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Method Development and Validation of Gemifloxacin by using Gas Chromatography

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ABSTRACT

The aim of present research work to development of simple, rapid and cost-effective method for the estimation Gemifloxacin by using gas chromatography with flame ionization detector (GC-FID).The solutions of standard and the sample were prepared in DMSO and Dichloromethane and Isopropyl were used as residual solvents. GC separation was performed by 30m x 0.53mm ID fused silica coated with 6% cyanopropyl 94% dimethylpolysiloxane (DB 624 of SGE make is suitable).Nitrogen was used as carrier gas at a flow-rate of 4.18 ml/min. After injection of the sample at inlet temperature 1250o c, the temperature of the GC oven was as follows: initial temperature was 40oc, held for 5 min, increased to 125oC at a rate of 8oc min-1 held for 5 min, and finally to 225oc at a rate of 14oc min-1 and held for 10 min. Detector temperature is 250oc. 1.5 µl was injected in split less mode. Calibration curves were linear between the concentration range 2.5-1.5µg ml-1. The method was validated for specificity, linearity, precision, accuracy and limit of quantitation. Also, the method was applied to directly and easily to the analysis of the pharmaceutical preparation of Gemifloxacin tablet.

Keywords: Gemifloxacin, Gas chromatography, DMSO, Pharmaceutical preparation

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1. Introduction

Gemifloxacin is an oral broad-spectrum quinolone antibacterial agent used in the treatment of acute bacterial exacerbation of chronic bronchitis and mild-to-moderate pneumonia. Gemifloxacin acts by inhibiting DNA synthesis through the inhibition of both DNA gyrase and topoisomerase IV, which are essential for bacterial growth. It is used to For the treatment of bacterial infection caused

by susceptible strains such as *S. pneumoniae, H. influenzae, H. parainfluenzae,* or *M. catarrhalis,S. pneumoniae* (including multi-drug resistant strains [MDRSP]), *M. pneumoniae*, *C. pneumoniae*, or *K. pneumoniae*.

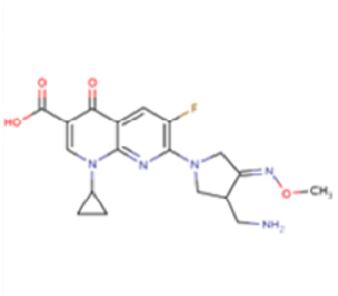


Fig 1: Chemical Structure of Gemifloxacin

From the literature survey conducted, it was found that there are no analytical methods reported for the quantitative estimation of residual solvents by gas chromatography in Gemifloxacin tablets. The main objective of the present work is to develop an analytical method for the quantitative estimation of residual solvents by gas chromatography in Gemifloxacin tablets.

2. Methodology

Instruments used:

Gas chromatography consist of AGILENT 6890 N Series with Chromeleon Software and containing 30m x 0.53mm ID fused silica coated with 6% cyanopropylphenyl 94% dimethylpolysiloxane column (DB 624 of SGE make is suitable or equivalent)with Flame ionization Detector.

Chemicals Used:

The standard drug of Gemifloxacin was purchased from Micro Labs Ltd. Bangalore, Karnaktaka. And GC grade Dimethyl sulfoxide (DMSO), Dichloromethane and Isopropyl alcohol were used in this study.

Optimized Chromatographic Conditions:

Column : 30m x 0.53mm ID fused silica coated with 6%cyanopropyl 94% dimethylpolysiloxane (DB 624 of SGE make is suitable)

Carrier gas : Nitrogen

Injector temperature: 125°C

Detector temperature: 250°C

Split ratio : 1:5

Purge flow : 4.18ml/min (linear velocity of 30cm/sec)

