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Novel analytical method development and validation for the determination of residual solvents in amlodipine besylate by gas chromatography

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ABSTRACT

The purpose of this research study was to develop and optimize an accurate and precise GC method for the determination of Residual solvents (n-Hexane, Methanol, Isopropyl Alcohol, Toluene, O-Xylene, DMF & Acetic acid) in Amoldipine besylate using the DB-FFAP, 30 m x 0.53mm ID, 1.0 µm column as stationary phase. The injection volume of samples taken is 1 µl. The split ratio of the injection was 1:10. The temperature maintained at the injector and detector was to be 220ºC and 260ºC respectively. Nitrogen gas having make up flow 40 ml/ minute and having column flow 2.8 ml/minute used as mobile phase and the detection was by FID. The flow of hydrogen and Air was maintained at 30ml/min and 300ml/min respectively. The diluent used is Dimethyl Sulfoxide. All solvents well resolved each other with diluents peak RT at 21 min in total run time of 24.25 min. The RTs observed for the Residual solvents n-Hexane, Methanol, Isopropyl Alcohol, Toluene, O-Xylene, DMF & Acetic acid are 3.03, 6.20, 6.87, 10.27, 14.57, 17.49 &19.54 respectively. The method was validated as meets the all regulations of System suitability, Specificity, Method Precision, Linearity, LOD & LOQ, Precision of LOQ and Accuracy/Recovery under ICH specifications.

Key words: GC, Residual solvents, Amoldipine besylate, DB-624 stationary phase.

INTRODUCTION

Chromatography is defined as a procedure by which solutes are separated by a dynamic differential migration process in a system consisting of two or more phases. One of which moves continuously in a given direction and in which the individual substances exhibit different motilities by reason of differences in adsorption, partition, solubility, vapour pressure, molecular size, or ionic charge density. The individual substances thus obtained can be identified or determined by analytical methods, presently in the pharmaceutical industries, special importance given for residual solvent testing. The residual solvents are potentially undesirable substances, they either modify the properties of certain compounds and also hazardous to the health of the individual. OVI's (Organic Volatile Impurities) also affect physicochemical properties like crystallinity[1,2] of the bulk drug, as a difference in the crystal structure may lead to change in dissolution properties and problems with formulations of the finished product. Also residual solvents may create odour problem and colour change in the finished products[3,4]. The

safety of the drug is determined by its pharmacological, toxicological profile and adverse effects[5,6]. The content of residual solvents in APIs analysed by using gas chromatography[7,8].

Amlodipine besylate 3-Ethyl 5-methyl (4RS)-2-[(2- aminoethoxy) methyl]-4- (2- chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate benzenesulphonate [Fig.1]. It is a dihydropyridine calcium antagonist (calcium ion antagonist or slow-channel blocker). It is used for the treatment of hypertension.

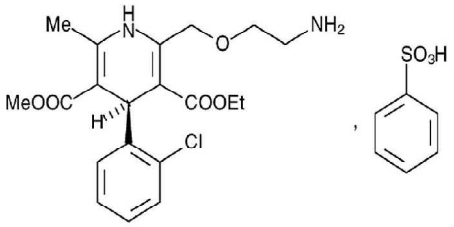


Fig.1: Structure of Amlodipine besylate

From the literature review many analytical methods have been reported for the determination of Amlodipine besylate such as spectrophotometry[9-32], spectrofluorimetry[33,34], HPLC[35-52], LC-MS/MS[53-56], HPTLC[57-59] and Voltammetry[60,61]. There is no reported method for the determination of Residual solvents in Amlodipine besylate by Gas chromatographic method. The objective of this work is report a simple, precise, accurate and cost effective method.

