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METHDOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF INDAPAMIDE AND AMLODIPINE BESYLATE BY RP-HPLC METHOD

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ABSTRACT

An isocratic Simultaneous estimation by RP-HPLC Method was developed and validated for the quantification of Indapamide and Amlodipine Besylate in tablet dosage form. Quantification was achieved by using the mobile phase (Phosphate buffer PH3: Acetonitrile: Methanol) in the ratio of 40:20:40. A C18 column contains octadecylsilane chemically bonded to porous silica particles was used as stationary phase. The flow rate was 1.0 ml/min. Measurements were made at a wavelength of 215nm. The average retention time for Indapamide and Amlodipine Besylate were found to be 2.07 min and 4.047. The proposed method was validated for selectivity, precision, linearity and accuracy. The assay methods were found to be linear from 9-21 µg/ml for Indapamide and 30-70 µg/ml for Amlodipine Besylate. All the validation parameters were found to be within the acceptable range. The developed method was successfully applied to estimate the amount of Indapamide and Amlodipine Besylate in tablet dosage form.

KEYWORDS

Indapamide (IND), Amlodipine Besylate (AB), RP-HPLC method, Hypersil 3V ODS and Acetonitrile (ACN).

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INTRODUCTION

Amlodipine

Amlodipine is a long-acting 1, 4-dihydropyridine calcium channel blocker (CCB). It acts primarily on vascular smooth muscle cells by stabilizing voltage- gated L-type calcium channels in their inactive dependent myocyte contraction and vasoconstriction. A second proposed mechanism for the drug's vasodilatory effects involves pH-dependent inhibition of calcium influx via inhibition of smooth muscle carbonic anhydrase.

Some studies have shown that amlodipine also exerts inhibitory effects on voltage gated N-type calcium channels[1]. N-type calcium channels located in the central nervous system may be involved in nociceptive signaling and pain sensation. Amlodipine is used to treat hypertension and chronic stable angina[2].

Mechanism of action

Amlodipine decreases arterial smooth muscle contractility and subsequent vasoconstriction by inhibiting the influx of calcium ions through L-type calcium channels. Calcium ions entering the cell through these channels bind to calmodulin. Calciumbound calmodulin then binds to and activates myosin light chain kinase (MLCK). Activated MLCK catalyzes the phosphorylation of the regulatory light chain subunit of myosin, a key step in muscle contraction. Signal amplification is achieved by calcium-induced calcium release from the sarcoplasmic reticulum through ryanodine receptors. Inhibition of the initial influx of calcium decreases the contractile activity of arterial smooth muscle cells and results in vasodilation. The vasodilatory effects of amlodipine result in an overall decrease in blood pressure. Amlodipine is a long-acting CCB that may be used to treat mild to moderate essential hypertension and exertion-related angina (chronic stable angina). Another possible mechanism is that amlodipine inhibits vascular smooth muscle carbonic anhydrase I activity causing cellular pH increases which may be involved in regulating intracelluar calcium influx through calcium channels[3]**.**

Medical uses

- Antihypertensive Agents
- Vasodilator Agents
- Calcium Channel Blockers
- Antianginals

INDAPAMIDE

A benzamide-sulfonamide-indole. It is called a thiazide-like diuretic but structure is different enough (lacking the thiazo-ring) so it is not clear that the mechanism is comparable.

Categories

Antihypertensive Agents, Diuretics.

Mechanism of action

Indapamide blocks the slow component of delayed rectifier potassium current (IKs) without altering the rapid component (IKr) or the inward rectifier current. Specifically it blocks or antagonizes the action the proteins KCNQ1 and KCNE1. Indapamide is also thought to stimulate the synthesis of the vasodilatory hypotensive prostaglandin PGE2 [4-5].

