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Quantification of organic volatile impurities in Oseltamivir phosphate drug substances by head space gas chromatography

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Abstract

Background: The present work is aimed at quantification of organic volatile solvents like Methanol, Ethanol, Acetonitrile, Isopropyl alcohol (IPA), Dichloromethane (DCM), Methyl tert-butyl ether (MTBE), Hexane fractions, Ethyl acetate (EA), Tetra hydro furan (THF), 1,4-Dioxane, Dimethylformamide (DMF), and Toluene in Oseltamivir phosphate Active pharmaceutical ingredient (API) and pharmaceutical dosage forms. The method was developed using a thermal gradient elution program associated with a column having dimensions are DB-1,60 m × 0.32 mm × 5.0 µm with a flow rate of 1.0 mL/min and Nitrogen (N_2) as a carrier gas. A flame ionization detector was used as a detector, and its temperature is at 290 °C whereas the injector temperature is at 180 °C. The total run time is 60.0 min. The developed method was validated according to International Council for Harmonization (ICH) guidelines.

Results: The linearity of the calibration curve for twelve impurities in the concentration range of Limit of Quantification (LOQ) to 150% was good. The curve was linear for twelve residual impurities of Oseltamivir phosphate. Relative standard deviation values for twelve residual impurities are not more than 15%. Limit of detection (LOD) and LOQ for twelve residual impurities were found to be very low-level concerning specification level. We found good results for all validated parameters for twelve residual impurities.

Conclusions: To quantify the residual organic volatile solvents (organic volatile impurities) in the Oseltamivir phosphate API, a novel, specific, and elevated sensitive headspace gas chromatography method was developed to separate twelve solvents that are accompanying with fifteen peaks. Out of fifteen peaks, critical components are Hexane fractions, Ethyl acetate, MTBE, and DCM. So, our method has capable of separating and simultaneous quantification such critical components. So, it can be successfully applied for routine analysis of quantification of these twelve residual impurities in Oseltamivir phosphate bulk and pharmaceutical dosage forms.

Keywords: Oseltamivir phosphate, Organic volatile impurities, Method development, Method validation

Background

Residual solvents in pharmaceuticals are defined here as organic volatile chemicals that are used or produced in the manufacture of drug substances or excipients, or the preparation of drug products. The solvents are not completely removed by practical manufacturing techniques. Appropriate selection of the solvent for the synthesis of drug substance may enhance the yield, or determine characteristics such as crystal form, purity, and solubility. Therefore, the solvent may sometimes be a critical parameter in the synthetic process. Since there is no therapeutic benefit from residual solvents, all residual solvents should be removed to the extent possible to meet product specifications, good manufacturing practices, or other quality-based requirements. Drug products should

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contain no higher levels of residual solvents than can be supported by safety data. The organic volatile impurity specifications are set by the ICH guidelines, regulatory authorities, and those guidelines are established based on respective toxicology data of solvents, and it is varying from a low-level part per million (ppm) to thousands of ppm based on the classification of residual solvents by risk assessment. To quantify the organic volatile impurities gas chromatographic instrument connected with the headspace instrument is being used more effectively, and it is playing a vital role in the pharmaceutical industry [1–6]. To enhance the quality as well as quantity of Oseltamivir Phosphate API, the following solvents were used as part of the synthetic process. To make affordable Oseltamivir Phosphate API, Hexane fractions are being used instead of n-Hexane solvent. Which is accomplished with mainly four fractions, and which is a critical part of development since these fractions are being co-eluted with MTBE as well as Ethyl acetate solvents, hence the efforts were made to separate the critical components like Hexane fractions, MTBE, Dichloromethane, Ethyl acetate along with other solvents. Many papers are published to quantify the residual solvents in the drug substance, but no method is readily available to separate such critical solvents.

Oseltamivir phosphate is a white crystalline solid with the chemical name (3R,4R,5S)-4-acetylamino-5-amino-3(l-ethylpropoxy)-1-cyclohexene-l-carboxylic acid ethyl ester, phosphate. The chemical formula is $C_{16}H_{28}N_2O_4$ (free base), and the molecular weight of Oseltamivir is 312.4 gr/mol. The chemical formula is $C_{16}H_{31}N_2O_8P$ (salt), and the molecular weight of Oseltamivir is 410.4 gr/mol for Oseltamivir phosphate salt. The structural formula is shown in Fig. 1.

Different organic solvents are used in the synthetic process of Oseltamivir phosphate API. The organic solvents like Methanol, Ethanol, Acetonitrile, Isopropyl alcohol, Dichloromethane, MTBE, Hexanes, Ethyl acetate, THF, 1,4-Dioxane, DMF, and Toluene. As per ICH Q3C (R6) Ethanol, Isopropyl alcohol, MTBE, Ethyl acetate, and DMF were grouped under class-3 organic solvents while Methanol, Acetonitrile, Dichloromethane, Hexanes, THF and 1,4-Dioxane, and Toluene were grouped under class-2 organic solvents [7].

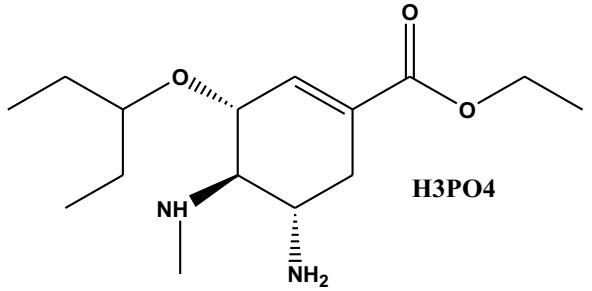


Fig. 1 Chemical structure of Oseltamivir phosphate API

Organic volatile impurities (OVI) or solvents of class-2 have inherent toxicity to human wellbeing, and chemical solvents of class-3 are less harmful. Therefore, the organic solvents utilized in Oseltamivir phosphate API must be regulated. The ICH Q3 specification levels opt for twelve organic volatile impurities in Oseltamivir phosphate API. Developed a novel and highly sensitive method to quantify the organic volatile impurities such as Methanol, Ethanol, Acetonitrile, Isopropyl alcohol, Dichloromethane, MTBE, Hexanes, Ethyl acetate, THF, 1,4-Dioxane, DMF, and Toluene. This is a novel method since the key component is to separate the critical solvents such as MTBE, Dichloromethane, Ethyl acetate, and Hexanes (Fraction-1,2,3 and 4) are in a single analysis. So, our proposed method should be separate these combinations along with other solvents, and we got good resolution between twelve organic volatile impurities accomplished with fifteen peaks and then validated as per ICH grainlines and Association of Official Agricultural Chemists (AOAC) guidelines. This method is very novel, simple, accurate, and precise.

