Available online at **www.joac.info**

ISSN: 2278-1862

Journal of Applicable Chemistry

2023, 12 (1): 42-52

(International Peer Reviewed Journal)

Method of Validation for Residual Solvents in Brimonidine tartrate by GC-HS

Sunil Lakhmapure[1], Sandip A Telavane[1], Seema Kothari[1] and Manohar V. Lokhande[2]*

1. Department of Chemistry PAHER University, Udaipur-313003, Rajasthan, **INDIA**
2. Department of Chemistry, Sathaye College, Mumbai-400057, Maharashtra, **INDIA**

Email: manohar2210@gmail.com

Accepted on 20th January, 2023

ABSTRACT

GC-HS is vital method for recognition, development and valuation of drugs in pharmaceutical analysis. Aim of this article was to check the progress and validation of the method employed for the Residual Solvents in Brimonidine Tartrate by Gas Chromatography Headspace technique. The objective of this protocol is to validate a GC-HS method of analysis for detection and Quantification of Residual Solvents Acetone, Benzene, Dichloromethane, Ethyl Acetate, Toluene, Isopropyl alcohol and bromobenzene in Brimonidine Tartrate. In the pharmaceutical industry, validation policy is more important for documentation for types of validation and validation policy. The method was developed accurately and validation parameters were explained. Chromatographic condition was Clarus 600 with parkin Elmer instrument, column: 30m x 0.53 mm-lD, 3.0 pm GS-TEK-624 column or equivalent (G-35) or equivalent and column temperature was 45°C (hold 15 min) to 250°C @ 40°C min^{-1}, hold at 250°C for 3 min. The parameters such as Specificity, Limit of detection (LOD) and Limit of quantitation testing with residual solvent such as Acetone, Benzene, Dichloromethane, Ethyl Acetate, Toluene, Isopropyl alcohol and Bromobenzene. All validation parameters are used in the routine and stability analysis.

Keywords: Brimonidine tartrate, GC-HS, Specificity, LOD and LOQ.

INTRODUCTION

Brimonidine is an alpha-adrenergic agonist and 2-imidazoline derivative initially familiarized in 1996 [**1**]. It is further careful for alpha-2-adrenergic receptors [**2**] than clonidine or apraclonidine, which are similarly alpha-2-adrenergic agonists glaucoma. As glaucoma is known globally as a communal source of blindness, primary treatment and regulate the glaucoma, which is mostly related through condensed intraocular pressure is significant [**3, 4**]. Chemically, brimonidine tartrate is 5-bromo-6-(2-imidazolidinylideneamino) quinoxaline L-tartrate. It is a water-soluble α2 adrenergic agonist that works by reducing the formation of aqueous humour and increasing the outflow of aqueous humour through the trabecular network. Brimonidine binds extensively and reversibly to melanin in ocular tissues without any adverse effects [**5**]. Recent studies suggest that brimonidine may promote the survival of injured retinal ganglion cells by activating the α2-adrenoceptor in the retina and/or optic nerve [**6**]. Brimonidine tartrate is therefore a potential anti-glaucoma agent. Patients on permanent brimonidine generally suffer from subclinical conjunctivitis and ocular allergy [**7, 8**]. Since

brimonidine tartrate is only available on the market in the form of a drop solution (Alphagan Z 0.1%), research should continue for better drug delivery options that allow slow and sustained release of the drug. In such circumstances, non-ionic surfactant vesicles could be a possible alternative ocular drug delivery system. Therefore, the objectives of our research to validate the parameters such as Specificity, Precision, Linearity, Limit of detection (LOD), Limit of quantitation (LOQ), and linearity testing with residual solvent such as Acetone, Benzene, Dichloromethane, Ethyl Acetate, Toluene, Isopropyl alcohol and Bromobenzene.