MATERIALS AND METHODS

Method Development :

Chromatographic separation was performed on a Perkin Elmer chromatographic system (Model- Clarus 500) equipped with FID detector. DB-FFAP, 30 m x 0.53mm ID, 1.0 µm was the column used for separation. Mobile phase (carrier gas) used was Nitrogen gas with detection at 260ºc. Amlodipine Besylate standard pure drug was supplied by Arch pharma laboratories private limited, Hyderabad. Isopropyl alcohol, n-Hexane, Acetic acid, Toluene, Dimethyl formaide, o-Xylene, Dimethyl sulfoxide, Sodium carbonate, Methanol and Water-milli q were of AR grade. Optimized chromatographic conditions are listed in Table.1&2

Table.1: Optimised Chromatographic conditions

Column	DB-FFAB, 30 m x 0.53mm, 1.0 µm
Detector	Flame ionization Detector
Carrier gas	Nitrogen
Injection mode	Split (1:10)
Column flow	2.8 ml/min
Purge flow	3.0 ml/min
Injector temperature	220^0C
Detector temperature	260^0C
Equilibration time (Oven)	0.2min
Hydrogen Flow	30ml/min
Air Flow	300 ml/min
Nitrogen Flow	40ml/min
Injection volume	1µl
Run Time	24.25min

Table.2: Column oven temperature programme

Rate (°C/min)	Temperature (^{0}C)	Hold time (min)
----	45.0	5.0
5.0	85.0	0.0
12.0	220.0	0.0

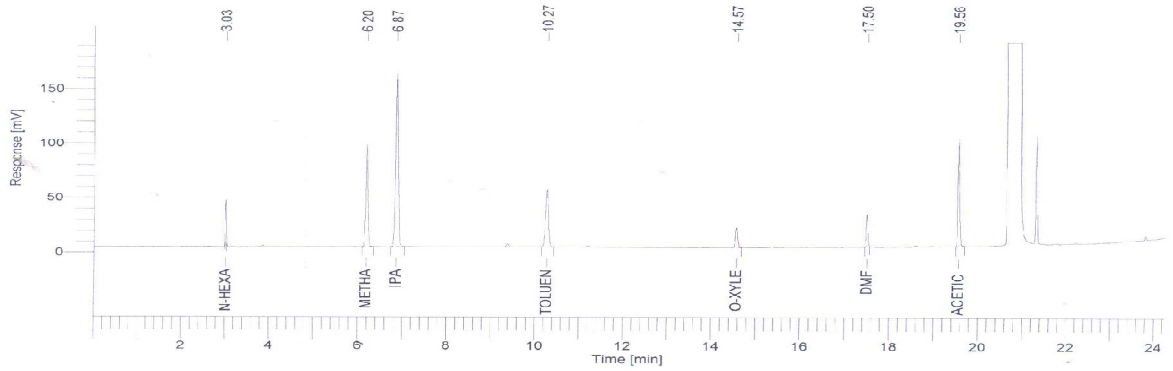


Fig.2: A Representative chromatogram for optimized method

Preparation of standard stock Solution:
Weighed accurately about 300.28mg Methanol, 500.24mg Isopropyl alcohol, 21.38mg n-Hexane and 89.76 mg of Toluene, 500.98 mg of Acetic acid&88.36 mg of DMF into a 100 ml volumetric flask containing about 10 ml of Dimethyl Sulfoxide and make up to volume with Dimethyl Sulfoxide.

Preparation of Standard solution:
Pipette out 5.0 ml of stock solution into a 50 ml volumetric flask add 10.0g of Sodium carbonate. Mix and diluted to the mark with Dimethyl Sulfoxide.

Preparation of test solution:
Take about 500 mg of Amlodipine besylate sample in to a 5 ml volumetric flask, dilute to the mark with Dimethyl Sulfoxide. Prepare the sample solution in duplicate.

Method Validation :
The analytical method validation was carried out as per ICH method validation guidelines. The validation parameters addressed were specificity, precision, linearity, limit of detection, limit of quantitation, Accuracy and system suitability.

Specificity
The specificity of the analytical method was determined by injecting stock & blank solution of pure Dimethyl sulphoxide solution under the same experimental conditions. The individual Retention times of residual solvents were noted (Fig.2). No peak was observed from the chromatogram obtained by injecting 1µL of DMSO as a blank. Table.3 & Table.4.