Literature survey

Few chromatographic methods have appeared in the literature. Those are the development and validation of the Reversed-phase High-performance liquid chromatography (RP-HPLC) method for the determination of Oseltamivir phosphate in bulk drug and dosage forms [8]. Stability indicates the liquid chromatography (LC) method for Oseltamivir phosphate [9]. The degradation behavior of Oseltamivir phosphate under various stress conditions was used stability-indicating HPLC method [10]. A literature search revealed that nobody has reported the quantification of Organic volatile impurities also. We have well separated Dichloromethane, Ethyl acetate MTBE with Hexanes fractions combination. So, our proposed gas chromatography-headspace (GC-HS) method is novel and very sensitive.

Methods

Chemicals and reagents

Oseltamivir phosphate API was taken from GVK Biosciences Pvt. Ltd. (Hyderabad, India).

The solvents viz. methanol, ethanol, acetonitrile, isopropyl alcohol, Dichloromethane, MTBE, Hexanes, ethyl acetate, THF, 1,4-Dioxane, DMF, and Toluene (all procured from Merck, India).

Instruments

Agilent GC 6890 N system, Agilent headspace G1888 N system, Flame ionization detector system, Waters United

States of America (USA) Empower version 3 software were used for the quantification of opted Twelve organic volatile solvents in Oseltamivir phosphate API.

Chromatographic conditions

Column	:	DB-1, 60 m, 0.32 mm, and 5.0 µm
Injector temperature	:	180°centigrade(°C)
Detector	:	Flame ionization detector (FID)
Detector temperature	:	290 °C
Initial oven temperature	:	40 °C
Hold time-1	:	10.0 min
Ramp rate-1	:	5.0 °C/min
Oven temperature	:	80 °C
Hold time-2	:	10.0 min
Ramp rate-2	:	4.0 °C/min
Oven temperature	:	150 °C
Hold time-3	:	5.0 min
Ramp rate-3	:	40.0 °C/min
Oven temperature	:	280 °C
Hold time-4	:	6.25 min
Carrier gas	:	Nitrogen
Mode of injection	:	Split
Constant Flow	:	1.0 mL/min
Split ratio	:	1:5
Makeup	:	30 mL/min
Hydrogen	:	40 mL/min
Air	:	400 mL/min
Run time	:	60.0 min
Diluent	:	N-methyl-2-pyrrolidone (NMP)

Headspace parameters

Oven temperature	:	90 °C
Loop temperature	:	160 °C
Transfer line temperature	:	165 °C
GC cycle time	:	75 min
Injection time	:	5.0 min
Loop equilibration time	:	0.05 min
Loop fill time	:	2.0 min
Pressurization	:	2.0 min
Vial equilibration	:	30.0 min
Shaking	:	High
Vial pressure	:	~15 Pounds per square inch (psi) (Nitrogen)

Specifications of organic twelve volatile impurities

Methanol	:	Not more than 3000 ppm
Ethanol	:	Not more than 5000 ppm
Isopropyl alcohol	:	Not more than 5000 ppm
Dichloromethane	:	Not more than 600 ppm
Hexanes	:	Not more than 290 ppm
Ethyl acetate	:	Not more than 5000 ppm
THF	:	Not more than 720 ppm
Toluene	:	Not more than 890 ppm
DMF	:	Not more than 880 ppm
1.4-Dioxane	:	Not more than 380 ppm
Acetonitrile	:	Not more than 410 ppm
MTBE	:	Not more than 5000 ppm

Standard and sample solution preparations

Blank preparation Transferred 2.0 Milley Letter (mL) of diluent into a 20 ml headspace vial. Immediately crimped the vial with a septum and a cap.

Standard stock preparation

Weighed and transferred about 600 mg of Methanol, 1000 mg of Ethanol, 82 mg of Acetonitrile, 1000 mg of IPA, 120 mg of DCM, 1000 mg of MTBE, 58 mg of Hexanes, 1000 mg of Ethyl acetate, 144 mg of THF, 76 mg of 1,4-Dioxane, 176 mg of DMF, and 178 mg of Toluene into a 50 mL volumetric flask contained 20 mL of diluent dissolved and diluted to volume with diluent.

Standard preparation Transferred 1.0 mL of the stock solution into 100 mL volumetric flask containing 50 mL of diluent, dissolved, and diluted to volume with diluent.

Preparation of standard vial Transfer 2.0 mL of standard stock solution in a headspace vial and seal with an aluminum septum and crimp the cap.

Preparation Oseltamivir phosphate sample

Accurately weighed and transferred 80 Milly gram (mg) of Oseltamivir phosphate into a headspace vial. Then add 2.0 mL of diluent and immediately seal with an aluminum septum and crimp the cap.

Pharmaceutical sample preparation

Twenty tablets were weighed and powdered. Accurately weighed and transferred an amount of powder equivalent to 80 mg of Oseltamivir phosphate to a 20 mL headspace vial then added 2.0 mL of diluent and immediately seal with an aluminum septum and crimp the cap.