MATERIALS AND METHODS

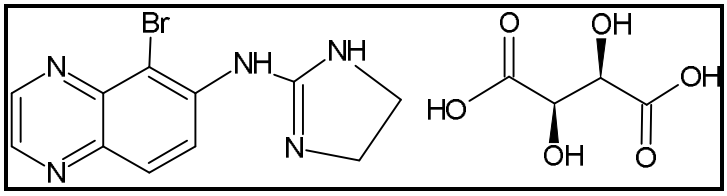
Material:

Common Name: Brimonidine tartrate

IUPAC Name : 5-bromo-*N*-(4, 5-dihydro-1*H*-imidazol-2-yl) quinoxalin-6-amine; (2*R*, 3*R*)-2, 3-dihydroxybutanedioic acid.

Molecular Formula: $C_{15}H_{16}BrN_5O_6$

Structure:



Methods:

Table 1. Chromatographic conditions

Instrument	Clarus 600
Instrument Make	Perkin Elmer
Injector Temperature	150°C
Column	30m x 0.53 mm-lD, 3.0pm GS-TEK 624 column or equivalent(G-35)
Initial Column Oven temp	40 °C
Hold Time	15 Min
Ramp Rate	25°C Min^{-1}
Final Column Oven Temp	210°C
Hold Time	2.0 min
GC Run Time	23.80 min
Carrier Gas	Nitrogen
Carrier gas Flow rate	2.0 mL min^{-1}`
Detector Type	FID
Detector Temp	250 °C
Detector Sensitivity	Range 1; Attenuation-5
*Detector sensitivity Programme	At 18.0 min Attenuation ATT1=2. At 18.1 min Autozero A/Z1. At 25.0 min Attenuation ATT1=-5.

Preparation of solutions:

i) **Preparation of Solution 1(Benzene 1000 ppm):** weigh accurately about 100 mg of Benzene in a 100 mL standard volumetric flask containing sufficient DMSO. Dilute to volume with DMSO.

ii) **Preparation of Solution-2 (Benzene10ppm, Dichloromethane 3000 ppm, Toluene 4450 ppm):** weigh accurately about 300 mg of Dichloromethane and 445 mg of Toluene in a 100 mL standard volumetric flask containing sufficient DMSO. Add to it 1.0 mL solution-1 and dilute the volume with DMSO.

iii) **Preparation of Standard solution (Benzene 1.0 ppm, Dichloromethane 300 ppm, Ethyl Acetate 2000 ppm, Toluene 445 ppm):** Weigh accurately about 200 mg gr Ethyl acetate in a 100 mL standard volumetric flask containing sufficient DMSO. Add to it 10 mL solution-2 and dilute the volume with DMSO.

Table 2. Head Space Conditions.

Instrument	Turbomatrix 110HS
Instrument Make	Parkin Elmer
Vial Oven Temperature	80 ºC
Vial Conditioning Time	For 30 min
Needle Temperature	85 ºC
Transfer Line Temperature	90 ºC
Vial Pressurization time	For 30.0 min
Programmable Pneumatic Control Pressure	20psi
Injection Volume	1.5 mL in 0.15min
Cycle Time	33 min

Preparation of vials:

i) **Preparation of a Blank vial:** Pipette 1.0 mL of DMSO each into two separate Clean Dry Vials. Add to them 5.0 mL of water and seal them.

ii) **Preparation of Standard solution vials:** pipette 1.0 mL of standard solution each immediately and accurately in six separate vials. Add to them 5.00 mL water and seal the vials.

iii) **Preparation of a Test vial:** Weigh accurately about 500 mg of the test solution under analysis into a vial. Add 1.0 mL of DMSO to the sample to dissolve the sample. Add 5.0 mL of water and seal the vials.

Procedure: The GC-HS system shall be set with the chromatographic conditions as mentioned above, the vials of the respective solutions will be injected as below;

Injection	Solution	Number of Vials
1	Blank	1.0
2	Standard	6.0
3	Blank	1.0
4	Test	1.0

The Standard solution chromatograms will be evaluated for the following for each of the solvent

a) RSD of Area for each residual solvent.
b) Tailing Factor for each solvent
c) Theoretical Plates for each solvent
d) Resolution between each solvent

System suitability criteria to be evaluated:

i) %RSD of Area for each residual solvent NMT 15 0%
ii) Tailing factor for each solvent NMT 2 0.
iii) Theoretical plates for each solvent NMT 5000
iv) Resolution between each solvent NLT1