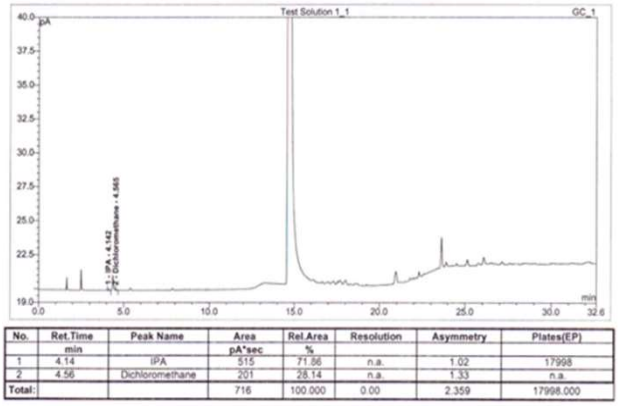
Column temperature programme

Step	Rate (°C/min)	Temperature (°C)	Time (min)
Initial	---	40	5
1	8	125	0
2	10	175	0
3	14	220	10

Run time : About 33 minutes

Head Space Conditions:

Cycle	:	HS-Inj
Sample Volume	:	1.0 ml (as gas)
Incubation Temperature	:	105.0°C
Incubation Time	:	45 minutes
Agitation speed	:	250rpm
Syringe Temperature	:	110.0°C
Fill Speed	:	500 µl/second
Pull up delay	:	1.0 second
Pre inject delay	:	200 µl/second
Pst inject delay	:	200 µl/second
Syringe flushing	:	3.00min
GC cycle time	:	45 min



No.	Ret.Time min	Peak Name	Area pA*sec	Rel.Area %	Resolution	Asymmetry	Plates(EP)
1	4.14	IPA	515	71.86	n.a.	1.02	17998
2	4.56	Dichloromethane	201	28.14	n.a.	1.33	n.a.
Total:			716	100.000	0.00	2.359	17998.000

Fig 2: Optimized Chromatogram

Preparation of analytical solutions:

Preparation of diluent: Dimethylsulphoxide (DMSO) was used as diluent.

Preparation of stock solution of Dichloromethane (Methylene chloride): About 120mg of methylene chloride was accurately weighed and it was transferred into a 100ml volumetric flask and diluted to volume with DMSO.

Preparation of stock solution of isopropyl alcohol: About 1000mg of isopropyl alcohol was accurately weighed and transferred into a 100ml volumetric flask and diluted to volume with DMSO.

Preparation of Gemifloxacin Standard solution:

About 740mg of placebo powder of Gemifloxacin mesylate Tablets 320mg was accurately weighed and transferred into a 25ml volumetric flask, 15ml of DMSO was added to dissolve the content and made up to the volume with DMSO. 1 ml of this solution was transferred into a dry and clean vial and it was closed with a septum. The cap was crimped and subjected into auto sampler.

Preparation of Gemifloxacin Sample solution:

About 1060mg of tablet powder equivalent to 320mg Gemifloxacin mesylate was accurately weighed and transferred into a 25ml volumetric flask, 15ml of DMSO was added to dissolve the content and made up to the volume with DMSO. 1 ml of this solution was transferred into a dry and clean vial and it was closed with a septum. (Teflon coated Butyl rubber septum). The cap was crimped and subjected into auto sampler. Like this another two vial were prepared.

3. Results and Discussion

Method Validation: Method validation was done by according to ICH guidelines Q2R1. The validation parameters like specificity, linearity, accuracy, precision, quantification limits, robustness and system suitability.

Specificity:

Specificity is the ability to asses unequivocally the analyte in the presence of impurities, degradants, matrix etc (components) which may be expected to be present.There shall be minimum no interference of the peaks observed from blank and placebo at the retention time of the peak obtained due to Dichloromethane and Isopropyl alcohol.

Linearity:

The linearity of the analytical method for each residual solvents was demonstrated (i.e. Dichloromethane and isopropyl alcohol) by injecting the various concentrations of standard solution prepared in the range of 10% to 150% with respect to the test concentration covering 7 different concentrations. A plot between the concentrations in ppm vs. peak response of residual solvents was drawn. The slope, intercept and regression coefficient from the plot was reported.