The Organic volatile impurity content (ppm) was calculated by the following formula:

$$\text{Calculation (ppm):} \frac{\text{Impurity area in test solution}}{\text{Impurity area in standard solution}} \times \frac{\text{Standard concentration (mg/mL)}}{\text{Sample concentration (mg/mL)}} \times 10^6$$

GC-HS method development

The adopted solvents for the Oseltamivir phosphate API are associated with class-II and class-III solvents by ICH guidelines. To develop an elevated sensitive and novel method, one needs to choose the best suitable solvent and column for Oseltamivir phosphate. The method should be robust to determine the trace levels of residual solvents in the drug substance as well as the drug product. As part of method development, following the quality by design principles to have a better control method for residual solvents in Oseltamivir phosphate.

Diluent selection

This method development was started with the selection of diluent. We have sought different diluents (DMF, NMP, and Dimethyl sulfoxide). For its ability to dissolve a broad range of organic solvents and will not impede with chosen solvents, analyzed by gas chromatography, NMP was exploited as the standard as well as sample diluent.

Column selection

The Column selection for GC-HS analysis was also a very important task in the method development process. This study utilized a chromatographic basic rule "like attracts like" and focused on the polarity matching among column stationary phase and mobile phase. In this study, many columns were screened i.e., VF-1(30 m × 0.32 mm × 0.45 µm), DB-624(30 m × 0.53 mm × 3.0 µm), DB-1, 60 m, 0.32 mm × 5.0 µm and DB-624 (30 m × 0.25 mm × 0.25 µm), DB-17, DB-5, DB-Vax columns. Out of seven columns , DB-1 column gave better resolution between Ethyl acetate, MTBE, Dichloromethane, and Hexanes whereas in other columns VF-1, DB-624, DB-5, DB-Vax either MTBE peak is co-eluting with Hexanes first peak or with DCM peak. In some columns, Ethyl acetate is co-eluting with Hexane's fraction 3. The GC-HS parameters were first optimized to achieve good retention time, acceptable resolution, and better peak shapes for the MTBE, Hexanes, Dichloromethane, and Ethyl acetate in Oseltamivir phosphate and its formulations. The DB-1 eluted sharp peaks with good peak tailing and good resolution between all peaks. It demonstrated that the DB-1 column was closely matched. Hence, the DB-1 60 m, 0.32 mm × 5.0 µm column was selected for this study.

Column oven temperature optimization:

Four different column oven temperature fluxes were tried. (1) maintained for 11 min at 40 °C and then continued to upsurge to a temperature close of 240 °C at a rate of 20 °C/min and retained for 30 min; (2) maintained for 10 min at 40 °C and then raise 80 °C with Ramp rate 5.0 °C/min hold for 10 min and again raise 150 °C with Ramp rate 4.0 °C/min hold for 5 min continued to upsurge to a temperature close of 280 °C at a rate of 40 °C/min and retained for 6.25 min. (3) maintained for 5 min at 40 °C and then continued to upsurge to a temperature close of 240 °C at a rate of 10 °C/min and retained for 35 min; (4) maintained for 11 min at 40 °C and then continued to upsurge to a temperature close of 240 °C at a rate of 15 °C/min and retained for 30 min;

Better separation with the good resolution was obtained with 1st oven program [maintained for 10 min at 40 °C and then raise 80 °C with Ramp rate 5.0 °C/min hold for 10 min and again raise 150 °C with Ramp rate of 4.0 °C/min hold for 5 min continued to upsurge to a temperature close of 280 °C at a rate of 40 °C/min and retained for 6.25 min]. In 1st, 3rd, and 4th oven temperature fluxes, the peaks of all the nine organic solvents were closely eluted. Nitrogen, as a carrier gas, with a flowing stream of 1.0 mL per min and 2 mL per min were tested. A 1.0 mL per min flow stream was optimized. The remaining optimized parameters were 180 °C temperature at injector port; 290 °C temperature at detector port; split mode injection in 1:5 ratio; air flow and hydrogen flow were 400 mL/min and 40 mL/min, respectively. Figure 2 displays the chromatogram acquired using configured parameters.

Results

Method validation

The GC-HS method was validated as per ICH guidelines [11]. The following validation parameters are done. Those are specificity, repeatability, method precision, LOD and LOQ, linearity, accuracy, ruggedness, and robustness.

Specificity and selectivity

Injected separately the individual standard solutions of each solvent, sample solution, and then selectivity solution. No peak from blank at Methanol, Ethanol, Acetonitrile, Isopropyl alcohol, DCM, MTBE, Hexanes,

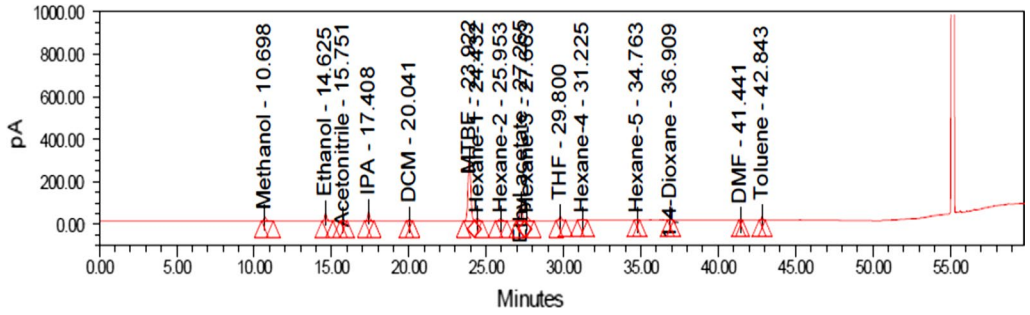


Fig. 2 Typical chromatogram for Twelve organic volatile impurities

Ethyl acetate, THF, 1,4-Dioxane, DMF, and Toluene Retention times. Methanol, Ethanol, Acetonitrile, Isopropyl alcohol, DCM, MTBE, Hexanes, Ethyl acetate, THF, 1,4-Dioxane, DMF, and Toluene solvents should be eluted at different retention times. Reported the resolution between all solvent peaks in specificity solution and reported the retention times of all solvents in individual solutions and selectivity solution. The resolution was obtained between the all peaks was not less than 1.0, and the typical chromatograms and retention times are shown in Fig. 3a–d and Table 1.