Pharmacodynamics

Indapamide is an antihypertensive and a diuretic. It contains both a polar sulfamoyl chlorobenzamide moiety and a lipid- soluble methylindoline moiety. Indapamide bears a structural similarity to the thiazide diuretics which are known to decrease vascular smooth muscle reactivity. However, it differs chemically from the thiazides in that it does not possess the thiazide ring system and contains only one sulfonamide group. Indapamide appears to cause vasodilation, probably by inhibiting the passage of calcium and other ions (sodium, potassium) across membranes. This same effect may cause hypocalcemia in susceptible individuals. Indapamide has also been shown to cause uterine myometrial relaxation in experimental animals. Overall, indapamide has an extra-renal antihypertensive action resulting in a decrease in vascular hyperreactivity and a reduction in total peripheral and arteriolar resistance[6].

MATERIALS AND METHOD

Instruments

The chromatographic technique performed on a Shimadzu LC20-AT Liquid chromatography with SPD-20A prominence UV-visible detector and Spinchrom software, reversed phase C18 column (HYPERSIL 5µ, 250 mm × 4.6 mm) as stationary phase, Electron corporation double beam UV-visible spectrophotometer (vision pro-software), Ultrasonic cleaner, Shimadzu analytical balance AY-220, Vacuum micro filtration unit with 0.45µ membrane filter was used in the study[7-9].

Materials

Pharmaceutically pure sample of Indapamide and Amlodipine Besylate were obtained as gift samples from Chandra lab, Prashanthinagar, Kukatpally, Hyderabad, India. The purity of the drug was evaluated by obtaining its melting point and ultraviolet (UV) and infrared (IR) spectra. No impurities were found. The drug was used without further purification.

HPLC-grade Acetonitrile and Methanol were obtained from standard reagents pvt ltd. KH_2PO_4 & NaH_2PO_4 (AR grade) were from Merck.

A tablet formulation of Indapamide and Amlodipine Besylate (1.5mg and 5mg label claims) was obtained from local market (Natrilam, Serdia Pharmaceutical Company, India).

Determination of Working Wavelength (λmax)

10 mg of the Indapamide standard drug is taken in a 10 ml volumetric flask and dissolved in methanol and volume made up to the mark, from this solution 0.1ml is transferred into a 10 ml volumetric flask and made up to the mark with the methanol to give a concentration of 10 µg/ml. The above prepared solution is scanned in uv between 200-400 nm using methanol as blank. The λmax was found to be 210nm (Figure no. 2.1).

10 mg of the Amlodipine Besylate standard drug is taken in a 10 ml volumetric flask and dissolved in methanol and volume made up to the mark, from this solution 0.1ml is transferred into a 10 ml volumetric flask and made up to the mark with the methanol to give a concentration of 10 µg/ml. The above prepared solution is scanned in uv between 200-400 nm using methanol as blank. The λmax was found to be 237m. (Figure no. 2.2).

The Isosbestic Point of Indapamide and Amlodipine Besylate was found to be 215nm (Figure No: 2.3).

Preparation of mobile phase

Buffer Preparation: 2gm of sodium dihydrogen phosphate and 1.5gm of potassium dihydrogen phosphate were dissolved in 100ml distilled water. Then the volume was made up to 1000ml with water. The solution was filtered through 0.45µm nylon membrane filter and degassed. Adjusted the P^H to 3 with orthophosphoric acid.

Mobile phase: phosphate buffer: ACN: Methanol (40:20:40) $P^{H:}$ 3.0.

Analysis of formulation

Preparation of standard solution

Weighed accurately 50 mg of AB and 15 mg of IND and transferred to 100ml volumetric flask, added few ml of mobile phase to dissolve and made up the volume with mobile phase to 100ml.From the above solution 1.0ml was taken and transferred to 10ml volumetric flask and the volume was made up to the mark with the mobile phase. It Contains 50 µg/ml of AB & 15µg/ml of IND.

Preparation of sample solution

Twenty tablets were accurately weighed and powdered. A quantity of powder equivalent to 50mg of AB and 15mg of IND was taken and transferred to 100ml volumetric flask and the volume was made up to the mark with the mobile phase, filtered. From the above solution 1.0ml was taken and transferred to 10ml volumetric flask and the volume was made up to the mark with the mobile phase.