Calculation for Content of each residual solvent:

$$\text{Content of each residual solvent (ppm)} = \frac{\text{Aspl}}{\text{Astd}} \times \frac{\text{Wstd}}{\text{Wspl}} \text{ D.F} \times 10^6$$

Where, Aspl= Area of the individual solvent under study in test $ample Astd = Average Area of the individual solvent under study from Standard solution injections. Wstd = Weight of respective standard (mg) Wspl = Weight of the test sample (mg). D.F. = Dilution Factor

Limit of Residual solvent impurities:

Benzene	NMT 2 ppm
Dichloromethane	NMT 600 ppm
Ethyl acetate	NMT 5000 ppm
Toluene	NMT 890 ppm

RESULTS AND DISCUSSION

Specificity is the ability to accurately and specifically measure the analyte of interest in the presence of other expected components in the sample matrix [**9**]. Selectivity is checked by examining chromatographic blanks (from a sample that is known to contain no analyte) in the expected time window of the analyte peak [**10**], and the raw data for selectivity will be recorded in the raw data in approved formats (Table 1-3 and figure 1-3).

Validation Parameters

Solution	Solvent	Weight(mg)	Volume (mL)	Dilution (mL)
Solution-1	Benzene	101.1		100.00
Solution-2	Dichloromethane	300.8	1.0 (Solution-1)	100.00
	Toluene	445.9		
Standard Solution	Ethyl Acetate	201.1	10.0(Solution-2)	100.0

Preparation of Specificity solutions:

Specificity solutions	Solvent	Weight (mg)	Dilution (mL)
1	Acetone	101.2	100
2	Benzene	101.4	100
3	Bromobenzene	101.8	100
4	Dichloromethane	101.3	100
5	Ethyl acetate	100.9	100
6	Isopropyl alcohol	101.2	100
7	Methanol	100.9	100
8	Toluene	101.5	100

Preparation of vials:

Solution	Pipette (mL)	Volume Added (mL)	Vials Prepared
Blank	1.0	05	02
Standard	1.0	05	06
1	1.0	05	1
2	1.0	05	1
3	1.0	05	1
4	1.0	05	1
5	1.0	05	1
6	1.0	05	1
7	1.0	05	1
8	1.0	05	1

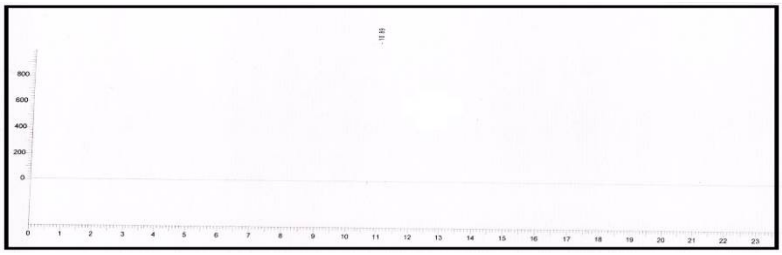
The result obtained are tabulated as follows:

Solvent	RT	Relative RT Bromobenzene Peak
Blank	ND	--
Acetone	07.12	0.33
Benzene	16.98	0.78
Bromobenzene	21.85	1.00
Dichloromethane	08.55	0.39
Ethyl acetate	14.58	0.67
Isopropyl alcohol	07.60	0.35
Methanol	04.58	0.21
Toluene	19.66	0.90

Preparation of Specificity solutions:

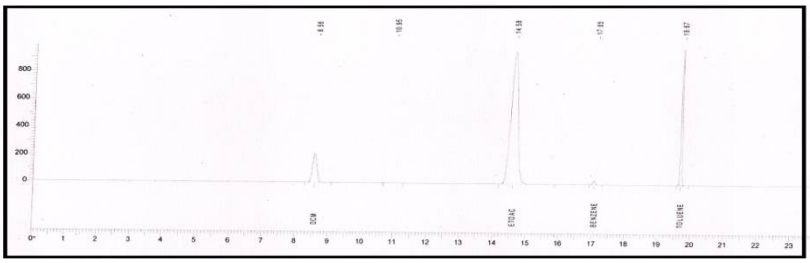
Solvent	Weight (mg)	Dilution (mL)
Benzene	101.4	100
Dichloromethane	101.3	100
Toluene	101.5	100
Ethyl acetate	100.9	100
Acetone	101.2	100
Bromobenzene	101.8	100
Isopropyl Alcohol	101.2	100
Methanol	100.9	100