System precision

The system precision of this method was expressed in terms of the % relative standard deviation (RSD) of the data. System precision has been demonstrated by six replicate injections of standard solutions. The % RSD was found out to be not more than 15%. The data of system precision are shown in Table 2.

Method precision

The method precision of the proposed method is expressed in the term of % RSD of the data. Method precision has been demonstrated by separately analyzing sample six preparations as per the method. The %RSD was found to be less than 15%. The data of method precision are shown in Table 3.

LOD and LOQ

Limit of Detection and Quantitation was established by the signal-to-noise ratio. During the LOD determination, the s/n ratio of methanol and acetonitrile was observed as 10.5 and 27.1, in LOQ determination as 10.1 and 33.1, respectively, while the remaining solvents are meeting the acceptance criteria. United states pharmacopeia (USP) signal to noise (s/n) ratio for LOD should be Greater than or equal (≥) to 3 and for LOQ should be ≥ 10 for each specified solvent. The data of LOD and LOQ are presented in Table 4.

Linearity

The linearity solutions were prepared for each organic volatile impurity over the range of LOQ, 50, 80, 100, 120, and 150%. To draw the linearity graph between concentration and area of organic volatile impurities. Finally, the correlation coefficient (r) was obtained not less than 0.99 for twelve organic volatile impurities. The correlation is calculated based on $Y=MX+c$. The method was linear from LOQ% level to 150% level concerning the Oseltamivir phosphate API concentration. The linearity data and typical graph are presented in Table 5 and Fig. 4a, b.

Accuracy

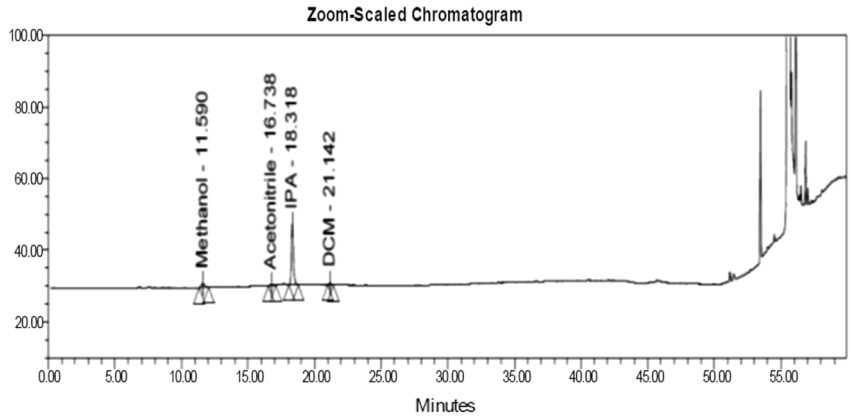
Accuracy is performed by the known amount of organic volatile impurities that standard solution was spiked to Oseltamivir phosphate API at three different concentrations (50,100,150, and LOQ%). From these accuracy data, the % recovery of organic volatile impurities was obtained 100 ± 20% at 50% Level to 150% Level and 100 ± 30% at LOQ Level. The results are shown in Table 6.

System precision at LOQ

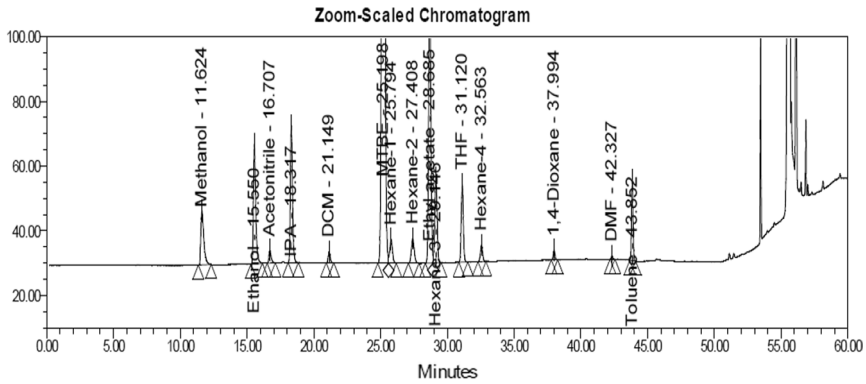
The system precision of this GC-HS method is expressed in the term of % RSD of the data. System precision at LOQ concentration has been demonstrated by injecting the six replicates of standard solutions. The obtained %RSD was not more than 15%. All values and chromatograms are shown in Table 7.

Robustness

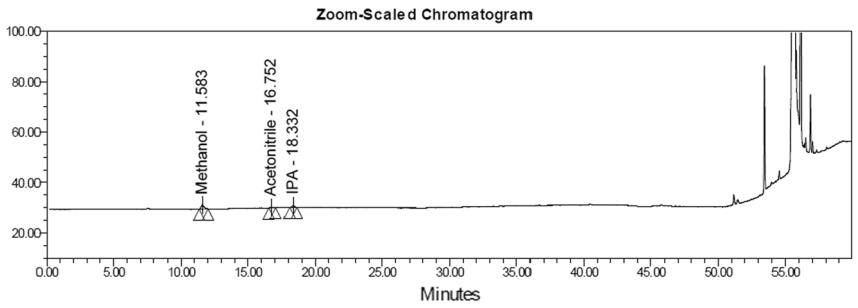
To determine the robustness of this GC-HS method, the %RSD was checked, to change any two method parameters from the initial conditions. Those parameters are column oven temperature 35 °C and 45 °C,