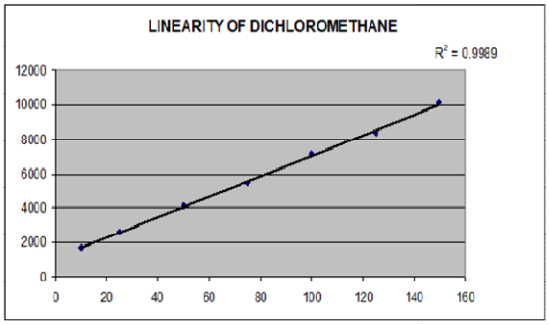


Fig 3: Linearity graph of Dichloromethane

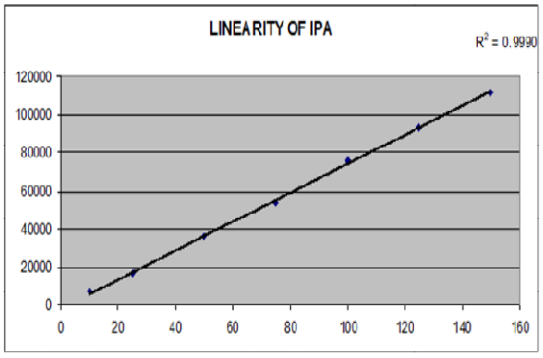


Fig 4: Linearity graph of IPA

Accuracy:

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The recovery studies were performed by adding known quantities of each solvent to placebo preparation in the range of with respect to test concentration.The recovery should lie between 85% and 115% for each level solvent peak. The relative standard deviation (RSD) of all recovery values should not be more than 15.0%.

Precision:

Precision can be defined as "the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogenous sample. The repeatability of the analytical method was established by estimating the content of Dichloromethane and Isopropyl alcohol for six different sample preparations of the same batch of Gemifloxacin measylate Tablets 320mg.The content of Dichloromethane and Isopropyl alcohol for all six-sample preparations was calculated and the RSD for the same was reported.

Limit of Detection:

A series of lower concentration from standard solution to achieve appropriate concentration were prepared at which the signal to noise ratio of solvent shall be more than or equal to 3.0. 1.0ml of the blank, standard solution and different LOD solution separately injected into the chromatograph. The chromatograms were recorded and the peak responses were measured. LOD of dichloromethane was found to be **0.50**µg/ml and Isopropyl alcohol was found to be **0.42**µg/ml.

Limit of Quantification:

A series of lower concentration from standard solution were prepared to achieve appropriate concentration at which the signal to noise ratio of solvent shall be more than or equal to 10.0. LOD of dichloromethane was found to be **1.9**µg/ml and Isopropyl alcohol was found to be **1.5**µg/ml.

Robustness:

The robustness of the analytical methoddetermined by estimating the content of residual solvents in Gemifloxacin mesylate Tablets 320mg under deliberately modified chromatographic conditions and the %RSD of results of the same was reported. The actual chromatographic conditions of flow rate specified under the methoddeliberately modified on lower and higher side of the actual value .The residual solvents in Gemifloxacin mesylate Tablets 320mg under these deliberately modified chromatographic conditions were determined.

System suitability:

To determine the suitability of chromatographic system described for the method of analysis by establishing system suitability parameters like resolution between adjacent peaks, tailing factor and the relative standard deviation for peak response for each solvent peak obtained from replicate injections of standard preparation.

Table 1: Specificity results for standard solution

IPA & DCM Standard Preparation				
S.No	**Retention Time of Isopropyl Alcohol**	**Area of Isopropyl Alcohol**	**Retention Time of Dichloromethane**	**Area of Dichloromethane**

1	4.13	51750	4.57	4033
2	4.13	51992	4.58	4054
3	4.14	51677	4.57	3992
4	4.13	50012	4.57	3787
5	4.13	51471	4.57	3941
Average	4.14	51445	4.58	4036
SDEV	0.01	787.30	0.01	106.62
RSD	0.11	1.53	0.10	2.64

Table 2: Specificity results for sample solution

IPA & DCM Sample Preparation				
S.No	**Retention Time of Isopropyl Alcohol**	**Area of Isopropyl Alcohol**	**Retention Time of Dichloromethane**	**Area of Dichloromethane**
1	4.13	515	4.57	210
2	4.14	460	4.58	273
3	4.13	506	4.57	254
Average	4.13	493.66	4.57	245.67
SDEV	0.01	29.51	0.01	32.31
RSD	0.11	0.05	0.10	0.13

