ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 15, Issue 2, 2022

Online - 2455-3891
Print - 0974-2441

Research Article

QUANTITATIVE DETERMINATION OF RESIDUAL SOLVENTS IN PALONOSETRON API BY HS-GC METHOD

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Received: 22 November 2021, Revised and Accepted: 10 January 2022

ABSTRACT

Objectives: Palonosetron is an antidote to 5-HT_3 in the prevention and treatment of chemotherapy-induced nausea and vomiting (CINV). The presence of residual solvents in pharmaceutical drug substances or products, as well as excipients, can have a detrimental effect on the product's quality and stability. These substances must be evaluated for safety and efficacy. The primary purpose of this work is to establish a method for validating and quantifying residual solvents in palonosetron API using Head Space Gas Chromatography (HS-GC).

Methods: In the proposed HS-GC technique for the quantifying residual solvents - ethanol, acetone, methanol, acetonitrile, and isopropyl alcohol (IPA) in Palonosetron API, the headspace equilibrium was achieved at 100°C and analyzed by DB-624 column (30 m × 0.24 mm, 1.8 µm) with injector and detector temperature set at 200°C and 230°C respectively. The dissolving solvent was dimethyl sulfoxide (DMSO). After the initial holding time of 5mins, the temperature was increased to 120°C from 40°C in 20mins at a rate of 10°C/min using a flow rate of 10 ml/min and a split ratio of 1:25 with nitrogen as carrier gas. The approach created has been validated and quantified as per International Conference on Harmonization's (ICH) guidelines.

Results: All the results obtained were within the ICH specified limits. The validation results for repeatability studies (%RSD values) were found to be <10; recovery studies values were in the range of 90–110% and for the selected linearity range 25–150 µg/ml the correlation coefficients(γ^2) for all the solvents were observed to be >0.99.

Conclusion: A sensitive, simple, precise, and economic HS-GC method with Flame Ionization Detector (FID) was developed and validated to quantitatively determine the residual solvents in Palonosetron API.

Keywords: Palonosetron, Residual solvents, Headspace gas chromatography, International conference on harmonization.

 DOI: http://dx.doi.org/10.22159/ajpcr.2022v15i2.43669. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Palonosetron, chemically known as (3aS)-2-[(3S)-Quinuclidin-3-yl]-2,3,3a,4,5,6-hexahydro- 1H-benzo[de]-isoquinolin-1-one; is 5-HT_3 receptor antagonist suggested for the treatment of nausea and vomiting associated with moderately-emetogenic cancer chemotherapy and post-operative nausea and vomiting. The anti-emetic effect is achieved by inhibiting $5HT_3$ receptors in both the central nervous system (medullary chemoreceptor zone) and peripheral nervous system (gastrointestinal tract) [1]. The FDA approved this medication in 2014 for use in combination with netupitant to treat CINV [2].

Residual solvents (RS) are frequently used in the manufacture of pharmacological compounds, excipients, and finished pharmaceutical products. Their presence is undesirable in the final product as it may affect the quality and stability of the drug which can be unpleasant for the patients. Even after applying various techniques to remove them, some solvents are still retained in small quantities. The need to test and control RS in pharmaceutical products was recognized in the late '70s [3]. Pharmaceutical products must be tested for 'RS when production or purification of processes known to produce or purify solvents' according to ICH harmonized guidelines set up by the European Union, Japan, and the United States for the registration of pharmaceutical products [4]. These guidelines specify the analytical procedures used to identify and quantify residual solvents, as well as the permitted acceptable concentration limits. Table 1 summarizes the RS concentration limitations for palonosetron.

Gas chromatography (GC) is a choice of analytical method for RS determination, as they have relatively low boiling points and are thermally stable. Different aspects of the GC method, such as injection systems, columns, and/or detectors, must be taken into account when developing it. Analyte retention times and detection limits can be reduced using the right system [5]. The headspace analysis extracts volatile and semi-volatile components [6]. A single sample of gas is collected and heated in a sealed vial before being delivered to the GC in a static headspace. Once the gas and liquid have equilibrated, the gas sample is obtained. This approach is the optimum choice when the pharmaceutical samples are soluble in solvents such as water, DMSO, dimethylformamide, dimethylacetamide, dimethyl isosorbide, or benzyl alcohol [7]. Separation is accomplished using capillary and wide-bore columns are used. The most often used stationary phases are polysiloxanes and polyethylene glycols [8]. When the analytes present in the sample are known or suspected, the flame ionization detector (FID) is indicated for GS- RS analysis. Due to its low detection limits, large linear dynamic range, and overall reliability and utility, FID has become the most widely employed detector in GC [9].

