RESEARCH ARTICLE

A Novel Validated Gas Chromatography Headspace (GC-HS) Method for the Simultaneous Quantification of Six Organic Volatile Impurities in Biperiden HCL Pure and Pharmaceutical Dosage Forms

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ABSTRACT

A novel, simple, and sensitive Gas Chromatography Headspace method for simultaneous quantification of six organic-volatile impurities in Biperiden HCL API and its pharmaceutical dosage forms using ZB-624 30 m × 0.53 mm, 3.0 µ column with Flame Ionized detector at 250°C. The Injector temperature is maintained at 225°C. The nitrogen gas was used as a carrier gas with a 3.0 mL/min flow rate. The method involved a thermal gradient elution. The total run time is 25.0 minutes. The method was linear over the concentration range of the limit of quantification to 150% of each impurity. The limit of quantification was found 57 ppm for Methanol, 107 ppm for Isopropyl alcohol, 143 ppm for Di-isopropyl ether, 19.5 ppm for Tetrahydrofuran, 0.1 ppm for Benzene, and 14.6 ppm for 1,4-Dioxane. The calculated recoveries were obtained should be 85-115%.

Furthermore, verified precision, ruggedness, robustness, Solution stability, and pharmaceutical analysis were done. All the results are found within acceptable limits. The suggested GC-HS method can quantify six organic-volatile impurities in Biperiden HCl API and its pharmaceutical dosage forms.

Keywords: 1,4-Dioxane, Benzene, Biperiden HCl API, Di-isopropyl ether, Isopropyl alcohol, Methanol, Method development and Validation, Tetrahydrofuran.

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INTRODUCTION

Biperiden hydrochloride [Figure 1] is chemically known as (1RS)-1- [(1RS,2SR,4RS)-Bicyclo [2.2.1] hept-5-en-2-yl]-1-[phenyl-3-(piperidin-1-yl) propan-1-ol hydrochloride. Chemical formula is $C_{21}H_{30}ClNO$ and Molecular weight is 347.9 gr/mole.[1] And it is an anticholinergic drug. The Organic volatile impurities (OVI'S) are used in produced during the synthesis of Biperiden HCl drug substances and in excipients used in the production of drug formulations. Many of these OVI generally can't be completely removed by standard manufacturing processes, preferably at low levels. These organic-volatile impurities are encounter during the manufacture and storage of active pharmaceutical ingredients. The OVI'S in the active pharmaceutical ingredients or from other drugs manufacturing processes can be harmful for human health.[2-4] The first problem facing the simultaneous quantification of these organic volatile impurities analysis of Biperiden HCl in quality control was the inability of the present official methods.[5]

The organic volatile impurities specifications were set following the toxicity of solvents vary from a low ppm to thousands of ppm. Generally, organic-volatile impurities are divided into three classes. Those are class-1, class-2 and class-3. So in the process of Biperiden HCl drug methanol (class-3), isopropyl alcohol (class-3), Di-isopropyl ether (class-3), tetrahydrofuran (Class-2), benzene (Class-1), and 1,4-Dioxane(Class-2) were used as organic-volatile impurities. After the drying process, the analysis needs to be performed to check if the amounts of solvents used at any step of the production do not

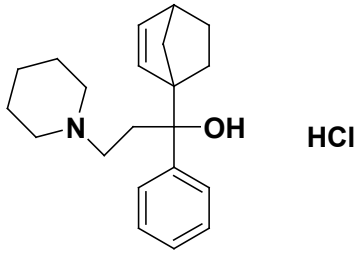

Figure 1: Chemical Structure of Biperiden HCl

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exceed acceptable limits. The static GC-HS quantification of OVI'S is now a day's mature technique well established in pharmaceutical analysis.[6] So we aim to simultaneously quantify these six organic volatile impurities in a single method by using GC-HS with the flame ionized detector. The specifications of the six organic volatile impurities are 3000 ppm for Methanol, 5000 ppm for Isopropyl alcohol, 5000 ppm for Di-isopropyl ether 500 ppm for Tetrahydrofuran, 2.0 ppm for Benzene, and 300 ppm for 1,4-Dioxane. The structures of six organic-volatile impurities are shown in Figure 2.

