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A Relative Study on Brands of Amlodipine Besylate by Using UV - Spectrophotometric Method

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ABSTRACT

A novel, simple, fast, and reproducible UV-spectrophotometric method was developed using 40% sodium benzoate as a hydrotropic agent for the analysis of amlodipine besylate in bulk and pharmaceutical dosage forms. Various organic solvents such as methanol, chloroform, dimethyl formamide, and acetonitrile have been employed for the solubilization of poorly water-soluble drugs for spectrophotometric analysis. The drawbacks of organic solvents include their high cost, toxicity, and environmental pollution. Amlodipine exhibits an absorption maximum at 243 nm, while sodium benzoate does not show any absorbance above 225 nm, thus ensuring no interference with the drug. Beer's Law was found to be obeyed in the concentration range of 2-10 µg/ml. This method shows no interference from common pharmaceutical additives and diluents. Various parameters such as accuracy, precision, linearity, and range were evaluated as per ICH guidelines. The method is accurate, precise, and economical.

KEYWORDS: Amlodipine besylate, UV spectrophotometric method, hydrotropy, sodium benzoate.

INTRODUCTION:

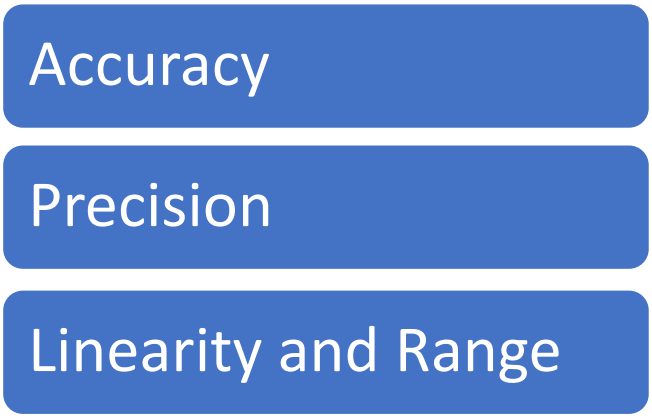
Amlodipine besylate is an important calcium channel blocker belonging to the dihydropyridine family. It is more selective for arterial vascular smooth muscle than for cardiac tissue and is approved for the treatment of hypertension and for variant and stable angina. Amlodipine besylate (AML) is (4R,S)-3-ethyl 5-methyl 2-(2-amino-ethoxy-methyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methylpyridine-3,5-dicarboxylate monobenzene sulpho-nate [1, 2].

The assay procedure listed in European Pharmacopoeia for amlodipine besylate describes the reversed phase high performance liquid chromatographic method [2] for determination of the drug in bulk and pharmaceutical formulations. The literature describes a number of methods for quantifying amlodipine, both in pure form and in dosage forms, among which chroma-tographic [3-8], electrochemical [9-13] and spectropho-tometric [14-19] methods are presented. In spectrophoto-metric methods for the quantitative determination of amlodipine besylate in the visible region of the spectrum various chemical reactions are used, among which the oxidation-reduction reactions and the reactions of complex formation with charge transfer are predominate. Also, few spectrophotometric methods for amlodipine besylate assay in dosage forms using sodium 1,2-naphthoquinone-4-sulphonate as a color reagent are described [20-21], but both methods are extractive and require more time and reagents.

Therefore, the aim of the presented work was to develop a highly sensitive, easy-to-use and economical method for quantitative determination of amlodipine besylate in the dosage forms based on the reaction with sodium 1,2-naphthoquinone-4-sulphonate, as well as validation of the developed methodology.

1. ANALYSIS OF METHOD VALIDATION PARAMETERS:

- METHOD VALIDATION PARAMETERS:



1. **ACCURACY**
    - ❖ Accuracy measures how close an analytical method's result is to the accepted reference value. It can be evaluated by comparing the recovery of analyte or spiking samples with known quantities.
2. **PRECISION**
    - ❖ Precision refers to the repeatability of results and is expressed as the percent relative standard deviation of multiple samples. It must be assessed at various levels as per ICH guidelines.
3. **LINEARITY & RANGE**
    - ❖ **LINEARITY**: The method's ability to produce results proportional to analyte concentration.
    - ❖ **RANGE**: The interval between the highest and lowest analyte levels that can be determined with accuracy, precision, and linearity.