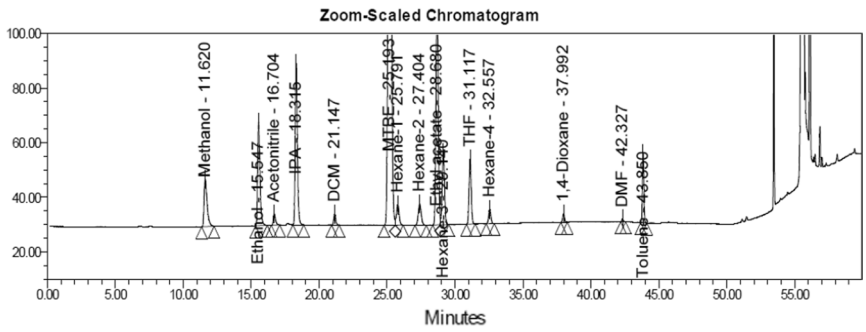
(a): Blank



(b): Standard solution



(c): Oseltamivir phosphate API



(d) Selectivity solution

Fig. 3 **a** Blank, **b** Standard solution, **c** Oseltamivir phosphate API, **d** Selectivity solution

Table 1 Specificity and Selectivity data

S. No	Name	Retention times of each solvent	Retention times of solvents in selectivity solution	Resolution between solvent peaks in selectivity solution
1	Methanol	11.61	11.62	–
2	Ethanol	15.55	15.55	10.9
3	Acetonitrile	16.7	16.7	3.8
4	IPA	18.31	18.32	5.5
5	DCM	21.15	21.15	10.1
6	MTBE	25.2	25.19	12.4
7	Hexane-1	25.79	25.79	1.6
8	Hexane-2	27.41	27.4	4.1
9	Ethyl acetate	28.68	28.68	3.6
10	Hexane-3	29.14	29.14	1.4
11	THF	31.12	31.12	6.3
12	Hexane-4	32.56	32.56	4.7
13	1,4-Dioxane	37.99	37.99	19.7
14	DMF	42.33	42.33	19.3
15	Toluene	43.85	43.85	7.3

Table 2 System precision data

No of injections	Peak areas						
	Methanol	**Ethanol**	**Acetonitrile**	**IPA**	**DCM**	**MTBE**	**Ethyl acetate**
1	286.66	454.05	46.85	471.52	36.17	4470.86	982.84
2	258.7	443.7	45.24	462.03	35.3	4406.22	964.7
3	260.6	451.7	46.03	469.01	35.7	4407.46	970.3
4	267.99	454.64	45.92	473.38	35.78	4498.06	976.72
5	282.98	463.53	47.53	482.04	36.54	4473.67	992.4
6	286.41	464.41	48.15	487.92	36.26	4421.39	984.88
Avg	**273.9**	**455.3**	**46.6**	**474.3**	**36**	**4446.3**	**978.6**
STDEV	**12.997**	**7.749**	**1.092**	**9.301**	**0.449**	**39.413**	**10.134**
%RSD	**4.7**	**1.7**	**2.3**	**2**	**1.2**	**0.9**	**1**

No of injections	Peak areas				
	THF	**1,4-Dioxane**	**DMF**	**Toluene**	**Hexanes**
1	266.16	27.79	8.37	192.18	613.03
2	262.23	27.2	7.67	187.52	607.53
3	263.57	27.42	7.44	188.88	606.39
4	265.19	27.62	8.03	191.38	627.26
5	268.5	28.33	7.99	194.33	608.18
6	265.31	28.98	9.87	199.53	604.22
Avg	**265.2**	**27.9**	**8.2**	**192.3**	**611.1**
STDEV	**2.158**	**0.658**	**0.865**	**4.285**	**8.434**
%RSD	**0.8**	**2.4**	**10.5**	**2.2**	**1.4**

Bold values indicate better results than other filtering methods

Table 3 Method precision data

No of injections	Peak areas						
	Methanol	**Ethanol**	**Acetonitrile**	**IPA**	**DCM**	**MTBE**	**Ethyl acetate**
1	3069.05	5104.35	430.81	7082.74	710.01	4878.18	5011.39
2	3137.88	5313.08	439.01	7281.68	720.92	4954.33	5122.94
3	2759.24	4859.6	408.87	6716.3	686.26	4839.57	4888.58
4	2796.26	4917.64	412.47	6724.73	685.49	4884.04	4897.69
5	2785.96	4913.64	414.77	6725.74	694.45	4910.43	4912.97
6	2764.73	4867.66	410.66	6519.87	674.61	4893.74	4886.57
Avg	**2885.5**	**4996**	**419.4**	**6841.8**	**695.3**	**4893.4**	**4953.4**
STDEV	**170.754**	**179.041**	**12.421**	**282.17**	**17.202**	**37.999**	**95.407**
%RSD	**5.9**	**3.60**	**3.00**	**4.10**	**2.5**	**0.80**	**1.90**

No of injections	Peak areas				
	THF	**1,4-Dioxane**	**DMF**	**Toluene**	**Hexanes**
1	712.97	391.13	965.73	913.78	283.1
2	727.4	401.8	1037.48	949.3	287.78
3	700.78	370.48	803.85	863.99	280.08
4	702.69	374.41	836.6	868.76	287.89
5	705.31	372.42	812.12	868.77	289.88
6	702.05	369.11	776.18	859.91	287.38
Avg	**708.5**	**379.9**	**872**	**887.4**	**286**
STDEV	**10.221**	**13.394**	**104.722**	**36.127**	**3.665**
%RSD	**1.4**	**3.5**	**12.0**	**4.1**	**1.3**

Bold values indicate better results than other filtering methods

Table 4 LOD and LOQ data

Name of OVI	LOD		LOQ	
	Conc. (ppm)	**S/N ratio**	**Conc. (ppm)**	**S/N ratio**
Methanol	16.52	10.5	54.06	10.5
Ethanol	14.53	4.5	48.6	4.5
Acetonitrile	20.78	27.1	66.49	27.1
IPA	6.01	4.3	20.03	4.3
DCM	18.04	3.3	54.13	3.3
MTBE	1.5	3.2	5.51	3.2
Ethyl acetate	6	3.3	20.01	3.3
THF	3.27	3.1	10.89	3.1
1,4-Dioxane	15.2	3.5	49.4	3.5
DMF	80.13	3.4	267.09	3.4
Toluene	4.05	4.7	13.49	4.7
Hexanes	0.88	4.4	2.94	4.4