Solvent	RT (min)	RRT (Bromo benzene)
Blank (DMSO)	ND	-
Benzene	16.98	0.78
Dichloromethane	08.55	0.39
Toluene	19.66	0.90
Ethyl acetate	14.58	0.67
Acetone	7.12	0.33
Bromobenzene	21.85	1.00
Isopropyl Alcohol	07.60	0.36
Methanol	04.58	0.21



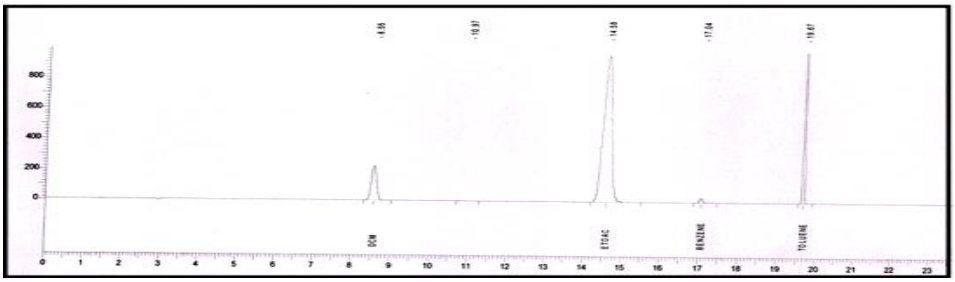
Name	RT	Area	Area %
SUM	10.89	18081	100.00
		10881	100.00

Figure 1. Blank Chromatogram.



Name	RT	Area	Area %	Tan Plates	Tailing factors	Resolution
Dichloromethane	8.56	2012507	10.29	16932	1.03	0.00
	10.95	21218	0.11	0.00	0.00	0.00
Ethyl acetate	14.58	14325382	73.24	20456	0.98	17.96
Benzene	17.05	155991	0.80	220556	1.03	8.93
Toluene	19.67	3044222	15.56	1062558	1.14	23.78
		19559320	100.00			

Figure 2. Standard solution.



Name	RT	Area	Area %	Tan Plates	Tailing factors	Resolution
Dichloromethane	8.55	2147226	10.14	17932	1.05	0.00
	10.97	24073	0.12	0.00	0.00	0.00
Ethyl acetate	14.58	14442686	72.22	21024	0.96	18.34
Benzene	17.04	170020	0.85	220648	1.04	9.00
Toluene	19.67	3214312	16.07	1072068	1.22	23.71
		19008317	100.00			

Figure 3. Standard solution

Preparation of Standard solution-1

Solvent	Weight (mg)	Volume Taken	Dilution (mL)
Benzene	101.1		100
Dichloromethane	300.9	1.0	100
Toluene	445.8		
Ethyl acetate	201.1	10.0	100

Table 3. Standard solution Results

Inj. #	Dichloromethane	Ethyl acetate	Benzene	Toluene
1	2012507	14325382	155991	3044222
2	2147226	14442686	170020	3214312
3	2094825	14235360	165209	3172099
4	2063992	14516121	162120	3109954
5	2138498	14197610	169057	3232603
6	2149463	14430889	169390	3223603
Mean	2101085	14430869	165298	3223440
SD	**55010**	**245886**	**5475**	**74937**
% RSD	**2.62**	**1.70**	**3.31**	**2.37**

Inj. #	Dichloromethane		Ethyl acetate (EA)		Benzene (B)		Toluene		Resolution EA & B
	Theoretical Plates	Tailing Factors	Theoretical Plates	Tailing Factors	Theoretical Plates	Tailing Factors	Theoretical Plates	Tailing Factors	
1	16932	1.03	20456	0.98	220558	1.03	1082556	1.14	8.93
2	17954	1.05	21024	096	220648	1.04	1072068	1.22	9.00
3	17552	1.05	20976	0.98	226432	1.01	1052480	1.24	9.04
4	17606	1.06	20862	0.96	223796	1.02	1059361	1.22	8.97
5	18325	1.04	20793	0.99	222362	1.02	1057382	1.23	8.98
6	17276	1.06	20556	0.98	230045	1.03	1069935	1.29	8.97
Mean	**17608**	1.05	20778	0.98	223974	1.03	1065630	1.22	8.98