Calculation

5 replicates of each of sample and standard solutions are injected and their average peak areas were taken. The amount of Indapamide and Amlodipine Besylate present in the formulation was found by using the formula given below, and results shown in Table No.6.

$$\%\ \text{Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

Where,

AS: Average peak area due to standard preparation
AT: Peak area due to assay preparation
WS: Weight of standard drug taken
WT: Weight of sample in assay preparation
DT: Dilution of assay preparation
DS: Dilution of standard preparation
AW: Average weight of 20 tablets
LC: Label claim
P: Purity of standard drug

Method Validation

Linearity

Linearity was studied by analyzing five standard solutions covering the range of 30-70 µg/ml for Amlodipine Besylate and 9-21 µg /ml for Indapamide.

From the primary stock solution 0.6ml, 0.8ml, 1.0ml, 1.2ml, 1.4ml of aliquots are pipetted into 10 ml volumetric flasks and made up to the mark with the mobile phase to give a concentrations of 30µg/mL, 40µg/mL, 50µg/mL, 60µg/mL and 70 µg/mL of Amlodipine Besylate and 9µg/mL, 12µg/mL, 15µg/mL, 18µg/mL, 21µg/mL of Indapamide [10]. Calibration curve (Figure No.3.1 and 3.2) with concentration verses peak areas was plotted by injecting the above prepared solutions and the obtained data were subjected to regression analysis using the least squares method.

Method precision (repeatability)

The precision of the instrument was checked by repeated injections and measurement of peak areas and retention times of solutions (n = 6) for 50µg/ml of Amlodipine Besylate and 15µg/ml of Indapamide without changing the parameter of the proposed chromatographic method.

Intermediate precision (reproducibility) The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 different days over a period of 1 week for 50µg/ml and 15µg/ml concentrations of standard solutions of Amlodipine Besylate and Indapamide .The result was reported in terms of relative standard deviation (% RSD).

Limit of detection and limit of quantification:

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations (2) and (3), respectively.

$$\mathbf{LOD = 3.3\ \delta/S \ldots\ldots\ldots\ (3)}$$

$$\mathbf{LOQ = 10\ \delta/S \ldots\ldots\ldots\ (4)}$$

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Accuracy (recovery study)

The accuracy of the method was determined by calculating the recoveries of Amlodipine Besylate and Indapamide by the standard addition method. Known amounts of standard solutions of Amlodipine Besylate and Indapamide were added at 20% concentration to pre quantified sample solutions of Amlodipine Besylate (40, 50, 60 µg/ml) and Indapamide (12, 15, 18µg/ml) (Figure No.5.1 and 5.2). The amount of Amlodipine Besylate and Indapamide recovered was estimated by using the following formulas [11].

$$\%\ Recovery = \frac{amount\ found}{Amount\ added} \times 100$$

$$Amount\ Found(mcg/ml) = \frac{\text{Mean test area}}{\text{Mean standard area}} \times \text{Standard concentration}$$

Specificity

In an assay, demonstration of specificity requires that it can be shown that the procedure is unaffected by the presence of impurities or excipients. In practice, this can be done by spiking the drug substance or product with appropriate levels of impurities or excipients and demonstrating that the assay results are unaffected by the presence of these extraneous materials [12]. There should be no interference of the diluents, placebo at retention time of drug substances (Figure No.7).

Robustness: Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Here the detection wavelength varied by ±2nm and the flow rate by ±0.2 ml/min. The results were shown in (Table no.4).

Ruggedness: The ruggedness of the method was studied by analyzing the sample and standard preparations by two analysts. The %RSD assay values between two analysts was calculated and is within the limit i.e., (<2%).

This indicates the method was rugged. The results were shown in Table no.5.