In the literature review, very few reports are present on Biperiden HCl. Some stability-indicating methods and Assay methods are available. Mohammadi *et al.* reported as Development and Validation of a Stability-Indicating High-Performance Liquid Chromatographic (HPLC) assay for Biperiden in Bulk Form and Pharmaceutical Dosage Forms.[7] We hope our method is novel and very sensitive from this literature survey.

To the best of our knowledge, there are no reports on the validated simultaneous quantification of six organic-volatile impurities in the Biperiden HCl pure and pharmaceutical substances by using GC-HS with the flame ionized detector.

MATERIALS AND METHODS

Chemicals and Reagents

Methanol, Isopropyl alcohol, Di-isopropyl ether, Tetrahydrofuran, Benzene, 1,4-Dioxane, and Dimethyl sulfoxide were purchased from Sigma-Aldrich. Biperiden HCl API is taken from a local well-known research Laboratory. Dimethyl sulfoxide is used as a diluent and blank.

Instrumentation and Chromatographic Conditions

Chromatography was performed on Shimadzu chromatographic system equipped with a Shimadzu GC-2010 system with FID. Samples were injected through a Teledyne tekmar HT3^{TM} Headspace. Data acquisition and integration were performed using GC solution software. The instrument parameters described below were set up to determine the Organic volatile impurities. The GC and HS method conditions are summarized in Table 1.

Preparation of Standard Solutions

Benzene Standard Stock Solution

Weigh and transfer about 500 mg of Benzene into 100 mL of the volumetric flask containing 70 mL of diluent and diluted to volume with diluent. Further taken 1.0 mL of above solution into 100 mL of volumetric flask and diluted to volume with diluent.

Standard Solution Preparation

Weighed and transferred about each 750 mg of Methanol, 1250 mg of Isopropyl alcohol, 1250 mg of Di-isopropyl ether, 125 mg of Tetrahydrofuran, and 75 mg of 1,4-Dioxane into a 100 mL of the volumetric flask containing 70 mL of diluent and diluted to volume with diluent. Further taken 5.0 mL of the above solution and 0.5 mL of Benzene stock solution into 50 mL of volumetric flask and diluted to volume with diluent.

The standard Headspace vials were prepared with 2 mL of the Standard solution and seal the vial with aluminum closure. (The standard solution concentration was prepared concerning Sample concentration).

Preparation of Biperiden HCl Sample Solution (250 mg/mL)

Accurately weighed about 500 mg of Biperiden HCl API into a 10 mL Head Space vial, added 2.0 mL of diluent, and immediately sealed with aluminum closure.

Preparation of Biperiden HCl Tablet Solution

Twenty tablets were weighed and powdered. An amount of powder equivalent to 500 mg Biperiden HCl was accurately weighed and transferred to a headspace vial, add 2 mL of diluent, and immediately sealed with aluminum closure.

RESULTS AND DISCUSSION

Method Optimization

The GC-HS method has been developed based on GC-HS chromatographic conditions. The column screening has been done for better peak resolution between six organic-volatile impurities. After selecting the column, fine-tune the method to change the Injection, Detector temperatures, and Oven program. The oven program tuning is beneficial to reduce the total run time with good resolution. The GC method optimization details are shown in Table-2.

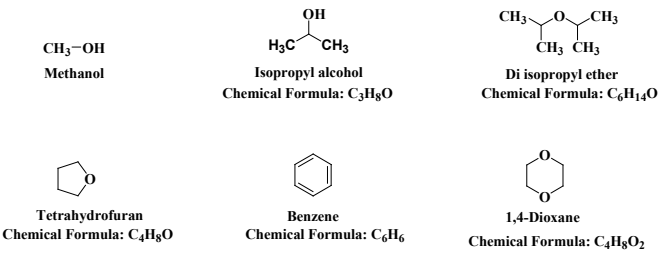

Figure 2: Chemical Structures of Six Organic Volatile Impurities

Table 1: GC and HS method conditions

Column	*ZB-624 (30 m × 0.53 mm, 3.0 μ)*
Flow	3.0 mL/min
Injector temperature(°C)	220°C
Detector temperature(°C)	260°C
Split ratio	20:1
GC Oven program	Ramp(°C/min) Temperature (°C) Hold time (min) 40 5 20°C/min 200 12
Total Run time (min)	25.0 min
Carrier Gas	N_2
HS-Vail temperature(°C)	90°C
HS-Oven temperature(°C)	100°C
HS-Transfer line temperature(°C)	110°C
HS-Vail equilibration time (min)	30 min
HS-Injection volume	1 mL
HS-Injection time (min)	1 min