Limit of Detection/Quantitation (LOD/LOQ): Limits of detection are usually evaluated for quantitative assays and impurities. ICH Q2 [**11**] defines LOD. Limit of Detection LOD is the lowest concentration at which a method can reliably detect (but not quantify) an analyte in a matrix. It is also defined as the lowest concentration that can be reliably separated from the background [**12**]. Detection limits are usually determined only for qualitative measurements/limit studies of impurities, but may also be required for quantitative measurements (Table 4-9). The LOQ known as the limit of quantitation is the lowest concentration of an analyte that can be reliably quantified by a method (Figure 4-7). Reliable means that sufficient accuracy and precision must exist and be proven. Quantitative impurity studies require determination of the limit of quantification. Like the LOD, the LOQ can be determined in a variety of ways, depending on whether the method uses an instrument [**13, 14**].

Solution	Solvent	Weight(mg)	Volume (mL)	Dilution (mL)
Solution-1	Benzene	100.8	-	100.00
Solution-2	Dichloromethane	301.4	1.0 (Solution-1)	100.00
	Toluene	445.9		
Standard Solution	Ethyl Acetate	200.8	10.0(Solution-2)	100.0

Table 4. Preparation of Level Solutions

Solution	Volume of standard solution	Dilution (mL)
Level-I (10%)	1.0	10
Level-II (20%)	20.	10
Level-III (30%)	3.0	10
Level-IV (40%)	4.0	10
Level-V (50%)	5.0	10

Table 5. Preparation of vials

Solution	Pipetted Volume	Volume of water added	Number of vials prepared
Blank	1.0	5.0	2
Standard	1.0	5.0	6
Level-I (10%)	1.0	5.0	3
Level-II (20%)	1.0	5.0	3
Level-III (30%)	1.0	5.0	3
Level-IV (40%)	1.0	5.0	3
Level-V (50%)	1.0	5.0	3

Preparation of Standard solution-1

Solvent	Weight (mg)	Dilution (mL)
Benzene	100.8	100.00

Preparation of Standard solution-2

Solvent	Weight (mg)	Volume (mL)	Dilution (mL)
Dichromethane	301.4	1.0	100
Toluene	445.9		

Table 6. Results of standard solution

Injection#	Area of			
	Dichromethane	Ethyl acetate	Benzene	Toluene
1	2257594	14966754	173498	3283669
2	2321690	15315524	179191	3362858
3	2348564	15279187	181720	3394886
4	2376137	15486318	183733	3403103
5	2465589	15486318	189920	3403103
6	2348273	15294218	179552	3482028
Mean	**2352975**	**15220667**	**181269**	**3378018**
SD	**68317**	**204859**	**5455**	**66622**
%RSD	**2.90**	**1.35**	**3.01**	**1.97**

Preparation of Standard solution-3

Solvent	Weight (mg)	Volume (mL)	Dilution (mL)
Ethyl Acetate	200.8	1.0	100.00

Table 7. Results of standard solution

Inj#	Area of								
	Dichromethane		Ethyl acetate		Benzene		Toluene		Resolution Between Ethyl acetate and Benzene
	Theoretical plates	Tailing Factor	Theoretical plates	Tailing Factor	Theoretical plates	Tailing Factor	Theoretical plates	Tailing Factor	
1	17350	1.04	21123	1.04	247735	1.01	1052819	1.28	9.20
2	16554	1.03	20612	1.07	217300	1.04	1054196	1.32	9.01
3	17547	1.03	20949	1.03	245187	1.01	1027242	1.36	9.20
4	16344	1.05	20921	1.08	237377	1.02	1038917	1.36	9.18
5	17134	1.04	21276	1.10	227663	1.01	998404	1.39	9.17
6	16803	1.04	21247	1.10	225995	1.00	1041552	1.30	9.19
Mean	**16936**	**1.04**	**21000**	**1.07**	**232824**	**1.01**	**1035020**	**1.36**	**9.15**