In general, static headspace GC analysis with FID detection is the most commonly used method for RS determination in pharmaceuticals. From the scheme of synthesis [10], residual solvents - ethanol, acetone, methanol, acetonitrile, and IPA were used in the synthesis of the palonosetron. The presence of this RS should be validated and quantified for ensuring the safety and efficacy of the drug. The present study created a unique HS-GC approach for the validation and quantification of RS in palonosetron API.

MATERIALS AND METHODS

Materials

Ethanol and acetone were obtained from Qualigens, Mumbai; methanol, acetonitrile, IPA, and DMSO were obtained from Sigma Aldrich,

Table 1: Concentration limits and boiling points for the RS present in Palonosetron

S. No.	Solvent	PDE (mg/day)	Concentration limit (ppm)	Boiling point (°C)
1	Ethanol	<50	5000	78
2	Acetone	<50	5000	56
3	Methanol	30	3000	65
4	Acetonitrile	4.1	410	82
5	IPA	<50	5000	83

*PDE is permitted daily exposure of the RS in mg/day as mentioned in USP General Chapter<467>Residual Solvents and ppm is parts per million.

Bengaluru. Palonosetron was a gift sample procured from Chandra labs, Hyderabad.

Instrument details

The study used Agilent infinity 7697A Head Space Gas Chromatography system equipped with a flame ionization detector and Open labs EZ chrome, as well as Mettler Toledo precision balance for weighing.

Method development

The selection of an appropriate GC system which includes a column, injection system, and detector, is a very crucial step in method development which will result in shorter retention times [5].

Selection of column

In order for the separation process to work, the stationary phase (SP) and the various chemical and physical properties of the injected sample must be in interaction [11]. In order to properly understand the compounds under investigation, chemistry must be considered [12]. This difference in analyte-phase interactions is responsible for the wide range of capillary column phases. Consequently, in Column I.D., efficiency and sample capacity can be balanced [13]. As the sample capacity increases, the efficiency of column I.D. decreases. Therefore, lower I.D. indicates higher efficiency. Coming to the next factor, film thickness; decreasing the thickness, a resolution is better but increased signal-to-noise is observed, and increasing the thickness will reduce the resolution by increasing column bleed. Considering all the parameters, film thickness should be selected. Mostly 0.25 mm I.D. columns have 0.25 or 0.50 μm film thickness [12]. Finally, column length: 30 m column provides the best balance of resolution, analysis time, and required column head pressure [14].

Selection of injection system

The sample injection technique will have a significant impact on the recovery and reproducibility of GC analysis. In most circumstances, the inlet temperature is maintained at 50°C above the lowest boiling point component of the sample mixture (Table 1). The autosampler tray is typically packed with sealed vials, and samples are injected one at a time. Operational software controls the injection volume, number of wash cycles, and injection sequence for standards and samples [15].

Selection of detector

FID is the most commonly used detector in GC for laboratory analysis. It has a wide dynamic range and is highly sensitive to all substances that contain carbon. The advantages of FID are easy to operate, simple, reliable, versatile, and gives no or little signal for common carrier gases or typical contaminants [16].

By considering all the above-mentioned factors, the following chromatographic conditions were optimized for method development of Palonosetron (Tables 2 and 3).

Optimization details

DB-624 Column (30 m*0.24 mm, 1.8 μm) was chosen for HS-GC analysis at a headspace equilibrium temperature of 100°C. A milliliter of standard and sample solutions was injected into an injection port at 230°C for an inlet temperature of 200°C, which was greater than the lowest boiling point component in the sample mixture. With a flow rate of 10ml/min and a split ratio of 1:25, the beginning oven temperature was set at 40°C, increasing by 10°C/min over a 5-min hold period until the final oven temperature 120°C. Nitrogen was used as the carrier gas, and the GC was performed for 20 min.

