelength was 237 nm for amlodipine and 226 nm for besylate [6, c.1].

2.3. Preparation of standard solution: A standard stock solution of amlodipine besylate was prepared by dissolving approximately 34.6 mg of standard powder in 25 mL of mobile phase to get a concentration of (1 mg/mL) [6, c.1].

2.4. System suitability: For the system suitability test four parameters namely relative standard deviation, tailing factor and theoretical plates and resolution were studied [7, c.1]. The standard solution of amlodipine besylate (1 mg/ml) was prepared and injected 5 times. Then the retention time and Area Under Curve (AUC) were recorded. The percent relative standard deviation (RSD %) was calculated for retention time.

2.5. Calibration Curve: the calibration curve solution contained 40% to 160% for amlodipine and besylate. Of each solution, 5 µl was injected in triplicate under the operating chromatographic conditions described above. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

2.6. Preparation of sample solution: 20 commercial tablets were weighed individually then grinded in a porcelain mortar. Equivalence sample of average

weight was taken and transferred into a 5 ml sample bottle. This was dissolved with 5 ml of mobile phase and sonicated in ultrasonic bath then filtered with syringe filter (0.45µm) and injected into the HPLC [8, c.204].

2.7. Method Validation:

The method was validated for parameters like precision, linearity, accuracy, specificity and robustness as per ICH guideline [7, c.1][9, c.1].

3. RESULTS AND DISCUSSION:

3.1. System suitability

The values obtained demonstrate the suitability of the system for this analysis as shown in table 1. Chromatogram of standard Amlodipine besylate is as shown in Fig 2.

Table 1: results of system suitability

Parameter	Amlodipine 237 nm	Besylate 226 nm
Theoretical plates/meter	7080	5690
Asymmetry factor	1.692	1.466
Tailing factor	1.921	1.694
RSD% (for retention time)	1.087	0.645
RSD% (for AUC)	0.720	0.629
Resolution	13.587	

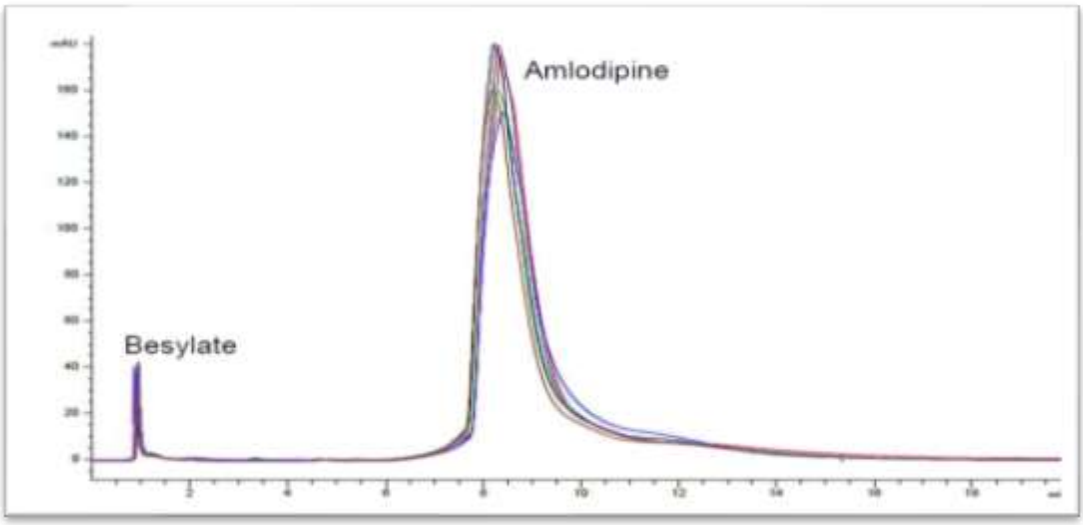

Figure.2. chromatogram of System suitability

3.2. Linearity

Both of amlodipine and besylate showed linearity in the concentration range of 40 - 160 % of the standard solution (as shown in figure 3). For HPLC method the linearity of calibration graphs and adherence of the system to Beer's law was validated by higher value of correlation coefficient (R^2 was 0.9997 for both amlodipine and besylate).