Precision
For the method precision six replicates of concentration of 100 ppm of mixed standard solution 1µL was injected into the chromatograph for each solvent from chromatogram peak areas standard deviation and relative standard deviation were calculated. For the precision of method and system the %RSD for six solvents complies with the acceptance criteria of less than 2%, hence the method and system is said to be précised.Table.16

Linearity
Prepare a series of solutions containing each solvent, [i.e. LOQ level- 50%, 75%, 100%, 125% and 150% with respect to the specification limit of each solvent]. Inject each concentration in duplicate injections. Plotted the calibration curves for each solvent at concentration ranges tested (i.e. LOQ to 150% of the specification level of each solvent) and the correlation coefficient for Residual solvents was within the limits i.e., not less than 0.9990. (n-Hexane, Methanol, Iso propyl alcohol, Toluene, O-Xylene, DMF and Acetic acid is 0.9938, 0.9929, 0.9944, 0.9945, 0.9934, 0.9959 and 0.9925).Table.5-11 & Fig.3

Accuracy/ Recovery study
Weighed accurately about 500mg of Amlodipine besilate test sample in 5.0ml volumetric flask, add 2.5, 3.75, 5.0, 6.25 and 7.5 of solution (a) and add 1.0g of sodium carbonate and made up to volume with diluent. It will produce

50%, 75%, 100%, 125% and 150% respectively. The % Recovery of each residual solvent should be in between 80-120 % for all four recovery levels studied (LOQ, 50, 100, and 150) of the target concentration.Table.12-15

RESULTS AND DISCUSSION

All the validated parameters were found to be within the limits. Linearity is performed from 50-150% and graph obtained was linear showing correlation coefficient $R^2 \geq 0.999\%$. Drug recovery should be 80-120%. System suitability for 6 injections %RSD was found to be NMT 15%.

Table.3: specificity (Individual RT's of Residual solvents)

S.No	Name of the solvent	RT	Resolution
1	n-Hexane	3.032	NA
2	Methanol	6.200	48.535
3	IPA	6.877	6.539
4	Toluene	10.275	27.939
5	O-Xylene	14.571	37.708
6	DMF	17.499	34.093
7	Acetic acid	19.547	27.851

Table.4: % RSD of area's for standard solution

S.No	Injections	n-Hexane	Methanol	IPA	Toluene	O- Xylene	DMF	Acetic Acid
1	Inj-01	56313	325097	683667	247190	71288	79961	295734
2	Inj-02	52265	306256	643618	232137	66831	76190	272885
3	Inj-03	52055	323720	682273	245471	70542	77793	279773
4	Inj-04	48381	313165	659726	235394	68149	76694	249239
5	Inj-05	48988	322640	671294	238365	68607	77175	293510
6	Inj-06	53038	350993	766653	279245	82301	88147	324228
AVG		51840	323645	684539	246300	71286	79326	285895
STDEV		2638.57	13924.10	39188.03	15649.11	5143.87	4122.14	23002.19
% RSD		5.09	4.30	5.72	6.35	7.22	5.20	8.05

Table.5: Area of n-Hexane in Linearity Solution

S/N	Sample ID	Conc. (ppm)	Injections	Area	Mean Area
1	LOQ Level	51.65	01	4962	4384
			02	3806	
2	50% linearity solution	1500	01	11238	11054
			02	10871	
3	75% linearity solution	2250	01	16835	15580
			02	14325	
4	100% linearity solution	3000	01	20246	20316
			02	20386	
5	125% linearity solution	3750	01	24587	25471
			02	26354	
6	150% linearity solution	4500	01	31752	31861
			02	31970	
Correlation coefficient value					0.9938

Table.6: Area of Methanol in Linearity Solution

S/N	Sample ID	Conn.(ppm)	Injections	Area	Mean Area
1	LOQ Level	17.77	01	51941	52553
			02	53165	
2	50% linearity solution	2500	01	168155	169594
			02	171033	
3	75% linearity solution	3750	01	234619	230451
			02	226283	
4	100% linearity solution	5000	01	263641	263116
			02	262591	
5	125% linearity solution	6250	01	304667	311941
			02	319214	
6	150% linearity solution	7500	01	361003	362849
			02	364695	
Correlation coefficient value					0.9929

Table.7: Area of Isopropyl alcohol in Linearity Solution

S/N	Sample ID	Conc.(ppm)	Injections	Area	Mean Area
1	LOQ Level	12.58	01	5146	5084
			02	5022	
2	50% linearity solution	205	01	288189	290211
			02	292233	
3	75% linearity solution	306	01	422759	434442
			02	446125	
4	100% linearity solution	410	01	616290	612548
			02	608807	
5	125% linearity solution	513	01	709422	693837
			02	678253	
6	150% linearity solution	615	01	839922	842608
			02	845293	
Correlation coefficient value					0.9944