From Table 2 method optimized data we have to observe, ZB-624 (30 m × 0.53 mm, 3.0 µ) the column gives better results than the remaining two columns as per USP specifications. So based on the above data we have selected ZB-624 (30 m × 0.53 mm, 3.0 µ) to quantify six organic-volatile impurities in Biperiden HCl API and its pharmaceutical dosage forms.

Headspace Method Optimization

The headspace method was optimized so that the maximum amount of the organic-volatile impurities present in the Biperiden HCl API gets evaporated for detection. For this, the standard and sample vials were heated at 70°C to 100ºC for 15 to 30 min with constant shaking. A combination of sample vial heating at 90ºC with 30 min shaking was suitable for getting a good response.

Method Validation

The GC-HS method validation was as per ICH guidelines.[8]

Specificity

The relative retention time of the six OVI's indicated that they were well separated from each other [Table 3]. The typical chromatograms of six organic-volatile impurities and Biperiden HCl are shown in Figure 3.

System Precision and Method Precision

System Precision was evaluated by injecting six replicates, and method Precision was evaluated by preparing the six different preparations of standard solution into the Chromatographic system as per the test method. The %RSD was calculated for the area of six OVI's. The %RSD of each Impurity is NMT 10.0%. Results are shown in Tables 4 and 5.

Linearity for Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ for the proposed method were determined using calibration standards and calculated using 3.3 σ /s and 10 σ/s formulae, respectively. The data and typical chromatograms are shown in Table 6 and Figure 4.

Linearity

The linearity of the method was determined over the concentration range of LOQ %, 50% 75% 100%, 125%, and 150%. The LOQ values are 1.90% for Methanol, 2.14% for IPA, 2.86% for Di-isopropyl ether, 3.90% for THF, 3.05% for Benzene and 4.85% for 1,4-Dioxane.The correlation coefficient

