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Der Pharma Chemica, 2013, 5(6):347-352
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Simultaneous estimation and forced degradation studies of amlodipine besylate and indapamide in tablet dosage form by RP-HPLC method

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ABSTRACT

The method developed for simultaneous estimation of Amlodipine besylate and Indapamide by using RP-HPLC method is simple, accurate, selective and economic by using c8 column (BDS Hypersil, 100×4.6mm,5µ particle size). Sample was analysed using water which is adjusted with ortho phosphoric acid buffer (OPA) to p^H 3: Methanol in the ratio 600:400 as a mobile phase at a flow rate of 1ml\min. Detection was performed with PDA detector at 248nm. The retention time of Indapamide and Amlodipine besylate is 1.45 & 3.30 respectively. The correlation coefficient R^2 value is found to be 0.999 for Amlodipine besylate and 0.999 for Indapamide. The LOD and LOQ values for Amlodipine besylate was found to be 0.00015 and 0.0005.The LOD and LOQ values for Indapamide was found to be 0.00028 and 0.00095 respectively. The proposed method was validated as per ICH guidelines with parameters like linearity, accuracy, Interday and precision, LOD, LOQ, robustness.

Keywords: Amlodipine besylate, Indapamide, RP- HPLC.

INTRODUCTION

Amlodipine besylate is chemically 3-ethyl 5-methyl (4RS)-2-[(2aminomethoxy) methyl]-4-(2-chlorophenyl) 6-methyl-1, 4 dihydropyridine-3, 5-dicarboxylate benzenesulphonate [1] (fig: 4). It is a dihydropyridine type calcium channel blocker and widely used as Antihypertensive Agents [2] [3]. Indapamide is chemically 4- chloro-N-(2-methyl-2, 3-dihydroindole -1-yl)-sulfomyl-benzamide (fig:5). It is a diuretic, belongs to the class of benzothiadiazines and also used as Antihypertensive agents [2] [4]. Amlodipine besylate is official in IP [5], BP [6] and Indapamide is official in IP [7], BP [8], USP [9]. Upon survey it was found that individually and in combination these drugs have been analysed by many methods such as HPLC [10-12], spectrophotometric [13] [14], HPTLC for Indapamide.For Amlodipine besylate spectrophotometric [15] [16], HPLC [17], chiral chromatography.

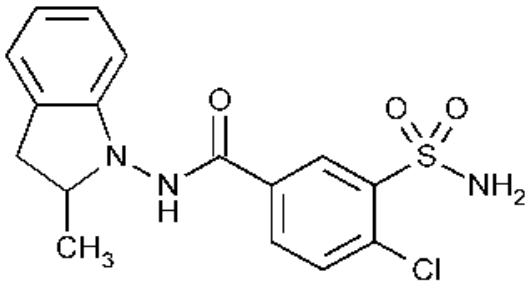

Figure – 1 Structure of Indapamide

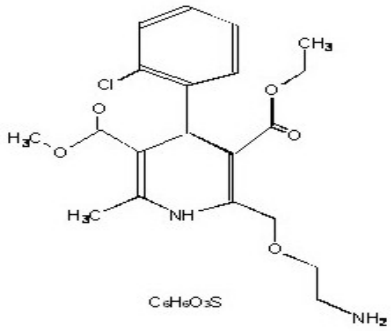

Figure – 2 Structure of Amlodipine Besylate

MATERIALS AND METHODS

Chemicals and reagent: Amlodipine besylate and Indapamide were kindly supplied as a gift of samples from lara drugs hyd. The pharmaceutical formulation (NATRIlAM) containing 5mg of Amlodipine besylate and 1.5mg of Indapamide were purchased from local pharmacy. All other reagents were used are of HPLC grade. The water used is also of HPLC grade.

Instrument: RP-HPLC instruments (WATERS) equipped with a photodiode array detector (PDA 2998), Auto sampler, BDS Hypersil C_8 column (100×4.6mm,5µ), digital balance(Satorious).