Table.8: Area of Toluene in Linearity Solution

S/N	Sample ID	Conc.(ppm)	Injections	Area	Mean Area
1	LOQ Level	6.90	01	10749	10892
			02	11035	
2	50% linearity solution	300	01	110967	111702
			02	112437	
3	75% linearity solution	450	01	162440	166781
			02	171122	
4	100% linearity solution	600	01	229695	228587
			02	227480	
5	125% linearity solution	750	01	259296	256869
			02	254442	
6	150% linearity solution	900	01	307979	309496
			02	311013	
Correlation coefficient value					0.9945

Table.9: Area of O-Xylene in Linearity Solution

S/N	Sample ID	Conc.(ppm)	Injections	Area	Mean Area
1	LOQ Level	26.71	01	2790	2755
			02	2720	
2	50% linearity solution	2500	01	30766	30873
			02	30981	
3	75% linearity solution	3750	01	44582	45546
			02	46509	
4	100% linearity solution	5000	01	64368	63859
			02	63350	
5	125% linearity solution	6250	01	72031	70799
			02	69566	
6	150% linearity solution	7500	01	85676	85804
			02	85932	
Correlation coefficient value					0.9934

Table.10: Area of DMF in Linearity Solution

S/N	Sample ID	Conc.(ppm)	Injections	Area	Mean Area
1	LOQ Level	16.64	01	2701	2747
			02	2793	
2	50% linearity solution	445	01	38395	37481
			02	36567	
3	75% linearity solution	668	01	55464	54438
			02	53413	
4	100% linearity solution	890	01	79420	78441
			02	77462	
5	125% linearity solution	1113	01	87741	89290
			02	90839	
6	150% linearity solution	1335	01	110031	109729
			02	109426	
Correlation coefficient value					0.9959

Table.11: Area of Acetic acid in Linearity Solution

S/N	Sample ID	Conc.(ppm)	Injections	Area	Mean Area
1	LOQ Level	16.64	01	13892	15058
			02	14641	
2	50% linearity solution	445	01	193756	213490
			02	233224	
3	75% linearity solution	668	01	322956	327705
			02	332454	
4	100% linearity solution	890	01	412543	419002
			02	425461	
5	125% linearity solution	1113	01	491227	500361
			02	509495	
6	150% linearity solution	1335	01	662109	666306
			02	670503	
Correlation coefficient value					0.9925

Accuracy/ Recovery studies:

Table.12: LOQ% Recovery of RS in spiked test solution

S.No	Sample Name	Injection	Areas	Avg (Area)	Obtained Value (ppm)	Theoretical value(ppm)	% Recovery
1	n-Hexane	1	8718	8553	27.21777	26	104.68
		2	8387				
2	Methanol	1	85562	87604	41.22333	43	95.87
		2	89647				
3	IPA	1	4184	4085	33.52929	38	88.23
		2	3987				
4	Toluene	1	10159	10294	49.67371	49	101.37
		2	10429				
5	O-Xylene	1	2911	2845	8.73911	10	87.39
		2	2780				
6	DMF	1	2775	2838	32.28857	30	107.63
		2	2901				
7	Acetic acid	1	9327	6921	65.0667	60	108.44
		2	4516				

Table.13: 50% Recovery of RS in spiked test solution

S.No	Sample Name	Injection	Areas	Avg (Area)	Obtained Value (ppm)	Theoretical value(ppm)	% Recovery
1	n-Hexane	1	14651	13521	145.6688	145	100.46
		2	12392				
2	Methanol	1	162813	164317	1695.255	1500	113.02
		2	165821				
3	IPA	1	273989	276403	2292.614	2500	91.70
		2	278818				
4	Toluene	1	111742	112588	423.7025	445	95.21
		2	113434				
5	O-Xylene	1	30632	30906	95.68683	95	100.72
		2	31180				
6	DMF	1	34993	35318	406.0583	440	92.29
		2	35643				
7	Acetic acid	1	124894	136737	2292.999	2500	91.72
		2	148580				