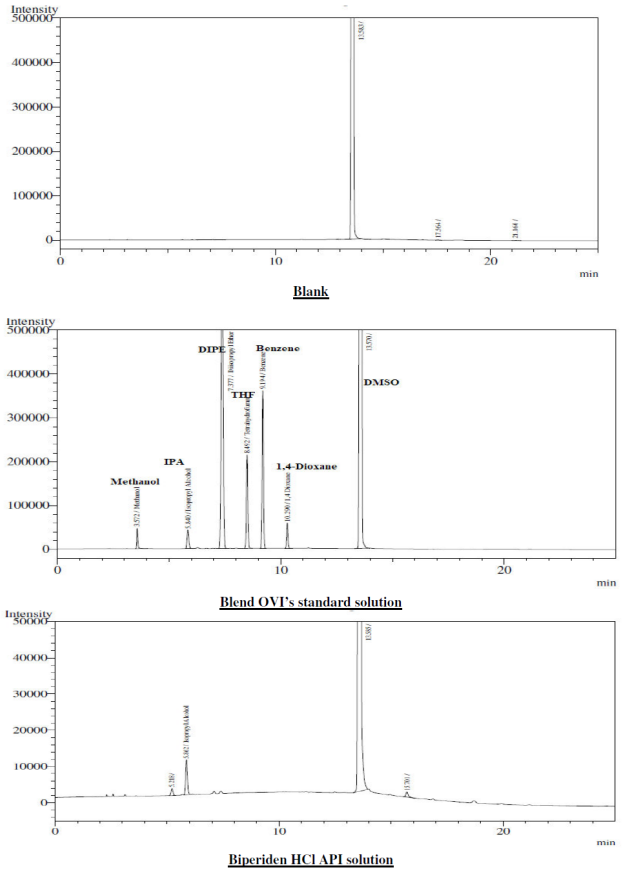

Figure 3: Typical chromatograms of Six OVI'S Standard and Biperiden HCl API

Table 2: GC-Method optimization details

Name of the column	*USP Resolution*	*USP Tailing factor*	*USP Plate count*
DB-1 (30 m × 0.53 mm, 1.5 µ)	Between 2.0 to 5.0	Most of the impurities are above 1.5	Plate count is good for all impurities (Between 2000 to 30000)
ZB-624 (30 m × 0.53 mm, 3.0 µ)	Between 5.0 to 20.0	All six impurities are below 1.5	Plate count is good for all impurities (Between 2000 to 150000)
ZB-Wax (30 m × 0.32 mm, 1.8 µ)	Between 3.0 to 8.0	Some impurities are above 2.0 and Three impurities are below 1.5.	Plate count is good for all impurities (Between 2000 to 50000)

Table 3: Specificity data for six organic-volatile impurities

S.No.	*Name of OVI'S*	*Retention time (min)*	*Theoretical Plates*	*Tailing Factor*	*USP Resolution*
1	Methanol	3.57	30139	1.28	
2	Isopropyl alcohol	5.84	26998	1.09	20.05
3	Di isopropyl ether	7.37	32863	1.01	10.07
4	Tetrahydrofuran	8.49	81429	1.07	7.92
5	Benzene	9.19	94064	1.07	6.02
6	1,4-Dioxane	10.29	147447	1.09	9.95

Table 4: System Precision data for six organic volatile impurities

No.of Injections	*Methanol*	*Isopropyl alcohol*	*Di isopropyl ether*	*Tetra hydro furan*	*Benzene*	*1,4-Dioxane*
1	369749	701586	12772550	207911	2214	23615
2	386897	745421	13129371	220334	2423	24253
3	385403	746081	12993170	213152	2214	23100
4	395539	796404	12855053	208834	2321	23336
5	369889	705647	12834163	209901	2215	24808
6	366751	702931	12732378	205160	2102	24575
ACVG	**379038**	**733012**	**12886114**	**210882**	**2248**	23948
STDV	11794	37375	148883	5314	110	697
% RSD	3.11	5.10	1.16	2.52	4.90	2.91

Table 5: Method Precision data for six organic volatile impurities

No.of Injections	*Methanol*	*Isopropyl alcohol*	*Di isopropyl ether*	*Tetra Hydro furan*	*Benzene*	*1,4-Dioxane*
1	350489	813813	12123543	216994	2225	23240
2	353970	815143	12204228	218798	2125	23390
3	336354	787640	12263998	216893	2066	22638
4	362012	854437	12052005	221205	2210	24265
5	373189	894055	12099833	212622	2120	24407
6	346263	822840	12008420	217101	2328	23233
ACVG	**353713**	**831321**	**12125338**	**217269**	**2179**	**23529**
STDV	**12771**	**37465**	**95028**	**2817**	**94**	**678**
% RSD	3.61	4.51	0.78	1.30	4.33	2.88

Table 6: LOD and LOQ data for six Organic volatile impurities

OVI'S	*LOD Con.(ppm)*	*LOQ Con.(ppm)*	*LOD Area*	*LOQ Area*
Methanol	18.9	57	2077	5363
Isopropyl alcohol	35.5	107	5577	17384
Di isopropyl ether	47	143	102279	321707
Tetrahydrofuran	6.5	19.5	2981	8792
Benzene	0.02	0.1	25	69
1,4-Dioxane	4.8	14.6	476	1529

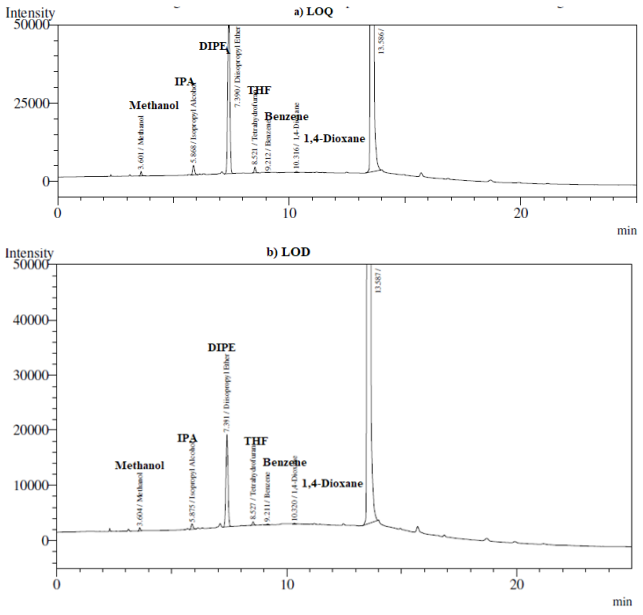

Figure 4: (a) LOQ and (b) LOD Chromatogram of Six OVI'S

(r^2) of each impurity is not less than 0.99. The linearity data and graphs is shown in Table 7 and Figure 5.