2. ASSAY IN UV SPECTROSCOPY

- ❖ Assay in UV spectroscopy determines the concentration of a specific analyte.

TYPES OF ASSAYS

1. **Quantitative assay**: determines the exact concentration.
2. **Limit test assay**: Checks if the analyte concentration is within a set limit.
3. **Identification assay**: Confirms the presence or absence of a compound.

3. AIM AND OBJECTIVE

AIM:

- ❖ To conduct a study on amlodipine besylate using UV-Spectrophotometric method.
- ❖ To analyze three different brands of amlodipine besylate using hydrotropic solvents.
- ❖ To estimate the assay of amlodipine besylate.

OBJECTIVES:

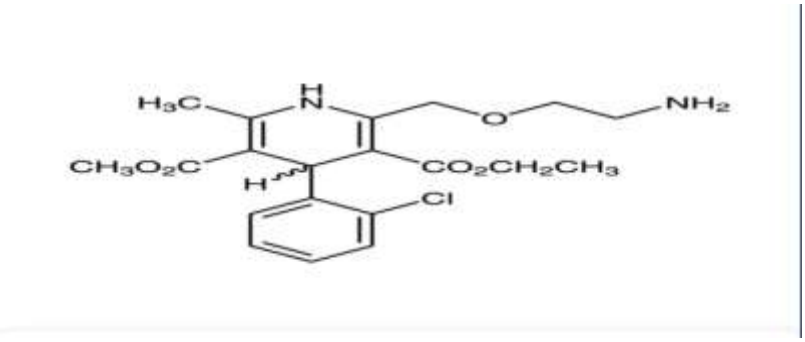
- ❖ Validate the UV-Spectrophotometric method for amlodipine besylate determination in pharmaceutical formulations.
- ❖ Compare the assay of three different brands of amlodipine besylate using UV-Spectrophotometry.
- ❖ Evaluate accuracy, precision, linearity, and range of the UV-Spectrophotometric method.

HYDROTROPY:

- ❖ Hydrotropes are water-soluble compounds that enhance the solubility of poorly water-soluble drugs.
- ❖ Hydrotropic solvents include:
    1. Sodium benzoate

2. Sodium citrate
3. Urea
4. Sodium salicylate
5. Sodium ascorbate
6. Sodium acetate
7. Caffeine

4. STRUCTURE OF AMLODIPINE BESYLATE



- Molecular weight of amlodipine besylate =408.879 g/mol.
- Molecular formula of amlodipine besylate = C26H31CIN2O8S.

5. MATERIALS REQUIRED:

S.No	Materials	Quantity
1.	Sample -1	5mg
2.	Sample -2	5mg
3.	Sample -3	5mg
4.	Sodium benzoate	40%
5.	Water	100ml

COLUMN : 1

6.INSTRUMENTATION:

S.No	Instruments
1.	UV- Spectrophotometer
2.	Mortar and pestle
3.	Digital balance

COLUMN : 2

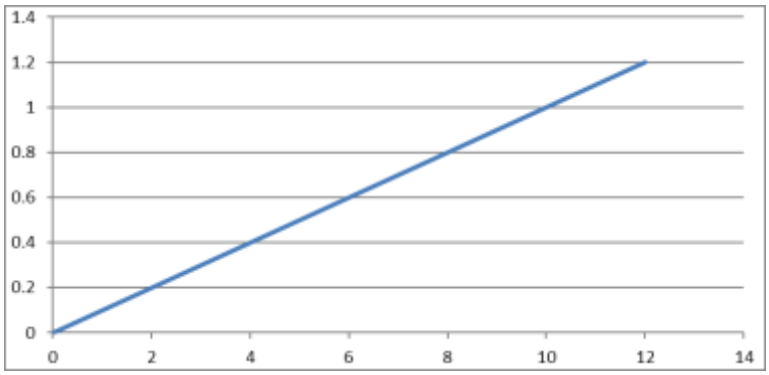
PREPARATION OF SODIUM BENZOATE STOCK SOLUTION:

- 40g of sodium benzoate was taken in 250ml beaker containing 70ml of distilled water and stirred well until it dissolved.Then transfer it to a 100ml standard flask and make up the volume upto the mark with distilled water.

PREPARATION OF LINEARITY CURVE FOR AMLODIPINE BESYLATE IN 40% SODIUM BENZOATE BY SOLVENT:

- 100 mg of amlodipine besylate was taken in 100ml of standard flask and add 70ml 40% sodium benzoate solution shake well until it dissolved.

- Then make up the volume upto the mark with 40%sodium benzoate solvent
- From the above solution pipette out 10ml and transfer it to a another 100ml standard flask and make up the volume upto the mark using 40% sodium benzoate solvent.
- From theabove solution series of aliquots portion of 2 to 10µg/ml.
- The different concentration are 2,4,6,8,10µg/ml.



Concentration µg /ml.

METHOD VALIDATION:

1. **Linearity and Range:** Create a calibration curve with the prepared concentrations.
2. **Accuracy:** Compare measured concentration to known values.
3. **Precision:** Test repeatability within and across

UV- SPECTRUM OF AMLODIPINE BESYLATE:

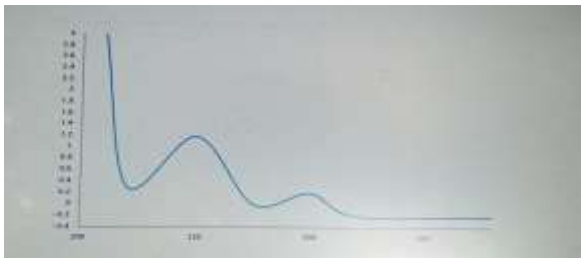


FIG NO :04

ACCURACY:

- Accuracy is the agreement between the data and true value or it refers to closeness of a single measurement to its true value.
- For analytical methods these are two possible ways of determining the accuracy. They are

1. Absolute method
2. Comparative method

1. **Absolute method**:

- An absolute method is a technique that directly measures the quantity of substance without the need for calibration or reference standards.

2. **Comparative method**:

- Comparative method is a technique that measures the quantity of a substance by comparing it to a known standard.

PRECISION:

- Precision is a measure of agreement among the values in a group of data, while accuracy is the agreement between the data and true value.

LINEARITY:

- It is the ability of attest to produce results that are directly proportional to the amount of a substance in a sample