Table 3: Linearity of Isopropyl alcohol and Dichloromethane

S.No	**Concentration**	**Concentration in ppm (IPA)**	**Area**	**Average Area**	**Concentration in ppm(DCM)**	**Area**	**Average Area**
1	25%	25	15783	16726	3	2529	2589
			16961			2628	
			17436			2610	
2	50%	50	34678	35975	6	4175	4163
			33616			4069	
			39630			4245	
3	75%	75	53573	53371	9	5488	5476
			53699			5439	
			52840			5501	
4	100%	100	76180	76040	12	7139	7142
			76214			7107	
			75727			7181	
5	125%	125	93665	93725	15	8317	8363
			94045			8348	
			93464			8423	
6	150%	150	109554	111391	18	10117	10107
			114611			10063	
			110007			10141	

Table 4: Accuracy results for Isopropyl alcohol

S.No	**Concentration**	**Area of IPA in Recovery**	**Average area of IPA in Recovery**	**ppm Recovered**	**Percentage Recovered**
1	25%	23940	24296	25.08	100.33
		24677			
		24271			
2	50%	49216	49719	52.33	100.65
		50481			
		49460			
3	75%	60452	73113	75.89	101.18
		78337			
		80550			
4	100%	103972	102977	100.93	98.90

		101152			
		103808			
5	125%	131482	129493	126.75	101.40
		129775			
		127223			
6	150%	159695	150633	152.95	101.96
		142574			
		149629			
Average					100.86
SD					1.03
%RSD					0.01

Table 5: Accuracy results for Dichloromethane

S.No	Concentration	Area of IPA in Recovery	Average area of IPA in Recovery	ppm Recovered	Percentage Recovered
1	25%	3095	3156	2.92	97.33
		3209			
		3164			
2	50%	5962	6082	6.13	102.17
		5919			
		6366			
3	75%	9640	9270	8.97	99.67
		9035			
		9134			
4	100%	12427	12554	12.23	101.72
		12698			
		12537			
5	125%	14909	15064	14.89	99.27
		15538			
		14745			
6	150%	17601	17333	18.31	101.72
		17015			
		17383			
Average					100.77
SD					2.09
%RSD					0.02

Table 6: Precision results for Isopropyl alcohol (IPA) of six different solutions

S.No	Sample weight(mg)	Area	Average Area	IPA in ppm
1	1060.2	4903	4908	106.05
		4913		
2	1060.5	4905	4903	106.08
		4900		
3	1060.2	4748	4868	105.26
		4987		
4	1061.9	4984	4989	107.90
		4993		
5	1062.3	4911	4917	106.40
		4922		
6	1061.5	4974	4964	107.40
		4953		
Average			4924	105.80
Standard Deviation			44.01	0.46
RSD			0.01	0.44

Table 7: Precision results for Dichloromethane (DCM)for six different solutions

S.No	Sample weight(mg)	Area	Average Area	DCM in ppm
1	1060.2	1883	1659	33.90
		1434		
2	1061.5	1585	1502	30.73
		1418		
3	1062.2	1651	1698	34.73
		1744		
4	1060.9	1693	1723	35.26
		1753		
5	1060.3	1473	1406	28.78
		1339		
6	1060.5	1668	1568	32.10
		1468		
Average			4924	33.12
Standard Deviation			44.01	2.11
RSD			0.01	6.36

Table 8: Robustness for Isopropyl alcohol: Change in flow rate(High)

S.No	Sample weight	Area	Average Area	Result in ppm
1	1060.4	4598	4431.5	104.31
		4265		
2	1061.7	4124	4349	102.45
		4574		
3	1063.4	4112	4337.5	102.16
		4563		
Average			4154.67	102.97
Standard Deviation			82.08	1.16
%RSD			0.019	1.13

Table 9: Robustness for Dichloromethane: Change in flow rate(High)