LOQ-Precision

To inject the six repeated injections of six organic-volatile impurities at LOQ concentration into the GC system. Then calculate the % relative standard deviation for six OVI's area. The %RSD has obtained not more than 15.0%. Results are summarized in Table 8.

Recovery

The %recovery was evaluated at 50%, 100%, 150% and LOQ% concentrations of six organic-volatile impurities are spiked with Biperiden HCl API. The acceptance criterion for accuracy was that it should be within 100 ± 15%. The results are indicated in Table 9.

Ruggedness and Robustness

The ruggedness of the method was evaluated by injecting the standard organic volatile impurities in six replicates with different analysts on different days. Robustness is performed by making small variations in the GC or HS method parameters.

Table 7: Linearity data with LOQ

Con.(%)	*Methanol*	*Isopropyl alcohol*	*Di isopropyl ether*	*Tetra hydro furan*	*Benzene*	*1,4-Dioxane*
LOQ Con.	5363	17384	321707	8792	69	1529
50	168286	341708	5981565	107185	1075	11428
75	256745	525025	8743768	161083	1666	17234
100	340445	695903	12817801	218480	2298	22630
125	402263	815893	14844431	267992	2774	26599
150	519833	1068870	18593710	330280	3493	33984
r2	**0.998**	**0.997**	**0.998**	**1.000**	**0.999**	**0.998**