Liquid chromatographic conditions: The following chromatographic conditions were used to quantity the Amlodipine besylate and Indapamide are

Column: BDS hypersil c8, 100×4.6mm, 5µ
Flow rate: 1ml/min
Auto sampler temperature: 25ºc
Column temperature: 40ºc
Detector wavelength: 248nm
Run time: 6min

Mobile phase: Methanol (40%v/v): water (60%v/v) adjusted to pH 3with orthophosphoric buffer (OPA)

Preparation of standard solution: A mixed standard solution of Amlodipine besylate and Indapamide was prepared by accurately weighing Amlodipine besylate (10mg) and Indapamide (15mg) in 10ml and 50ml volumetric flask respectively and diluted with methanol. From these solutions 0.5ml were transferred in 10ml volumetric flask and diluted with methanol upto the mark.

Preparation of sample solution: 10 tablets were weighed and powdered. Transfer 1027mg weight of sample into 50ml volumetric flask and add sufficient methanol, sonicate for 20minutes and make up the volume with methanol. From this solution 0.5m is transferred into 50ml volumetric flask and make up with methanol. The solution is filtered through whatman filter paper no.41.

Forced degradation studies: This study was done by treating the samples with 0.1M NaoH (base), 0.1M Hcl (acid), 1%H_2O_2, light, and water. The results were in the Table 4.

METHOD VALIDATION PARAMETRES: The developed method was validated for the assay of Amlodipine besylate and Indapamide as per ICH guidelines.

ACCURACY: Accuracy was performed by recovery studies. The recovery studies were carried out at three concentration levels 50%, 100%, 150%. The recoveries were found to be 99%-102% and 100%-101% for Indapamide and Amlodipine besylate. (Table 1)

LINEARTY: Linearity was determined by calculating regression line i.e area versus analyte concentration. It was determined for Indapamide in the range of 50-150% and co-relation coefficient was found to be 0.99 and for Amlodipine besylate in the range of 50-150% and the co-relation coefficient was found to be 0.99.(fig 3&4)

PRECISION: Instrument precision was performed by repeatable injections and the RSD values for Amlodipine besylate and Indapamide are 0.94and 0.57 respectively. The intra-day precision was carried out and the low RSD values indicate the method is precisie. (Table 1)

ROBUSTNESS: The method was found to be robust, as small changes in the parameters of the method have no differential effect on the method performance as shown in the (Table 3).

LOD AND LOQ: The LOD and LOQ were separately determined by using calibration curve. The detection limits of Amlodipine besylate and Indapamide are 0.00015 and 0.00028 and quantification limits of Amlodipine besylate and Indapamide are 0.0005 and 0.00095(Table 1)

SYSTEM SUTABILITY: The system suitability was checked by using the parameter such as tailing, resolution, theoretical plates, and retention time. These parameters were found to be within limit. (Table 1)

SPECIFICITY: Specificity is the ability to assess unequivocally the analyte in the presence of compound which may be expected to be present. The specificity of HPLC method was determined by complete separation of Amlodipine besylate and Indapamide by peak purity with a PDA detector. The results show that there were no interference peaks with the analyte peak. (Table 3)