Table 8. Limit of Detection/Quantitation

Inj#	Area of								
	Dichromethane		Ethyl acetate		Benzene		Toluene		Resolution Between Ethyl acetate and Benzene
	Theoretical plates	Tailing Factor	Theoretical plates	Tailing Factor	Theoretical plates	Tailing Factor	Theoretical plates	Tailing Factor	
1	17350	1.04	21123	1.04	247735	1.01	1052819	1.02	9.20
2	16554	1.03	20612	1.07	217300	1.04	1054195	1.03	9.00
3	17547	1.03	20949	1.03	245187	1.01	1027242	1.02	9.18
4	16344	1.05	20921	1.08	237377	1.02	1038917	1.01	9.19
5	17134	1.04	21276	1.10	227663	1.01	998404	1.01	9.18
6	16803	1.04	21247	1.10	225995	1.00	1041552	1.00	9.19
Mean	**16955**	**1.04**	**21021**	**1.07**	**233543**	**1.02**	**1035522**	**1.02**	**9.17**

Table 9. Dichloromethane chromatograms for LOD/LOQ

Level	Conc. of Dichloro methane (ppm)	Injection # No.	Area	Average Area
10%	30.1	1	275588	211614
		2	324947	
		3	274397	
20%	60.2	1	433013	463225
		2	446215	
		3	510448	
30%	90.3	1	592402	652576
		2	683794	
		3	681553	
40%	120.5	1	778956	780012
		2	779238	
		3	781842	
50%	150.8	1	818026	888403
		2	1005158	
		3	84025	

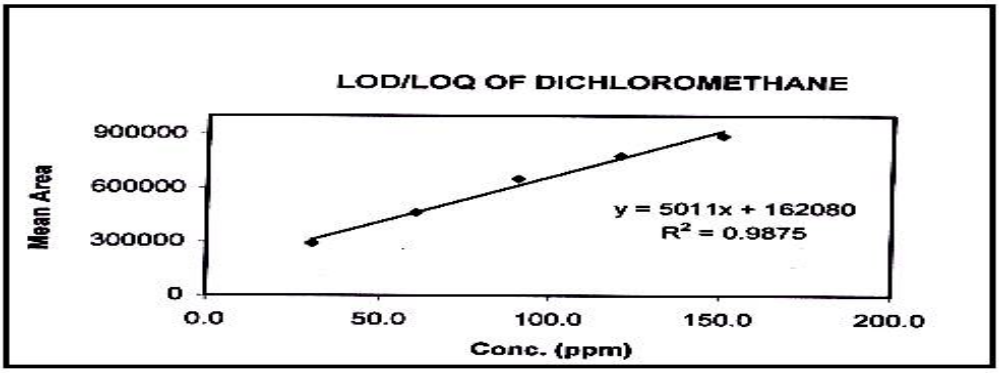
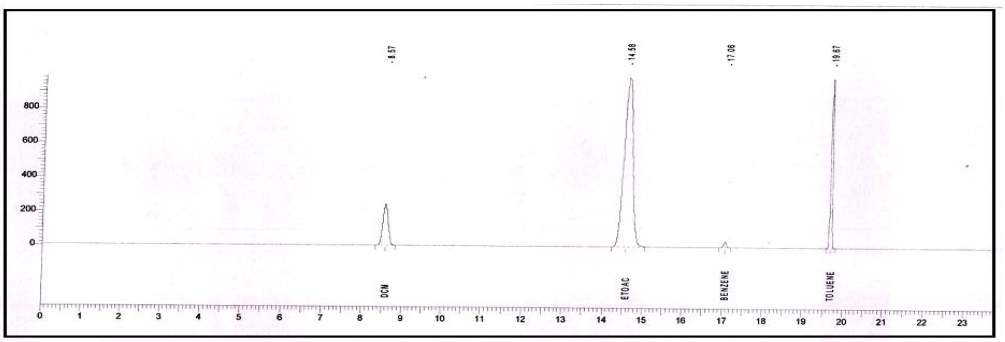
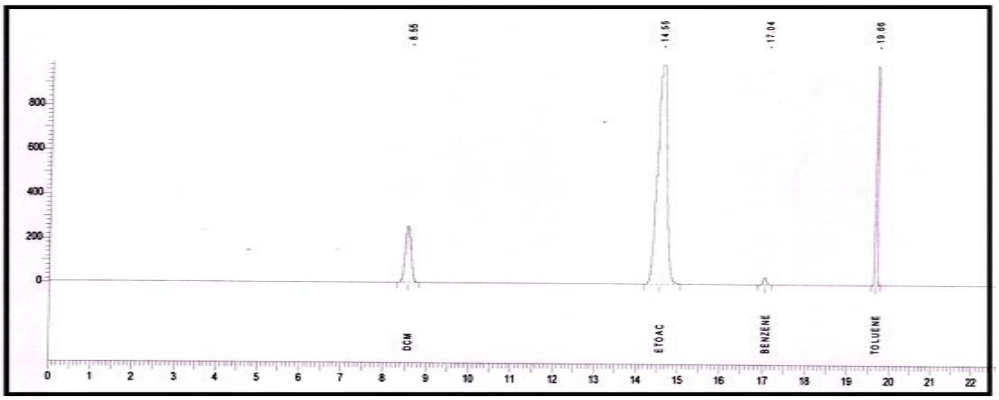