RESULTS AND DISCUSSION

In RP HPLC method, the primary requirement for developing a method for analysis is that the using different solvents, buffers and columns to get better retention time, theoretical plates, better cost effective and time saving method than the previously developed methods. The Isosbestic Point of Amlodipine Besylate and Indapamide was found to be 215nm (Figure No: 2.3) by scanning in UV region. The chromatographic method was optimised with mobile phase consisting of phosphate buffer: ACN: Methanol (40:20:40) and C18 HYPERSIL 3V ODS column. All the validation parameters were studied at a the wavelength 215nm. Accuracy was determined by calculating the recovery (Table No.3) and the results were in acceptable range (limit 98-102%). The method was successfully used to determine the amount of Amlodipine Besylate and Indapamide present in the Tablet. The results obtained were in good agreement with the corresponding labeled amount (Table No.3). The method was linear in the concentration range of 30 to 70 µg/ml for Amlodipine Besylate and 9 to 21 µg/ml for Indapamide (Figure no.1, Table No.1). Precision was calculated as repeatability and intra and inter day variations (% RSD) for the drug (Table No.7). Robustness and ruggedness results were in acceptable range (Table No.4 and Table No.5). Summary of all validation parameters for method is given in Table No.8. By observing the validation parameters, the method was found to be simple, sensitive, accurate and precise. Hence the method can be employed for the routine analysis Amlodipine Besylate and Indapamide in tablet dosage form

Table No.1: Linearity

AMLODIPINE BESYLATE		
S.No	**mcg**	**Area**
1	30.0	3902.902
2	40.0	4953.736
3	50.0	6253.353
4	60.0	7561.462
5	70.0	8810.648

INDA AMIDE		
S.No	**mcg**	**mcg**
1	9.0	425.029
2	12.0	536.041
3	15.0	770.725
4	18.0	904.71
5	21.0	1090.087

Table No.2: LOD and LOQ values from calibration curve

AMLODIPINE BESYLATE			
S.No	**mcg**		**Area**
1	30.0		3902.902
2	40.0		4953.736
3	50.0		6253.353
4	60.0		7561.462
5	70.0		8810.648
6	Std dev	15.81	1965
7	Slope	124.2	

INDAPAMIDE			
S.No	**mcg**		**Area**
1	9.0		425.029
2	12.0		536.041
3	15.0		770.725
4	18.0		904.71
5	21.0		1090.087
6	Std dev	4.74	269
7	Slope	55.72	

AMLODIPINE BESYLATE	**INDAPAMIDE**
mcg	**mcg**
0.85	0.28
1.27	0.42

Table No.3: Recovery data

% Standard	Amount of IND present (µg/ml)	Amount of IND added(µg/ml)	Avg. area of 3 recoveries	%Recovery
80	12	3	708.878	100.72
100	15	3	933.709	100.96
120	18	3	1056.044	100.05
			MEAN	100.57

% Standard	Amount of AB present (µg/ml)	Amount of AB added(µg/ml)	Avg. area of 3 recoveries	%Recovery
80	40	10	6155.781	99.41
100	50	10	7614.791	101.48
120	60	10	8695.288	98.57
			MEAN	99.82

Table No.4: Results of Robustness study

Chromatographic change		Retention time		Asymmetry	
		IND	AB	IND	AB
Flow rate (ml/min)	0.8	3.853	5.603	1.341	1.283
	1.0	2.833	4.107	1.355	1.279
	1.2	2.230	3.253	1.250	1.205
Wavelength (nm)	228	2.833	4.123	1.273	1.267
	230	2.833	4.107	1.355	1.279
	232	2.830	4.120	1.219	1.244

Table No.5: Results of Ruggedness

		Std Area	**Spl Area**	**%Assay**	**%RSD**
Analyst-1	INDAPAMIDE	704.511	743.656	99.56%	0.34%
Analyst-2		702.718	748.280	99.19%	
Analyst-1	AMLODIPINE BESYLATE	6178.967	6222.219	100.83%	1.48%
Analyst-2		5934.034	6312.074	98.23%	

Table No.6: Assay Results

		Indapamide	**Amlodipine**
Standard Area	1	698.106	6181.293
	2	694.029	5958.144
	3	683.82	5936.486
	4	689.305	5907.803
	5	690.537	5889.387
Average area		691.159	5974.623
Sample area	1	733.633	6359.624
	2	741.68	6360.287
	3	725.991	6251.943
	4	737.686	6275.122
	5	728.755	6242.301
Average area		691.159	6297.855
Tablet average weight		150.2 mg	150.2 mg
Standard weight		7.54 mg	25.01 mg
Sample weight		768.4 mg	768.4 mg
Label amount		1.5 mg	5 mg
std.purity		99.8	98.7
Cal:		**1.47** mg	**5.09** mg
% Assay		**98.06**	**101.72**