RESULTS AND DISCUSSION

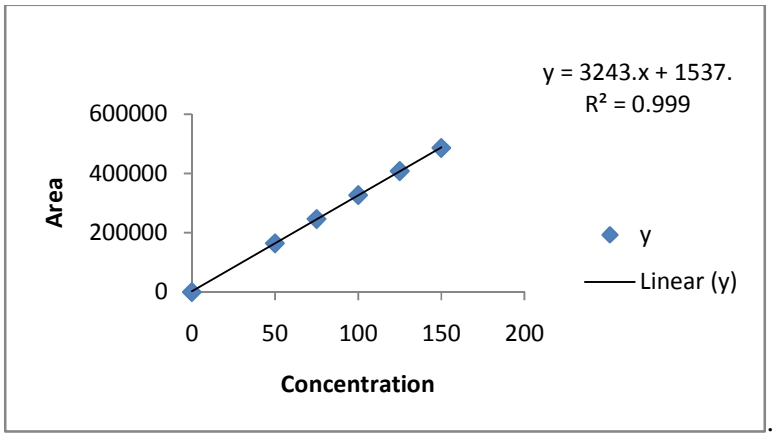

Figure – 3 Calibration curve of standard Amlodipine besylate

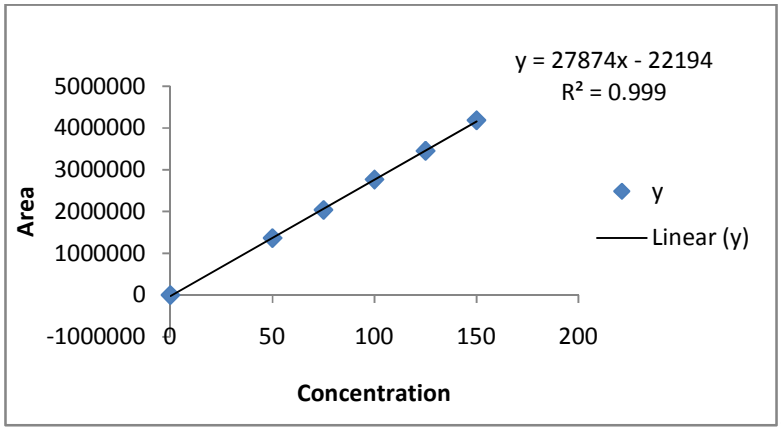

Figure – 4 Calibration curve of standard Indapamide

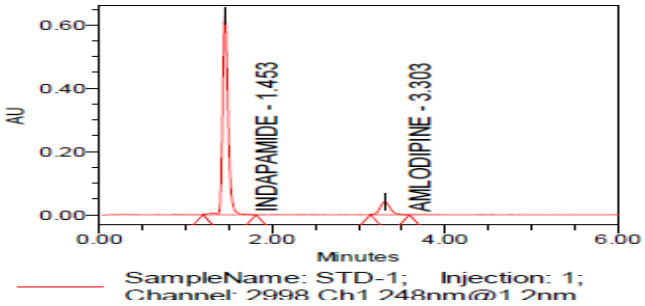

Figure-5 Standard chromatogram of Amlodipine besylate and Indapamide

TABLE-1 Validation parameters of Amlodipine besylate & Indapamide

Validation Parameter	Indapamide	Amlodipine besylate
Linearity(conc.)	**50-150%**	**50-150%**
Accuracy		
50%	99	101
100%	99	100
150%	102	101
Precision		
Repeatability(n=6), % RSD	0.57	0.94
Intraday	0.51	0.82
LOD	0.00028	0.00015
LOQ	0.00095	0.0005
System Suitability Parameters		
Retention time(min)	1.453	3.303
Tailing	1.27	1.14
Theoretical plate	2705	3817
%RSD	0.5	0.7

TABLE-2 Robustness data for Amlodipine besylate & Indapamide

Robust Condition	Retention Time		Tailing Factor		Theoretical plates	
flow rate (ml/min)	Indapamide	Amlodipine besylate	Indapamide	Amlodipine besylate	Indapamide	Amlodipine besylate
0.5	1.78	3.92	1.27	1.16	2777	4485
1	1.45	3.30	1.305	1.169	2661	3919
1.5	1.19	2.66	1.21	1.04	2714	3492
temp(ºC)						
35	1.43	3.19	1.26	1.11	2701	3770
40	1.45	3.30	1.3	1.16	2661	3919
45	1.44	3.22	1.25	1.08	2723	4211

TABLE-3 Specificity data for Amlodipine besylate & Indapamide

Specificity	Indapamide	Amlodipine besylate
RT of analyte	1.4	3.3
RT of placebo	ND	ND
RT of blank	ND	ND

Table-4 Forced degradation data of Amlodipine besylate & Indapamide

Sample treated	Amlodipine besylate		Indapamide	
	RT	Area	RT	Area
Acid	2.97	299852	1.41	2445766
Base	3.21	309217	1.43	2384081
Light	3.20	305752	1.43	2583264
Peroxide	3.19	309217	1.43	2481162
Water	3.203	313354	1.43	2621293
Mean		301559		2503113
%RSD		4.4		3.9

CONCLUSION

The results obtained from all the validation parameters, it is concluded that the developed method RP-HPLC is suitable for the simultaneous estimation of Amlodipine besylate and Indapamide in pharmaceutical preparation is very simple, accurate, precisie. Therefore the method is suitable for tablet formulation in quality control.

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