S.No	Sample weight	Area	Average Area	Result in ppm
1	1060.4	4598	4431.5	104.31
		4265		
2	1061.7	4124	4349	102.45
		4574		
3	1063.4	4112	4337.5	102.16
		4563		
Average			4154.67	102.97
Standard Deviation			82.08	1.16
%RSD			0.019	1.13

Table 10: Robustness for Isopropyl alcohol: Change in flow rate(Low)

S.No	Sample weight	Area	Average Area	Result in ppm
1	1060.3	3356	2958.5	76.14
		2561		
2	1060.2	2658	3010	77.53
		3362		
3	1060.4	3912	3656.5	94.17
		3401		
Average			3208.33	82.61
Standard Deviation			388.97	10.03
%RSD			0.12	12.14

Table 11: Robustness for Dichloromethane: Change in flow rate(Low)

S.No	Sample weight	Area	Average Area	Result in ppm
1	1060.3	-----	Not detected	Not detected

2	1060.6	-----	Not detected	Not detected

3	1060.5	-----	Not detected	Not detected

Table 12: Robustness for Isopropyl alcohol: Change in Column oven temperature (High)

S.No	Sample weight	Area	Average Area	Result in ppm
1	1060.3	3413	3509.5	106.96
		3606		
2	1060.8	3436	3624.5	110.56
		3813		
3	1060.1	3564	3407.5	103.92
		3251		
Average			3513.83	107.14
Standard Deviation			108.56	3.32
%RSD			0.03	3.10

Table 13: Robustness for Dichloromethane: Change in Column oven temperature (High)

S.No	Sample weight	Area	Average Area	Result in ppm
1	1060.3	-----	Not detected	Not detected

2	1060.8	-----	Not detected	Not detected

3	1060.3	-----	Not detected	Not detected

Table 14: Robustness for Isopropyl alcohol: Change in Column oven temperature (Low)

S.No	Sample weight	Area	Average Area	Result in ppm
1	1060.3	6883	5309.5	115.12
		3736		
2	1060.8	4135	4102.5	89.03
		4070		
3	1060.2	3267	3361	72.92
		3455		
Average			4257.66	92.36
Standard Deviation			983.57	21.30
%RSD			0.23	0.23

Table 15: Robustness for Dichloromethane: Change in Column oven temperature (Low)

S.No	Sample weight	Area	Average Area	Result in ppm
1	1060.3	6883	5309.5	115.12
		3736		
2	1060.8	4135	4102.5	89.03
		4070		
3	1060.2	3267	3361	72.92
		3455		
Average			4257.66	92.36
Standard Deviation			983.57	21.30
%RSD			0.23	0.23

Table 16: Results for system suitability

S.No	Areas		Resolution	Theoretical Plates		Tailing Factor	
	IPA	DCM		IPA	DCM	IPA	DCM
1	51750	4033	3.64	16787	26384	1.12	1.07

2	51992	4054	3.1	16812	26735	1.14	1.02
3	51677	3992	3.67	16858	26995	1.14	1.03
4	50012	3787	3.68	16852	21835	1.14	1.08
5	51471	3941	3.68	16858	26671	1.15	1.08
6	51445	4036	3.66	16832	26151	1.1	1.05
Average	51445	4036	3.57	16833.1	25795.16	1.13	1.055
SDEV	787.30	106.62	0.23	28.88	1961.95	0.02	0.03
%RSD	1.53	2.64	6.483	0.172	7.606	1.621	2.453

Acceptance criteria: a) The resolution between the two components should not be less than 1.0.
b) The tailing factor should not be more than 2.0.
c) The RSD for the area response of the replicate injections of the standard solution should not be more than 15% for each solvent peak.

4. Conclusion

A method was developed for the estimation of Residual solvents in Gemifloxacin tablets by gas chromatography. The developed method was validated as per ICH guidelines. System suitability test is established and recorded. The method found to be specific for Residual solvent of Gemifloxacin tablets. The method is found to be linear in the specified range. For the LOD & LOQ, method has been established. Hence, this method stands validated and can be used for routine analysis.

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