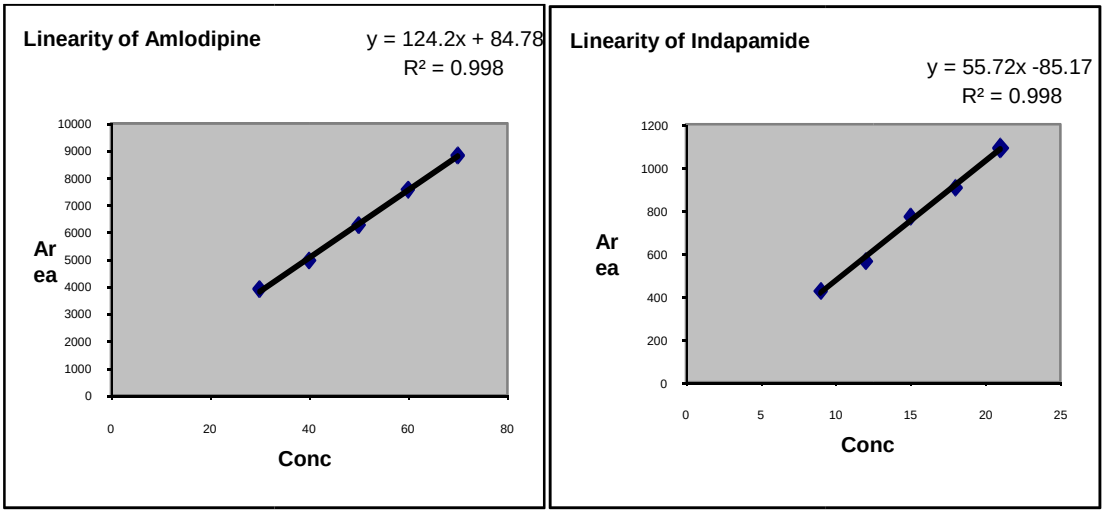
Table No.7: Method Precision (Repeatability)

Injection	INDAPA IIDE		AMLODIPINE	
	Retention Time	Area	Retention Time	Area
1	2.833	720.725	4.107	6175.362
2	2.817	714.973	4.087	6132.395
3	2.830	706.359	4.100	6080.999
4	2.780	690.499	4.047	5994.12
5	2.767	695.797	4.023	6025.236
6	2.807	699.665	4.110	6049.578
AVG	2.8057	704.670	4.079	6076.282
SD	0.0269	11.588	0.036	67.908
% RSD	0.96	1.64	0.88	1.12

Table No.8: Validation parameters of evaluated method

S.No	Parameter	Value Obtained of INDAPAMIDE	Value Obtained Amlodipine
1	ACCURACY(%Recovery)	100.05-100.96%	99.41-100.48%
2	Linearity concentrations Range(µ g/mL) Regression coefficient (R2 value)	9-21 µ g/mL 0.998	30-70 µ g/mL 0.998
3	LOD	0.85	0.28
4	LOQ	1.27	0.42
5	Precision (% RSD) Method precision(Repeatability) (%RSD, n = 6)	0.88	0.41-1.12%
6	Robustness(%assay)	98.06%	101.72%
7	Ruggedness (%RSD analyst to analyst variation)	0.34%	1.48%

a. SD=Standard deviation, b. LOD = Limit of detection, c. LOQ = Limit of quantification, d. RSD = Relative standard deviation