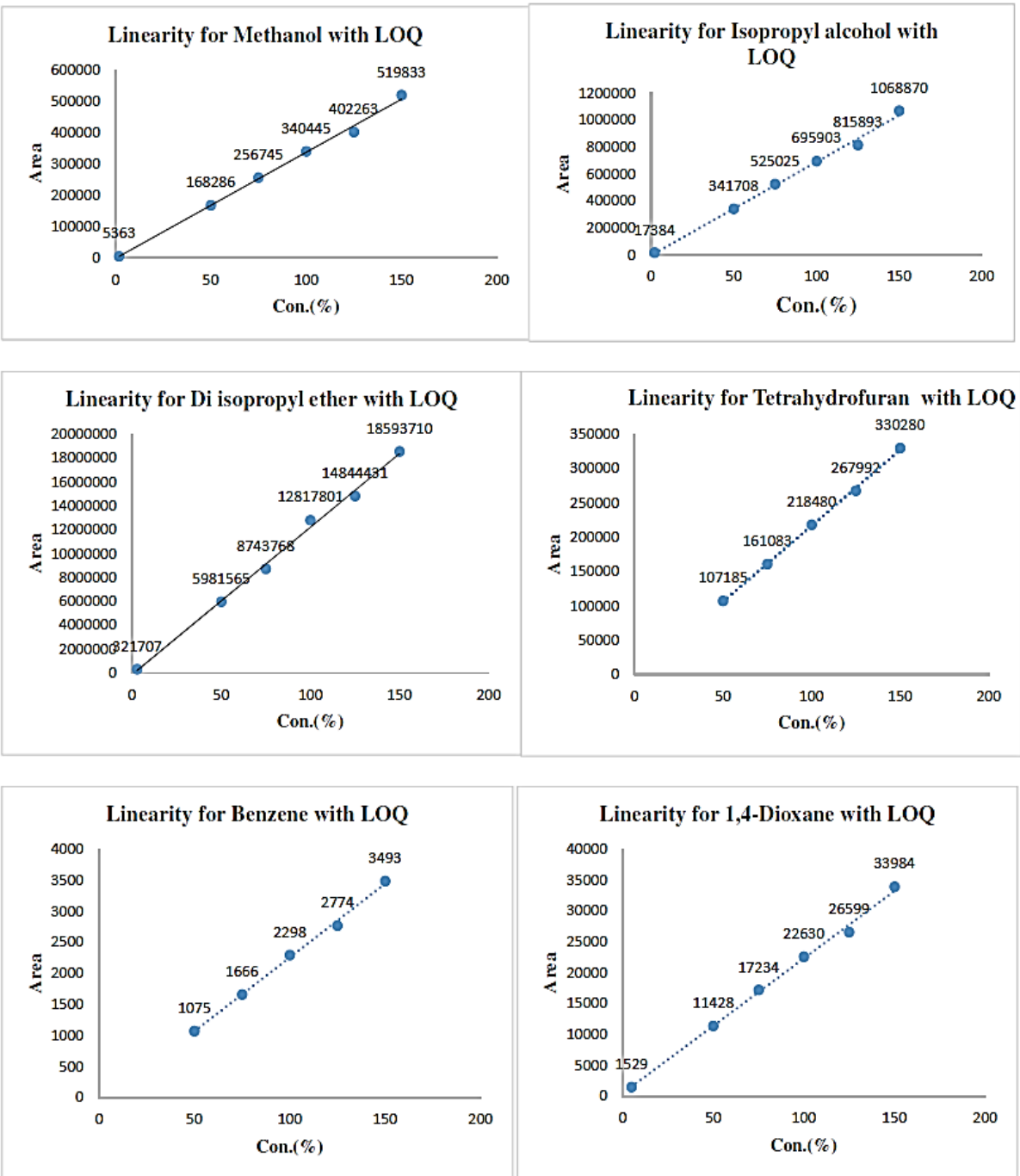

Figure 5: Correlation graphs of six OVI'S

Table 8: LOQ-Precision data

No. of Injections	*Methanol*	*Isopropyl alcohol*	*Di isopropyl ether*	*Tetra hydro furan*	*Benzene*	*1,4-Dioxane*
Run-1	5363	17384	321707	8792	71	1529
Run-2	5391	16445	321260	8832	69	1306
Run-3	5483	17951	328209	8951	68	1517
Run-4	5358	17868	324103	8788	75	1419
Run-5	5147	15749	320954	8453	79	1309
Run-6	5068	16620	328390	8950	62	1369
ACVG	5302	17003	324104	8794	**71**	**1408**
STDV	159	874	3434	183	5.9	98
%RSD	3.00	5.14	1.06	2.08	8.33	6.98

Table 9: Recovery data for six organic-volatile impurities

No of OVI'S	*Recovery at 50%*	*Recovery at 100%*	*Recovery at 150%*	*Recovery at LOQ%*
Methanol	88.11	89.52	89.83	98.39
Isopropyl alcohol	105.07	103.26	102.27	95.18
Di isopropyl ether	90.37	94.85	91.3	96.1
Tetrahydrofuran	100.82	104.69	103.12	99.97
Benzene	96.53	102.8	94.37	102.35
1,4-Dioxane	96.98	97.4	95.67	101.47

Table 10: Ruggedness data for six Organic volatile impurities

Different Days and Analysts		*%RSD for Methanol*	*%RSD for IPA*	*%RSD for Di isopropyl ether*	*%RSD for THF*	*%RSD for Benzene*	*%RSD for 1,4-Dioxane*
Day-1	Analyst-1 (n=6)	3.90	4.94	1.16	1.40	1.74	3.35
	Analyst-2 (n=6)	2.69	2.51	1.02	1.00	1.27	2.27
	Analyst-1 & 2 (n=12)	3.20	3.75	1.73	1.36	1.52	2.73
Day-2	Analyst-1 (n=6)	4.63	5.45	1.31	1.58	2.10	3.99
	Analyst-2 (n=6)	2.62	2.21	1.44	1.52	2.36	2.47
	Analyst-1 & 2 (n=12)	3.97	4.66	4.81	1.83	2.16	3.87
Analyst-1	Day-1&2 (n=12)	5.35	4.96	2.18	1.47	1.84	3.71
Analyst-2	Day-1&2 (n=12)	5.81	3.62	4.34	2.46	1.81	2.53