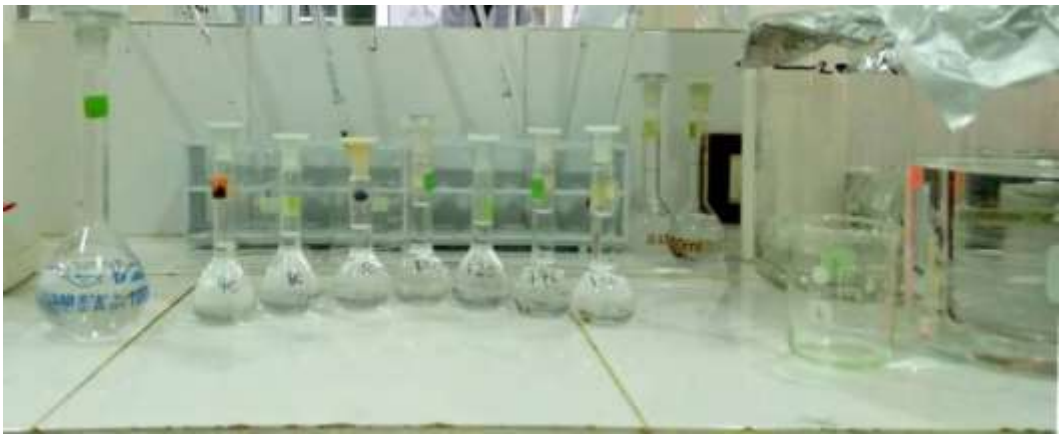

Figure.3: Preparation of standard calibration curve

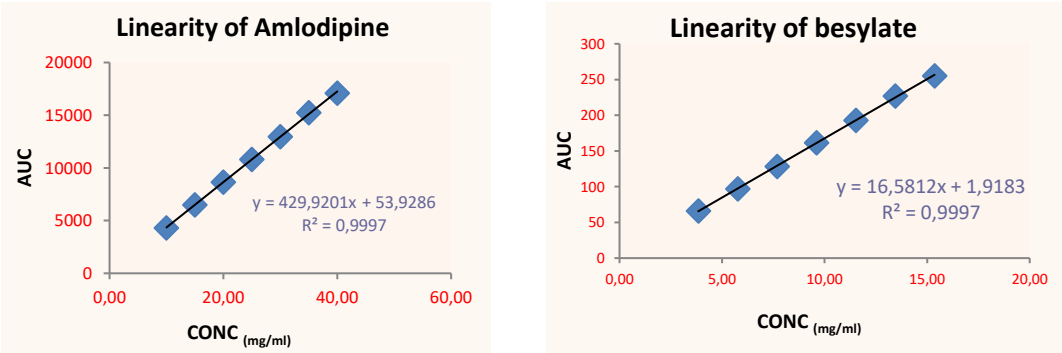

Figure.4: Linearity lines of amlodipine & besylate

3.3. Analysis of the marketed formulation

The peaks at t_R 9.342 (for amlodipine) and t_R 0.181 (for besylate) were observed in the chromatogram of the drug samples extracted from tablets. The drug content was found to be 101.12% and 98.62% for amlodipine and besylate respectively, and presented in table 2.

Table 2: results of dosage form analysis

Parameter	amlodipine	besylate
Label claim (mg)	5	1.92
Amount found (mg)	5.056	1.893
Drug content %	101.12%	98.62 %
RSD %	0.401	0.313

3.4. Precision

- **The intra-day precision (Repeatability)**

The precision evaluated at the repeatability of the method was studied by calculating the relative standard deviation (RSD %) for 6 determinations of 1 mg/mL performed on the same day and under the same experimental conditions. The RSD % value was 0.720 % and 0.629 % for amlodipine and besylate respectively. These results were summarized in Table 3.

- **The inter-day precision (Intermediate precision)**

The inter-day precision was assessed by analyzing 6 samples on 3 different days. The RSD % values obtained was 0.908 % and 0.131 % for amlodipine and besylate respectively. These results were summarized in Table 3.

Table 3: Intra-day Precision and Inter-day precision (n=6)

Precision type	Amount of Amlodipine %		Amount of Besylate %	
	Mean ± SD	RSD%	Mean ± SD	RSD%
Intra-day precision	103.340 ± 0.854	0.720	98.385 ± 0.121	0.629
Inter-day precision	103.082 ± 0.936	0.908	98.533 ± 0.129	0.131

3.5. Accuracy

Accuracy was evaluated by determining the analyte in solutions prepared according to the standard addition method and expressed in terms of percentage recovery amlodipine and besylate from the real samples. The mean recovery data was 101.169 % & 98.356 % for amlodipine and besylate respectively. Table 4 demonstrating that the method is accurate within the desired range.

Table 4: Recovery study results for amlodipine and besylate

Concentration %	Amlodipine	Besylate
80% (n=3)	102.36	97.54
100% (n=3)	101.12	98.62
120% (n=3)	100.03	98.91
Recovery %	101.169	98.356

3.6. Sensitivity (LOD, LOQ)

This method has high sensitivity by obtain low LOD and LOQ

Table 5: results of sensitivity

Parameter	Amlodipine	Besylate
LOD = 3:3*SD/Slope	0.754	0.967
LOQ = 10*SD/Slope	2.285	2.931

3.7. Robustness

The flow rate had been changed in our study ± 2%, then the five samples of the standard solution had been injected at each flow rate. The relative standard deviation of t_R and AUC was calculated.

Table 6: results of robustness

Flow rate (ml/min)	Amlodipine		Besylate	
	RSD% (t_R)	RSD% (AUC)	RSD% (t_R)	RSD% (AUC)
0.98	0.789	0.156	0.665	0.299
1	0.972	0.940	0.645	0.629
1.02	0.554	0.857	0.653	0.238

4. CONCLUSION

It can be concluded that the proposed newly developed method is a rapid, economical, accurate and precise method for the simultaneous determination of amlodipine and besylate in pharmaceutical preparations. This method can be used for routine analysis of the amlodipine.

5. **Acknowledgments:**

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