FigureNo.1: Linearity (calibration) curve of Amlodipine Besylate and Indapamide

Figure No.2: Determination of Working Wavelength (λmax) of Amlodipine Besylate

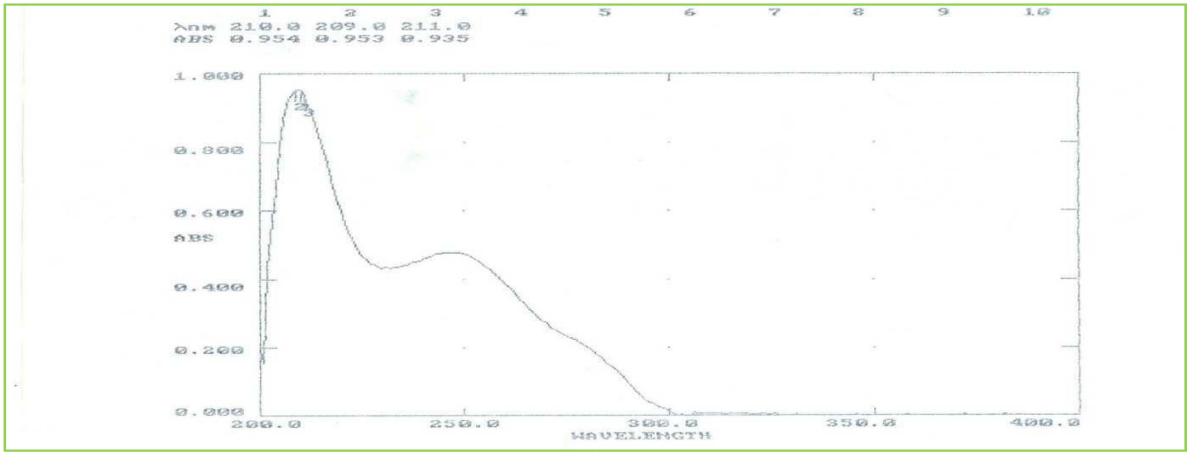


Figure No.2.1: Determination of Working Wavelength (λmax) of Indapamide

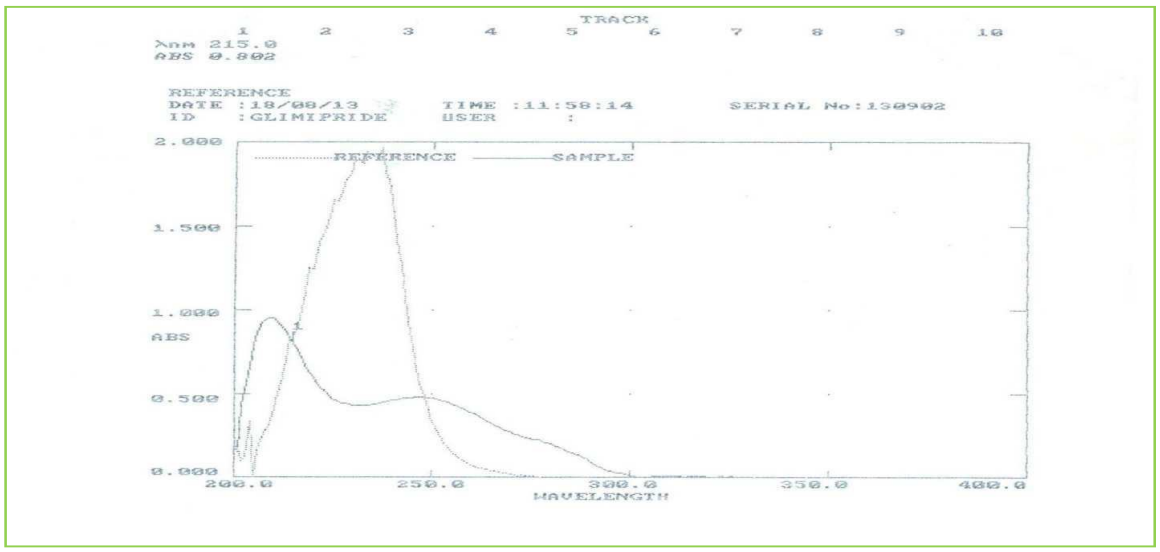


Figure No.3: Determination of Isosbestic Point

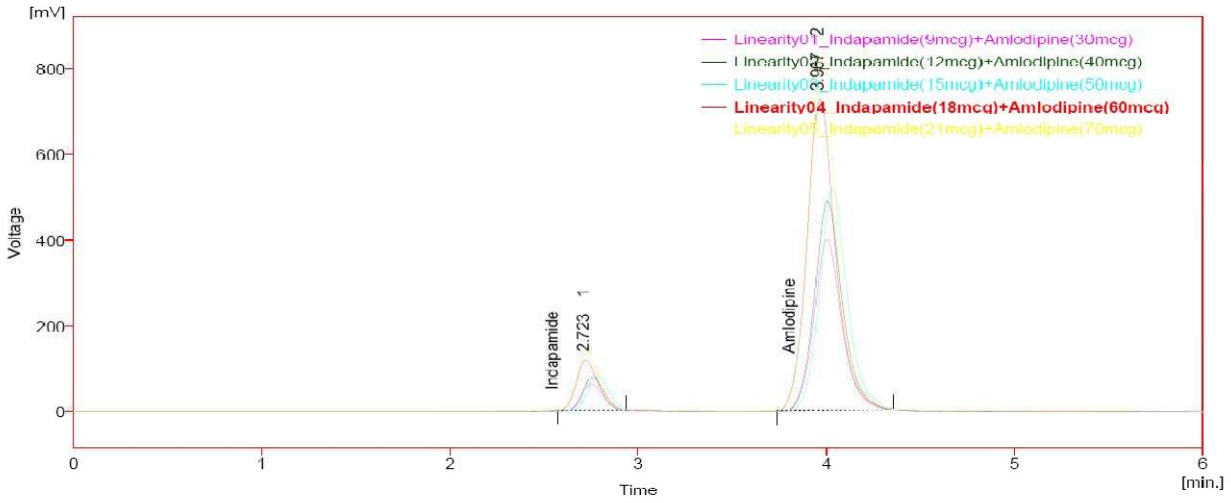


Figure No.4: Overlain chromatograms of Linearity

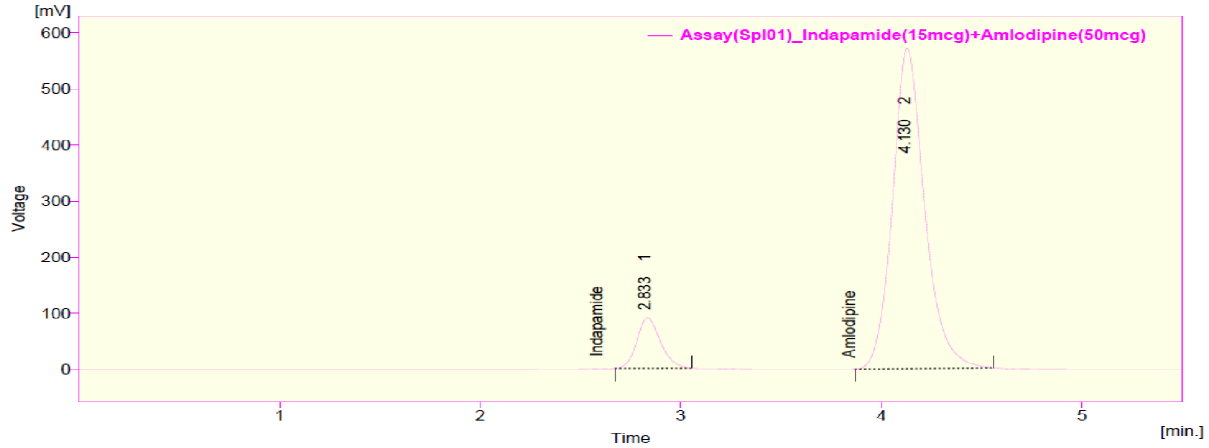