Figure 4. LOD/LOQ.



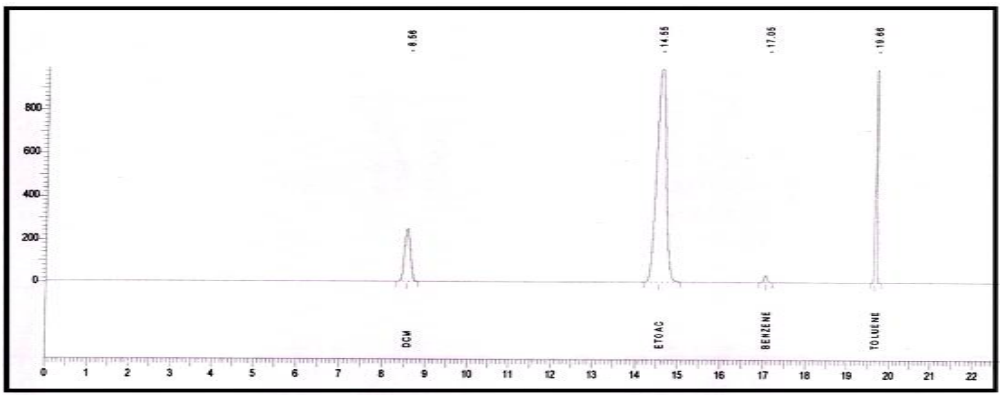
Name	RT	Area	Area %	Tan Plates	Tailing Factors	Resl'n
Dichloromethane	8.57	2257594	10.92	17350	1.04	0.00
Ethyl acetate	14.58	14966754	72.37	21123	1.04	18.18
Benzene	17.07	173498	0.84	247735	1.01	9.20
Toluene	19.68	3283669	15.89	1052819	1.28	24.47
Sum		20681515	100.0			

Figure-5. Chromatogram of LOD/LOQ.



Name	RT	Area	Area %	Tan Plates	Tailing Factors	Resl'n
Dichloromethane	8.55	24655589	11.40	17134	1.04	0.00
Ethyl acetate	14.55	15486318	71.62	21276	1.10	18.17
Benzene	17.04	173498	0.88	227663	1.01	9.18
Toluene	19.66	3283669	16.10	998404	1.39	23.59
Sum		20681515	100.0			

Figure-6. Chromatogram.



Name	RT	Area	Area %	Tan Plates	Tailing Factors	Resl'n
Dichloromethane	8.56	2348273	11.10	16803	1.04	0.00
Ethyl acetate	14.55	15294218	72.27	21247	1.10	18.07
Benzene	17.05	179552	0.85	225995	1.00	9.20
Toluene	19.66	3341562	15.79	1041552	1.30	23.73
Sum		21163605	100.01			

Figure 7. Chromatogram.