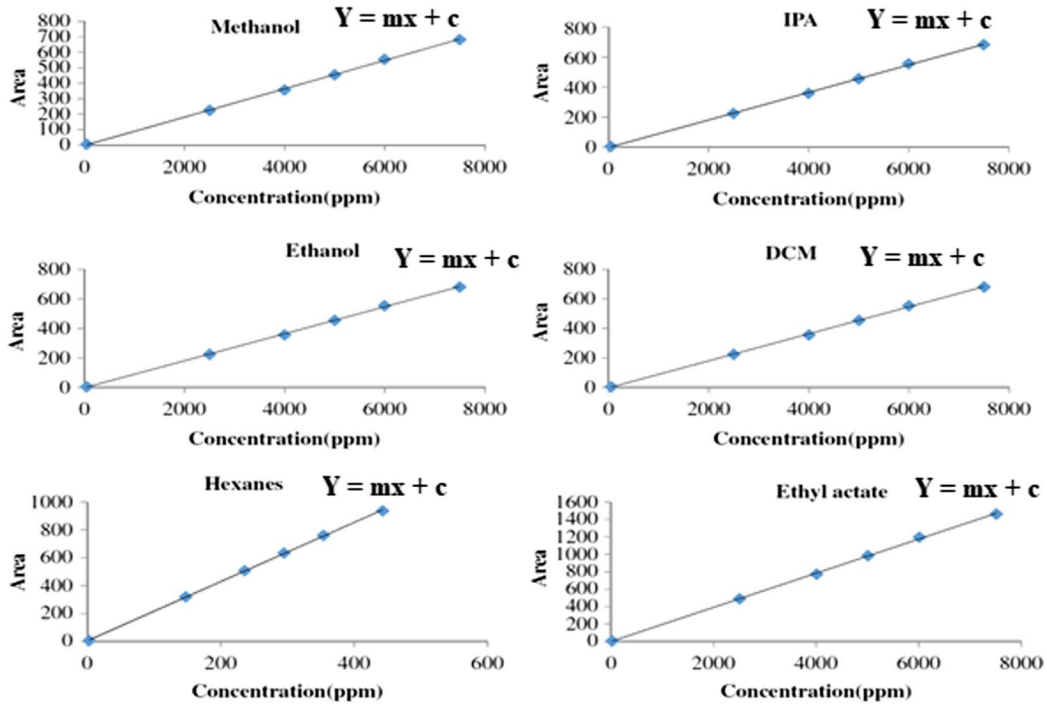
Table 5 Linearity data

Name of OVI	Correlation(r)
Methanol	0.9996
Ethanol	0.9998
Acetonitrile	0.9993
IPA	0.9998
DCM	0.9994
MTBE	0.9999
Ethyl acetate	0.9998
THF	0.9998
1,4-Dioxane	0.9997
DMF	0.997
Toluene	0.9997
Hexanes	0.9999

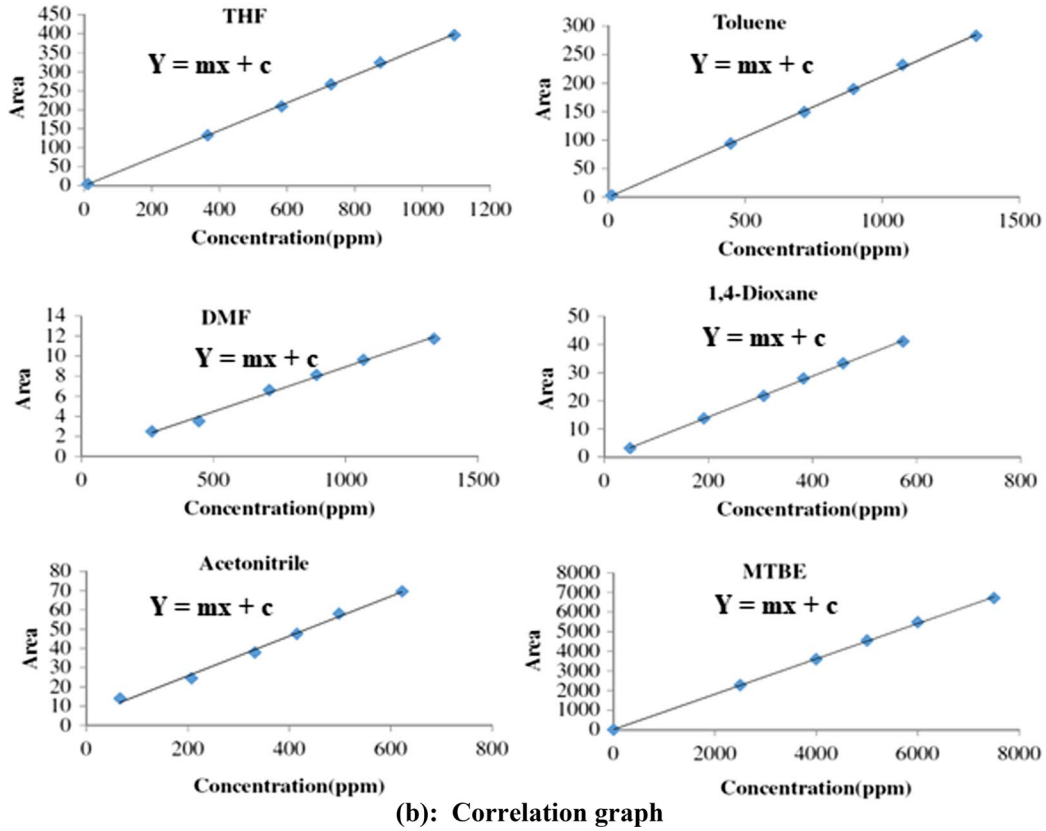
and injector temperature is 175 °C and 185 °C. The results are presented in Table 8. Finally, in two changed method parameters, the %RSD was not more than 15.0% for each organic volatile impurity.