Figure No.5: Chromatogram of Assay sample preparation

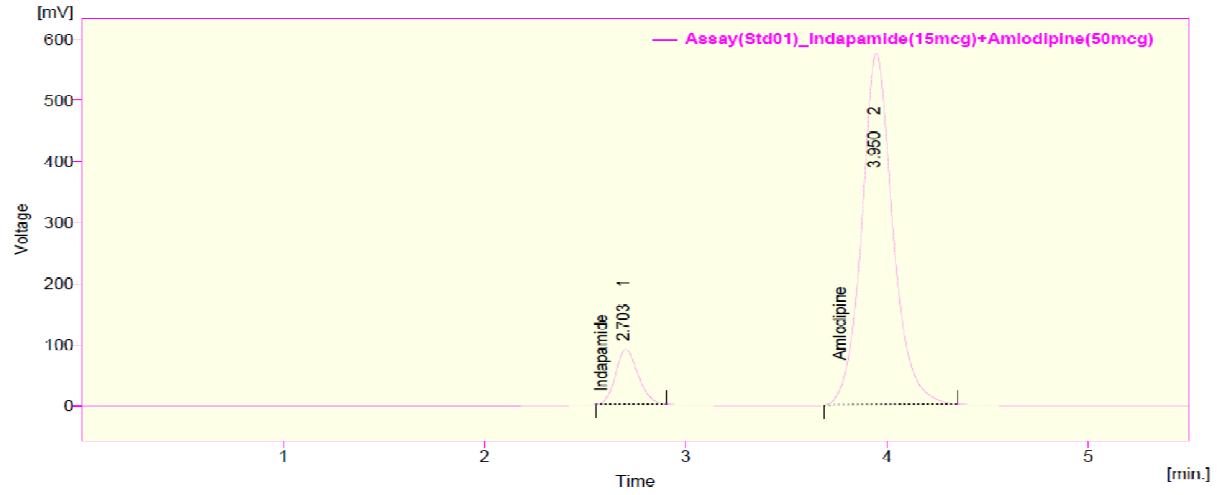


Figure No.6: Chromatogram of Assay standard preparation:

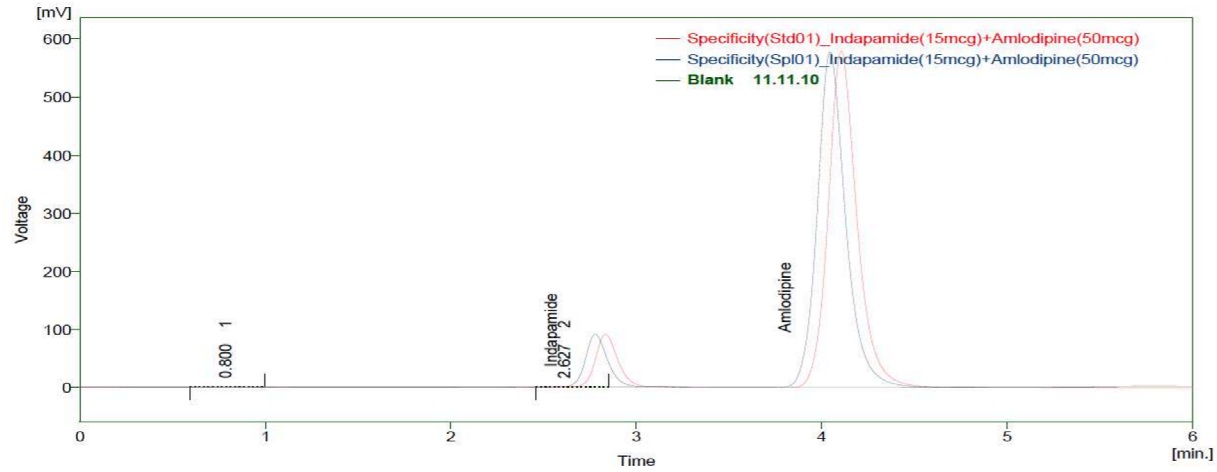


Figure No.7: Overlain chromatograms of Specificity (placebo,blank,sample and standard preparations)

CONCLUSION

The proposed Simultaneous Estimation by RP-HPLC method was found to be simple, sensitive, accurate and precise for determination of Amlodipine Besylate and Indapamide in tablet. The method utilizes easily available and cheap solvent for analysis of Amlodipine Besylate and Indapamide hence the method was also economic for estimation of Amlodipine Besylate and Indapamide from Tablet .The common excipients and other additives are usually present in the Tablet mixture do not interfere in the analysis of Amlodipine Besylate and Indapamide, hence it can be conveniently adopted for routine quality control analysis of the drug in pharmaceutical formulation.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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