Table.14: 100% Recovery of RS in spiked test solution

S.No	Sample Name	Injection	Areas	Avg (Area)	Obtained Value (ppm)	Theoretical value(ppm)	% Recovery
1	n-Hexane	1	34759	36161	389.3215	435	89.50
		2	37563				
2	Methanol	1	359619	358598	4209.55	4500	93.55
		2	357577				
3	IPA	1	819187	818657	6785.791	7500	90.48
		2	818127				
4	Toluene	1	329105	327879	1237.231	1335	92.68
		2	326652				
5	O-Xylene	1	90397	90250	279.9615	285	98.23
		2	90103				
6	DMF	1	104518	104533	1201.036	1320	90.99
		2	104547				
7	Acetic acid	1	509518	476154	7979.499	7500	106.39
		2	442790				

Table.15: 150% Recovery of RS in spiked test solution

S.No	Sample Name	Injection	Areas	Avg (Area)	Obtained Value (ppm)	Theoretical value(ppm)	% Recovery
1	n-Hexane	1	27170	27791	300.2771	290	103.54
		2	28411				
2	Methanol	1	254462	252862	2978.937	3000	99.30
		2	251263				
3	IPA	1	600619	597037	4966.492	5000	99.33
		2	593455				
4	Toluene	1	239863	238083	898.5819	890	100.96
		2	236303				
5	O-Xylene	1	65863	65512	203.4186	190	107.06
		2	65160				
6	DMF	1	77536	77471	893.2886	880	101.51
		2	77406				
7	Acetic acid	1	135752	136964	4987.287	5000	99.75
		2	138175				

Precision at LOQ:

Table.16: % RSD of areas of RS for Precision at LOQ solution

Injections	n-Hexane	Methanol	IPA	Toluene	O-Xylene	DMF	Acetic Acid
01	4962	57789	5386	10749	2892	2808	15053
02	4305	53165	5022	11190	2720	2793	13892
03	5065	59296	4848	11014	2731	2672	14641
04	4502	54402	5745	10974	2855	2729	15363
05	3818	44062	4485	10507	2731	2738	19017
06	3650	46605	5020	10917	2598	2745	12383
AVG	4384	52553	5084	10892	2755	2747	15058
STDEV	579.158	6068.8	436.019	236.52	105.635	48.7227	2211.6
% RSD	13.2117	11.548	8.5757	2.1715	3.835	1.7734	14.687

LOD & LOQ

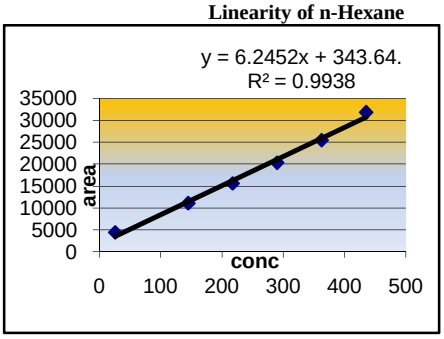
Table.17: Diluted concentrations of RS for LOD & LOQ

Concentration	Methanol in ppm	IPA in ppm	n-Hexane in ppm	Toluene in ppm	Acetic acid in ppm	O-Xylene in ppm	DMF in ppm
Level -1	60	100	5.8	17.8	100	3.8	17.6
Level -2	120	200	11.6	35.6	200	7.6	35.2
Level -3	180	300	17.4	53.4	300	11.4	52.8
Level -4	240	400	23.2	71.2	400	15.2	70.4
Level -5	300	500	29	89	500	19	88
Level -6	360	600	34.8	106.8	600	22.8	105.6

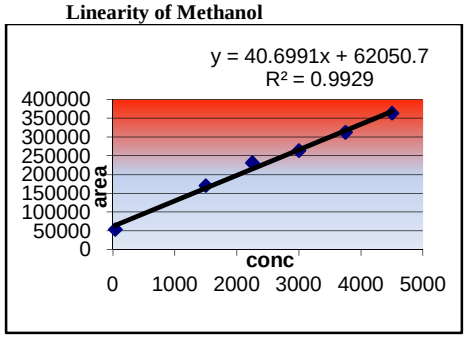
Table.18: Areas of each RS in LOD & LOQ solutions