Ruggedness

Ruggedness is the ability of the chemical measurement process to resist changes in the test results when subjected to minor changes in environmental and method procedural variables, laboratories, personnel,



(a): Correlation graph



(b): Correlation graph

Fig. 4 **a** Correlation graph, **b** Correlation graph

Table 6 Accuracy data

Name of OVI	LOQ recovery (%)	50% Recovery	100% Recovery	150% Recovery
Methanol	109.6	103.4	100.1	100.6
Ethanol	102.5	105.3	102.6	102.5
Acetonitrile	88.3	113.5	99.1	101.7
IPA	85.3	103.2	102.1	101.5
DCM	110.2	104.7	101.5	101.1
MTBE	89.5	105	101.7	99.5
Ethyl acetate	100.5	105.2	101.7	100.9
THF	94.4	103.7	100.3	99.3
1,4-Dioxane	110.7	105.5	101.3	100.7
DMF	108.8	100.4	96.1	97
Toluene	98.7	105.8	101.8	101.3
Hexanes	73.7	104.9	102.1	98.5

etc. Ruggedness has been established by separate six analyses of a single batch of the sample prepared by two different analysts on different days. Overall RSD of residual solvents was found out to be less than 15% as per AOAC guidelines and USP general chapter <1467>. According to this data, our method was rugged. All values are presented in Table 9.

Solution stability

Stability of six organic volatile impurities standard and Oseltamivir phosphate API sample prepared in NMP as a diluent. Three-time intervals (After 12 Hours (H), after 24 H and after 48 H) have been observed from the Initial time. The standard solutions were prepared on the same day and keep them at room temperature. Injected standard organic volatile solution at Initial, after 12 H, after 12 H and after 48 H. Then the calculated % of solution stability at each time point, compared with Initial time area of each organic volatile impurity. The % of solution stability is $100 \pm 15\%$ as per in-house established solution stability data. From these solution stability results; twelve organic volatile impurities standards were stable up to 48 h. The corresponding data are presented in Table 10.

Application of the proposed method (analysis of Oseltamivir phosphate tablet)

The proposed method was evaluated by the assay of commercially available Oseltamivir phosphate tablet (Oseltamivir 75 mg) for the quantitative deamination of organic twelve volatile impurities present in it. The obtained results should have within our proposed specifications. This revealed that the concentration of these twelve organic volatile impurities has presented

Table 7 System precision data at LOQ

No of injections	Peak areas						
	Methanol	Ethanol	Acetonitrile	IPA	DCM	MTBE	Ethyl acetate
1	9.9	4.35	13.44	4.64	3.43	4.73	4.76
2	11.13	3.53	14.29	5.74	4.06	4.62	4.54
3	10.78	3.49	13.71	5.51	3.76	4.58	4.94
4	10.29	3.93	13.7	5.11	3.21	4.46	4.24
5	10.03	3.37	13.32	6.13	3.15	5.01	4.47
6	10.84	3.55	15.14	5.06	3.49	4.79	4.27
Avg	**10.5**	**3.7**	**13.9**	**5.4**	**3.5**	**4.7**	**4.5**
STDEV	**0.493**	**0.369**	**0.679**	**0.535**	**0.344**	**0.192**	**0.274**
%RSD	**4.7**	**10**	**4.9**	**9.9**	**9.8**	**4.1**	**6.1**

No of injections	Peak areas				
	THF	1,4-Dioxane	DMF	Toluene	Hexanes
1	3.92	3.22	2.5	2.81	5.08
2	3.75	2.89	2.42	2.77	5.87
3	3.71	2.85	2.55	2.53	5.84
4	3.67	2.9	1.87	2.75	4.78
5	3.59	2.67	2.25	2.47	4.91
6	3.46	2.97	2.31	2.42	4.82
Avg	**3.7**	**2.9**	**2.3**	**2.6**	**5.2**
STDEV	**0.155**	**0.18**	**0.246**	**0.171**	**0.505**
%RSD	**4.2**	**6.2**	**10.7**	**6.6**	**9.7**

Bold values indicate better results than other filtering methods

Table 8 Robustness data

Name of organic volatile impurity	%RSD for different method parameters			
	Column oven Temp. 35 °C	Column oven Temp. 45 °C	Injector temp. 175 °C	Injector temp. 185 °C
Methanol	2.0	2.2	1.3	1.1
Ethanol	2.6	1.8	1.3	0.4
Acetonitrile	2.1	1.9	1.1	0.6
IPA	2.6	2.1	1	0.5
DCM	2.0	1.4	1	0.9
MTBE	1.0	0.7	1.7	1
Ethyl acetate	1.6	1.1	0.7	0.6
THF	1.4	0.8	0.8	0.6
1,4-Dioxane	3.9	2.7	1.9	1.1
DMF	13.5	10.3	5	5.2
Toluene	3.9	2.5	1.2	0.7
Hexanes	0.9	0.7	3.4	1.4

Table 9 Ruggedness data

Name of OVI	%RSD for different analysts and days		
	Day-1 Analyst-1&2 ($n=12$)	Day-2 Analyst-1&2 ($n=12$)	Analyst-1 Day-1&2 ($n=12$)
Methanol	1.5	2.6	6.2
Ethanol	2	0.6	2.7
Acetonitrile	12.62	12.1	13.9
IPA	2.2	0.8	3
DCM	2	0.5	2.1
MTBE	1.5	0.6	3.7
Ethyl acetate	1.5	0.2	1.8
THF	1.4	0.2	2.7
1,4-Dioxane	2.8	0.7	2.6
DMF	10.7	4.7	10
Toluene	2.4	0.4	3
Hexanes	1.5	1.4	5.3

Oseltamivir phosphate tablet in ppm levels. The corresponding data are presented in Table 11.