Table 11: Robustness data for six Organic volatile impurities

	Flow rate (mL/min)		*Vial Cond. Temperature (°C)*	
Name of OVI'S	*2.8 mL/min (%RSD)*	*3.2 mL/min (%RSD)*	*75°C (%RSD)*	*85°C (%RSD)*
Methanol	6.55	4.75	3.03	5.48
Isopropyl alcohol	7.46	6.32	3.35	6.37
Di isopropyl ether	0.73	1.11	1.34	2.00
Tetrahydrofuran	1.51	3.76	1.52	3.01
Benzene	1.82	2.03	2.25	2.08
1,4-Dioxane	6.18	6.13	3.10	5.12

The changed parameters are Column flow and Vial condition temperature. The % RSD for six organic-volatile impurities are not more than 10.0%. The results are summarized as shown in Table 10 and Table 11.

Pharmaceutical Application

The prepared Biperiden HCl tablet solution (250 mg/mL) was injected. The six organic volatile impurities content in Biperiden HCl tablets were found within the specified limits. The results are shown in Table 12.

Solution Stability

The six organic volatile impurities standard and Biperiden HCl API sample solutions were prepared in Dimethyl sulfoxide as a diluent. So we have to check whether these standard and sample solutions are stable or not. To prepare the standard and

Table 12: Six organic volatile impurities content in tablet analysis

Name of API	*API Label claim (mg)*	*Methanol (ppm)*	*IPA (ppm)*	*DIPA (ppm)*	*THF (ppm)*	*Benzene (ppm)*	*1,4-Dioxane (ppm)*
Biperiden HCl	2	Not detected	75	Not detected	Not detected	Not detected	Not detected

Table 13: Solution stability data for Six OVI'S and Biperiden HCl API

Methanol	*% Solution stability for standard*	*% Solution stability for API*
Initial hours	Not applicable	Not detected
After 12 h	97.13	Not detected
After 24 h	96.45	Not detected
After 36 h	93.19	Not detected
Isopropyl alcohol	*% Solution stability for standard*	*% Solution stability for API*
Initial hours	Not applicable	Not applicable
After 12 h	97.79	98
After 24 h	95.98	98.71
After 36 h	94.18	95.26
Di isopropyl ether	*% Solution stability for standard*	*% Solution stability for API*
Initial hours	Not applicable	Not detected
After 12 h	97.61	Not detected
After 24 h	96.63	Not detected
After 36 h	95.78	Not detected
Tetrahydrofuran	*% Solution stability for standard*	*% Solution stability for API*
Initial hours	Not applicable	Not detected
After 12 h	97.48	Not detected
After 24 h	97.01	Not detected
After 36 h	94.71	Not detected
Benzene	*% Solution stability for standard*	*% Solution stability for API*
Initial hours	Not applicable	Not detected
After 12 h	95.99	Not detected
After 24 h	93.06	Not detected
After 36 h	92.17	Not detected
1,4-Dioxane	*% Solution stability for standard*	*% Solution stability for API*
Initial hours	Not applicable	Not detected
After 12 h	96.54	Not detected
After 24 h	93.58	Not detected
After 36 h	92.97	Not detected

sample solutions for four-time intervals (Initial hours, after 12 hours, after 24 hours and after 36 hours) on the first day and kept at room temperature. These solutions are injected at Initial hours, after 12 hours, after 24 hours, and after 36 hours, then calculated the % of solution stability for the area at each time interval. We got a % of solution stability is 100 ± 10%. Based on these data, six organic volatile impurities standards and Biperiden HCl API solutions were stable for up to 36 hours. The corresponding data is presented in Table 13.

CONCLUSION

This is the novel GC-HS method for the simultaneous quantification of six OVI's in Biperiden HCl API. Methanol, Isopropyl alcohol, Di-isopropyl ether, Tetrahydrofuran, Benzene, and 1,4-Dioxane were well separated and quantified by the proposed method. Good results are obtained in each validation parameter as per ICH guidelines. We reported that the LOD and LOQ value was very low from this method. We have to prove that this GC-HS method is also suitable for quantifying organic-volatile impurities in pharmaceutical dosage forms. The proposed method was validated as per the ICH guidelines, and the results revealed that the method was scientific. This investigation may be helpful to the manufacturers for controlling and minimization of the organic-volatile impurities. And this method was found to be applicable for the routine analysis of the Biperiden HCl API in the pharmaceutical industry.

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