Concentration	n-Hexane	Methanol	IPA	Toluene	O-Xylene	DMF	Acetic acid
Level -1	ND	ND	ND	ND	ND	ND	ND
Level -2	328	10317	10233	3552	2195	4166	10484
Level -3	623	20961	25982	7713	2907	5484	14926
Level -4	1023	31122	36287	13353	4467	7237	19079
Level -5	2201	35219	56497	23445	6533	7749	33852
Level -6	2582	52234	64162	28356	7827	9604	31038

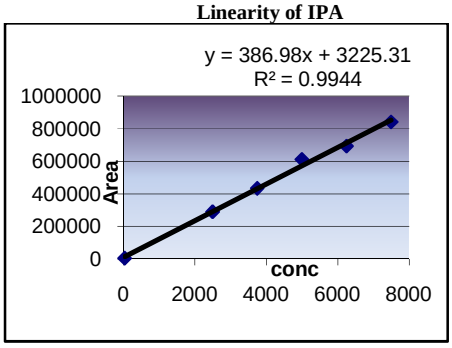
Linearity of n-Hexane



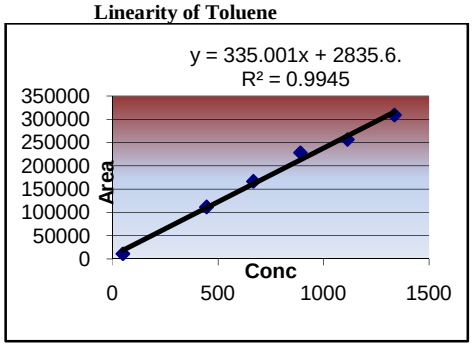
Linearity of Methanol



Linearity of IPA



Linearity of Toluene



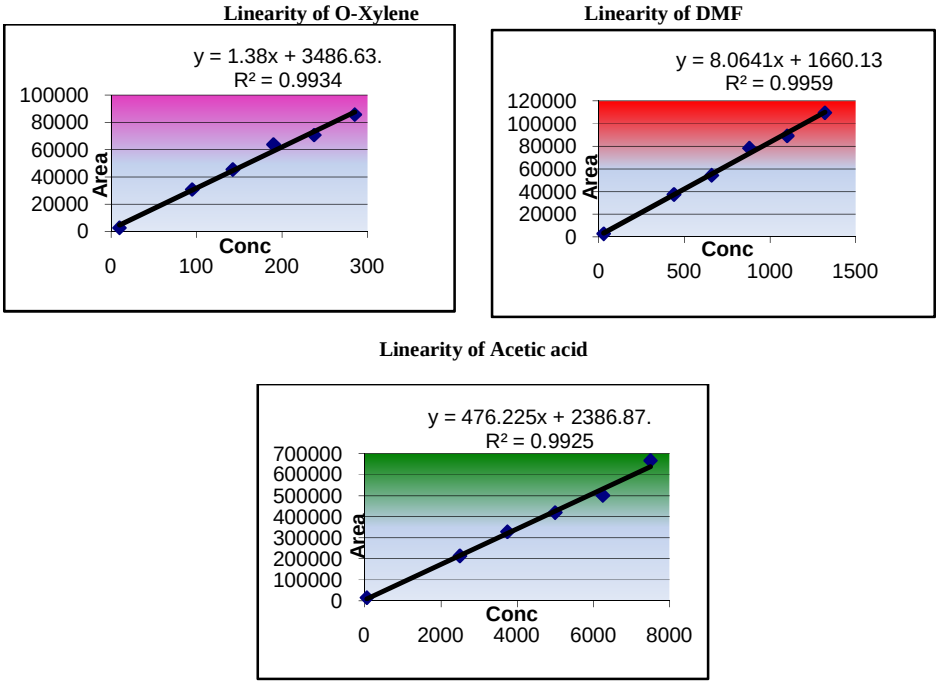


Fig.3: Linearity plots of Residual solvents

CONCLUSION

This study presents a simple and validated Gas chromatographic method for estimation of residual solvents in Amlodipine besylate. The developed method is simple, specific, accurate and precise. The amount of residual solvents present in the Amlodipine besylate was found to be within the ICH limits.

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