Discussion

The development of an analytical method for the determination of twelve organic volatile impurities (Methanol, Ethanol, Acetonitrile, IPA, DCM, MTBE, Hexane fractions, EA, THF, 1,4-Dioxane, DMF, and Toluene) in Oseltamivir phosphate API and pharmaceutical dosage forms by GC-HS with flame ionization detector has received considerable attention in recent years because of their importance in quality control of API drug and its impurities. The goal of this study was to develop a simple rapid accurate and precise GC-HS method for the simultaneous determination of twelve organic volatile impurities using the most commonly employed DB-1, 60 m, 0.32 mm, and 5.0 µm column with flame ionization detector.

We got well resolution between twelve OVI's. The numbers of theoretical plates obtained for six organic volatile impurities were more than 2000, respectively, which indicates the efficiency of the column. The % RSD was found not more than 15% for the system precision, Method precision, LOQ precision, Robustness, and Ruggedness. Linearity was observed over the concentration range of LOQ to 150% for twelve OVI's with a correlation coefficient ($r2=0.99$). From these precision and Linearity data, our proposed method is precise and linear. We have obtained LOD and LOQ results for the twelve solvents very low level with acceptable USP s/n ratio. From LOD and LOQ result indicates the sensitivity of the method. The recovery was found to be $100\pm15\%$ at 50%, 100%, 150%, and LOQ. We have reported the prepared standard, and sample solution in NMP is stable up to 48 h. Our method is also applied to pharmaceutical dosage forms. The above all validated data indicates that the proposed GC-HS method is highly sensitive and accurate.

Table 10 Solution stability

Time interval	Methanol % of variation	Ethanol % of variation	Acetonitrile % of variation	IPA % of variation	DCM % of variation	MTBE % of variation	EA % of variation
After 12 h	−0.4	−2.4	−1.6	−2.6	−1.7	−1.3	−1.7
After 24 h	−0.3	−1.4	−0.2	−1.9	−0.6	−1.1	−1.3
After 48 h	2	0.9	3.3	2.1	2.6	0.5	1.1

Time interval	THF % of Variation	1,4-Dioxane % of Variation	DMF % of Variation	Toluene % of Variation	Hexanes % of Variation
After 12 h	−1.5	−1.9	0.9	−2.4	−1.3
After 24 h	−1.3	−0.2	−5.2	−1.4	−1.5
After 48 h	0.4	−0.2	7.1	1.6	−1.2

Table 11 Data for Oseltamivir phosphate tablet analysis

Si. No	Name of OVI	Results (ppm)	Approved values (ppm)
1	Methanol	108	Not more than 3000
2	Ethanol	Not detected	Not more than 5000
3	Acetonitrile	112	Not more than 410
4	IPA	120	Not more than 5000
5	DCM	Not detected	Not more than 600
6	MTBE	Not detected	Not more than 5000
7	Ethyl acetate	Not detected	Not more than 5000
8	THF	Not detected	Not more than 720
9	1,4-Dioxane	Not detected	Not more than 380
10	DMF	Not detected	Not more than 880
11	Toluene	Not detected	Not more than 890
12	Hexanes	Not detected	Not more than 290

Conclusions

Reliable and effective gas chromatography coupled with flame ionization mode of detection dependent methodology to detect and quantify the residual chemical solvents Methanol, Ethanol, Acetonitrile, Isopropyl alcohol, Dichloromethane, MTBE, Hexanes, Ethyl acetate, THF, 1,4-Dioxane, DMF, Toluene in oseltamivir phosphate drug substance as well as in drug product. The validation parameters (linear, system suitability, quantification limit, detection limit, robustness, accuracy, precision, selectivity, ruggedness) for opted twelve residual chemical solvents were in line with ICH requirement and AOAC guidelines. Present results revealed that the quality of the oseltamivir phosphate sample can be evaluated using the methodology of gas chromatography proposed in this work. The method was found to be applicable for the routine analysis of the Oseltamivir phosphate API, and its pharmaceutical dosage forms in the pharmaceutical industry.

Abbreviations

MTBE: Methyl tert-butyl ether; IPA: Isopropyl alcohol; THF: Tetra hydro furan; DMF: Dimethylformamide; DCM: Dichloromethane; EA: Ethyl acetate; API: Active pharmaceutical ingredient; N_2: Nitrogen; ICH: International Council for Harmonization; LOQ: Limit of quantification; LOD: Limit of detection; Ppm: Parts per million; AOAC: Association of Official Agricultural Chemists; RP-HPLC: Reversed-phase high-performance liquid chromatography; LC: Liquid chromatography; GC-HS: Gas chromatography-head space; USA: United States of America; FID: Flame ionization detector; °C: Centigrade; NMP: N-methyl-2-pyrrolidone; Psi: Pounds per square inch; mL: Milley letter; mg: Milly gram; RSD: Relative standard deviation; USP: United states pharmacopeia; s/n: Signal to noise; $\geq$: Greater than or equal; OVI: Organic volatile impurities; H: Hours.

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Authors' contributions

All authors read and approved the final manuscript.

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Availability of data and materials

From the literature and GVK Biosciences.

Declarations

Ethics approval and consent to participate

Our proposed work is not related to Bio work. This is completely Impurity analysis by a new analytical technique (GC-HS). In the IRB guidelines like 21 CFR 50.24 and 45 CFR, Part 46" is mentioned Human, Bio, Clinical trials work. According to the above guideline, our work is not related to Human, Bio, Clinical trials work. This is completely new analytical method development work. So, I hope do need the "Ethics approval and consent to participate".

Consent for publication

Our proposed work is not related to Bio and Clinical trials work. So, "Consent for publication" is not applicable.

Competing interests

Not applicable.

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