CONCLUSION

Optimum chromatographic conditions for separating brimonidine tartrate from other impurities in the leaching liquor or pharmaceutical formulations have been achieved by using Chromatographic condition was Clarus 600 with parkin Elmer instrument, column: 30m x 0.53 mm-lD, 3.0 pm GS-TEK- 624 column or equivalent (G-35) or equivalent and column temperature was 45°C (hold 15 min) to 250°C @ 40°C min^{-1}, hold at 250°C for 3 min. The proposed method was validated in accordance with ICH guidelines with respect to Specificity limit of detection and quantitation. The validation by GC-HS method after all types of stress tests, indicating an excellent separation of brimonidine tartrate peak from other impurities. The measurement course could be completed within 10 min, which was very quick, effective and convenient. Overall, the proposed GC-HS method was suitable for routine quality control and drug analysis of brimonidine tartrate.

ACKOWLEDGMENTS

The author thanks Supriya Life Science Ltd, Mumbai, Pacific University for their cooperation and help to carry out this research work.

Conflict of Interest: We are authors declare that, there is no conflict of interest.

REFERENCES

[1]. L. B. Cantor, The evolving pharmacotherapeutic profile of brimonidine, an alpha 2-adrenergic agonist, after four years of continuous use, *Exp. Opi. Pharm.*, **2000**, 1(4), 815-34.
[2]. A. Galanopoulos, I. Goldberg, Clinical efficacy and neuroprotective effects of brimonidine in the management of glaucoma and ocular hypertension, *Clin. Ophtha*., **2009**, 3, 117-22.
[3]. L. B. Cantor, J. Burke, Brimonidine, *Exp. Opi. Inves. Drugs*, **1997**, 6(8), 1063-83.
[4]. D. S. Greenfield, J. M. Liebmann, R. Ritch, Brimonidine: a new alpha2-adrenoreceptor agonist for glaucoma treatment, *J. Glaucoma.*, **1997**, 6(4), 250-258.
[5]. S. J. McKinnon, L. D. Goldberg, P. Peeples, J. G. Walt, T. G. Bramley, Current management of glaucoma and the need for complete therapy, *Am. J. Manag. Care*, **2008**, 14, S20–S27.

[6]. J. N. Chang, L. P. Spada, W. M. Blanda, W. C. Orilla, J. A. Bruke, P. M. Hughes, Alpha-2-agonist-polymeric-drug-delivery-systems, *US Patent Application* 20060233860, **2006**.
[7]. A. P. Kamath, S.Satyanarayana, C. F. E. A. Rodrigues, Ocular surface changes in primary open angle glaucoma with long term topical antiglaucoma medication, *Med. J. Arm. Forces India*, **2007**, 63, 341–345.
[8]. J. C. Adkins, J. A. Balfour, Brimonidine: A review of its pharmacological properties and clinical potential in the management of open-angle glaucoma and ocular hypertension, *Drugs Aging*, **1998**, 12(3), 225-241.
[9]. A. Patel, N. Dwivedi, N. Kaurav, S. Bashani, S. Patel, H. S. Sharma, Chemical analysis of pharmaceuticals: a review, *J. Med. Pharm. Inno.*, **2016**, 3, 4-7.
[10]. M. V. Lokhande, S. K. Churi, Impurity Profiling of Pharmaceutical Drugs By Various Methods, *J. Appl. Chem.*, **2017**, 10(7), 27-34.
[11]. ICH Q2(R-1), Validation of analytical Procedure and methodology, **2005**.
[12]. M. V. Lokhande, S. A. Telavane, S. Kothari, Method of Validation for Residual Solvents in Bisoprolol Fumarate by GC Technique, *Asian J. Pharm. Res*., **2021**, 11(3), 147-155.
[13]. M. V. Lokhande, S. R. Ambadekar, B. K. Iyer, Validation of Pharmaceutical (API) Bulk Drug by HPLC Methods, *J. Appl. Chem*., **2018**, 11(2), 1-20.
[14]. M. V. Lokhande, C. P. Maurya, Characterization and validation of impurities related to pharmaceutical bulk drug (API) by using some analytical techniques, Inter, *J. Pharm. Sci. Res*., **2017**, 8(8), 3325-3340.