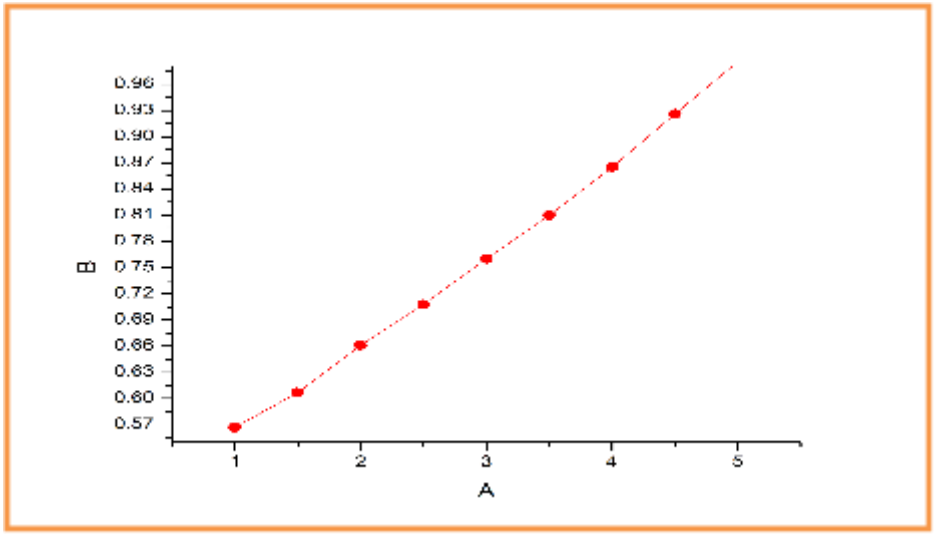


Fig no: 05 Beer's law plot

- The linearity of an analytical procedure is its ability to obtain test results which are proportional to the concentration of the analyte in the sample.
- The calibration curve of the amlodipine besylate was linear over the range of 2 -10µg /ml.

TABLE :1

S.NO	volume of AML Stock solution(ml)	Concentration of AML In Final solution(microgram/ml)	Absobance at 298 nm
1	1	5	0.567
2	1.5	7.5	0.607
3	2	10	0.661
4	2.5	12.5	0.708
5	3	15	0.76
6	3.5	17.5	0.81
7	4	20	0.865
8	4.5	22.5	0.926
9	5	25	0.986

OPTICAL CHARACTERISTICS OF AMLODIPINE BESYLATE FOR THE DEVELOPED METHOD :

TABLE :2

S.NO		PARAMETERS
1	Beers law limit	5-25µg/mL
2	Correlation coefficient	0.99863
3	Y=ax+C	Y=0. 0.02033333X+0.45877777
4	Molar absorptivity	$2.0333x10^4$.L/mol.cm.
5	Sandell's sensitivity	0.027890(µg/cm^2/0.001/ absorbance

7. RESULT OF ANALYSIS OF TABLETS:

TABLE :3

Brand name	Concentration (microgram/ml)	Absobance at 298 nm	% Label Claim	Active Content per tablet mg	Mean% label claim	Standard Deviation	Standard Error
Amlong	10	0.689	100	10	99.93	0.035	0.024748737
	15	0.675	99.86	14.98		0.035	0.024748737

Asomex	10	0.672	95.9	9.59	97.12	0.61	0.431335137
	15	0.688	98.34	14.75		0.61	0.431335137
Amlodac	10	0.687	99.4	9.94	99.7	0.15	0.106066017
	15	0.691	100	15		0.15	0.106066017

8.REPEATABILITY :

Repeatability expresses the precision under the same operating conditions over a short interval oftime. The precision of an analytical procedure is usually expressed as the standard deviation of a series of measurements. The reproducibility of the method was studied using three different concentrations of AML (10, 15 and 20µg/ml) which were prepared from stock solution. The absorbance was measured at 298nm against Sodium Benzoate as blank for three times and their mean values were calculated and the data isgivenintable.5. Theintra-dayandinter-day precision studiesof AML were carried out by estimating the corresponding responses three times on the same day and on three different days(1^{st},$2^{nd}$and 3^{rd} day) for three different concentrations of AML(10, 15 and 20µg/ml) and the results are reported in terms of relative standard deviation in table.5 and table.6.

Data For Repeatability;

TABLE : 4

Concentration (Microgram/Ml)	**Absobance At 298 Nm**	**Mean Value**	**Standard Deviation**	**Standard Error**	**Coefficient Of Variation**
	0.677				
10	0.679	0.680333333	0.003399346	0.001962614	0.499658943
	0.685				
	0.762				
15	0.765	0.680333333	0.002054805	0.001186342	0.268719006
	0.767				
	0.867				
20	0.869	0.680333333	0.00294392	0.001699673	0.338381642
	0.874				

Intra-day precision.

TABLE :5

No	**Concentration (microgram/ml)**	**Absorbance at 298 nm**			
		0 hr	1.5hr	3 hr	RSD %
1	10	0.672	0.666	0.662	0.6164414
2	15	0.757	0.735	0.725	1.80874494
3	20	0.865	0.863	0.851	0.7191639

INTRA-DAY PRECISION :

ABSORBANCE AT 298 nm

TABLE :6

NO	Concentration (microgram/ml)	1st Day	2nd Day	3rd Day	RSD %
1	10	0.669	0.665	0.665	0.282984205
2	15	0.758	0.73	0.727	1.890780279
3	20	0.862	0.863	0.856	0.359303312

9. ASSAY CALCULATION

BRAND :A

- **Weight of 20 tablets**: 3g (Average weight = 0.15g).
- **Weight to be taken**: 0.15g × 0.1 = 1.5g.
- **Absorbance**: Sample = 0.655, A1%1cm = 659nm.

S.No	Content	Absorbance (Nm)
1.	$A^{1\%}$1cm	659 nm
2.	Sample	0.655
3.	Blank	0

COLUMN : 3

- **Concentration of Amlodipine**: 0.09939gms.
- **Amount of Amlodipine**: 0.09939 × 0.15 / 1.5 = 0.0099939gms.
- **Percentage Purity**: (0.009939 × 100) / 0.010 = 99.39 %

BRAND : B

- **Weight of 20 tablets**: 3g (Average weight = 0.15g).
- **Weight to be taken**: 0.15g × 0.1 = 1.5g.
- **Absorbance**: Sample = 0.652, A1%1cm = 659 nm.

S.No	Content	Absorbance (Nm)
1.	$A^{1\%}$1cm	659nm
2.	Sample	0.652
3.	Blank	0

COLUMN : 4

- **Concentration of Amlodipine**: 0.09893gms.
- **Amount of Amlodipine**: 0.09893 × 0.15 / 1.5 = 0.009893gms.
- **Percentage Purity**: (0.009893 × 100) / 0.010 = 98.93 %.

BRAND : C

- **Weight of 20 tablets**: 3g (Average weight = 0.15g).
- **Weight to be taken**: 0.15g × 0.1 = 1.5g.
- **Absorbance**: Sample = 0.651, A1%1cm = 659 nm.

S.No	Content	Absorbance (Nm)
1	A 1%(1cm)	659 nm
2	Sample	0.651
3	Blank	0

COLUMN : 5

- **Concentration of Amlodipine**: 0.09878gms
- **Amount of Amlodipine**: 0.09878 × 0.15 / 1.5 = 0.009878gms.
- **Percentage Purity**: (0.009878 × 100) / 0.010 = 98.78 %.

10. RESULTS:

S.No	Various Brands	Absorbance (Nm)	% Purity
1	BRAND A	0.655nm	99.39%
2	BRAND B	0.652nm	98.93%
3	BRAND C	0.651nm	98.7%

COLUMN : 6

11. CONCLUSION:

- The assay of Amlodipine besylate in three different brands of tablets using UV Spectrophotometric method showed that all three brands met the acceptable purity standards.
- However, Brand -B, and Brand -C showed a slightly lower percentage purity value, when compared to Brand-A.

12. DISCUSSION:

- The percentage purity of Amlodipine in the three brands was found to be within the acceptable range of 90-110% Brand A showed higher percentage purity values, while Brand B and Brand C showed a slightly lower value.

13. SUMMARY:

- This study compares the assay and validation of Amlodipine in three marketed brands using UV spectroscopy. Sodium benzoate was used as a reference standard.
- The results showed variations in purity and potency among the brands. The study highlights the importance of quality control and validation of pharmaceutical products to ensure efficacy and safety.

Compliance with ethical standards:

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Disclosure of conflict of interest

There is no conflict of interest.

Mr N.Ramanadhan was responsible for the revision of the manuscript for important intellectual content. , JAYASHREE. S, JEEVA. V, JEEVARATHINAM. P, KARAN. V, KAVPRIYA. S contributed towards reviewing of the literature. All of us agreed on the final version of the manuscript.

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