Table 2: Optimized gas chromatographic conditions for palonosetron

GC parameter	Condition
Column	DB-624 Column (30 m*0.24 mm, 1.8 μm)
Inlet temperature	200°C
Detector temperature	230°C
Initial oven temperature	40°C
Final oven temperature	120°C
Carrier gas	Nitrogen
Flow rate	10 ml/min
Split ratio	1:25

Table 3: Optimized headspace conditions for palonosetron

Headspace parameter	Conditions
Oven temperature	100°C
Transfer line temperature	110°C
GC cycle time	20 min
Loop fill temperature	120°C

Preparation of blank, standard, and sample solution

All these studies were carried out at room temperature. DMSO was selected as a dissolving solvent in the determination of RS in the Palonosetron API.

For blank vail, 1 ml of DMSO was used. A standard stock solution in DMSO was prepared with each of the RS in Palonosetron API (ethanol, acetone, methanol, acetonitrile, and IPA) at a concentration of 2000 μg/ml. This is followed by the preparation of a sub-stock solution with a final concentration of 100 μg/ml. For the standard vial, 1 ml of the prepared sub-stock standard solution was taken and for the sample vial, approximately 10 mg of sample in 1ml DMSO as dissolving solvent was taken.

Method validation

Method validation is conducted by evaluating the following criteria, in accordance with ICH guidelines: specificity, linearity, precision, accuracy, the limit of detection (LOD), limit of quantification (LOQ), ruggedness, and system adaptability [17].

Specificity

The specificity is determined by injecting blank, standard, and specificity solutions (composite standard solutions of all residual solvents) and by checking out different parameters such as resolution, tailing factor, and theoretical plates for system suitability.

Linearity

A series of dilutions of the standard solutions between the concentrations of 25 and 150 μg/ml were made. A linear relationship between the concentrations and the responses of the solvents was represented in a calibration curve form and correlation coefficients with regression equations were calculated statistically.

Limit of detection (LOD) and Limit of quantification (LOQ)

Limit of detection refers to the smallest value of the analyte that the detector is capable of detecting and limit of quantification refers to the smallest amount of analyte the detector is capable of quantifying. Both values were calculated statistically using the following formulas:

$$LOD = 3.3*(\sigma/S)$$

$$LOQ = 10*(\sigma/S)$$

Where σ is standard deviation and S is a slope.

Precision
Six replicate injections of standard solutions of solvents were taken and analyzed for evaluating system precision according to the harmonized method and the chromatograms were obtained.

Ruggedness
Six replicate injections of standard solutions of solvents were taken and analyzed by a different analyst to test the ruggedness of the developed method.

Robustness
Solutions were taken in triplicate by varying temperatures - low, control, and high to determine the robustness of the system.

Accuracy
The accuracy was determined by spiking all the solvents in triplicates at three distinct concentrations: 75%, 100%, and 150% to the quantitation limit.

RESULTS

The quantitative determination of the residual solvents methanol, ethanol, acetone, acetonitrile, and IPA in palonosetron API has been developed and validated. Optimizing the chromatographic conditions during method development was critical in the development of this method and validated in accordance with ICH guidelines [17]. The obtained results were found to meet the acceptance criteria. This technique has demonstrated excellent linearity, recovery, and repeatability.

Specificity and system suitability
The system suitability is tested before starting the analysis by injecting the standard solutions of the solvents. From the data, for the parameters - retention times, resolution, theoretical plates, and tailing factor (Table 4), the values were found to be within the accepted limits and hence pass the test. Fig. 1 depicts a chromatogram of the standard solution. All the peaks were well resolved without any interference with solvent or API peaks and hence the method was found to be specific.

Linearity
To establish a linear relationship between residual solvent concentration and average peak area, a graph of concentration vs average peak area was produced and correlation coefficient, y-intercept, and slope of the regression was determined [18-21]. The linearity acceptance condition, γ^2 should be >0.99. Table 5 contains the linearity results.

LOD and LOQ
Using the above-mentioned statistical formulas, the values for LOD and LOQ were calculated. The acquired values were listed in Table 5.

Precision
The standard deviation and percentage relative standard deviation (percent RSD) were calculated in this method to determine the system's precision. Acceptance limits for method and system precision should be percent RSD NMT 10%. The data obtained were represented in mean(SD) values which indicate the reliability of this study. The %RSD values were found to be 2.51 for acetone, 3.40 for methanol, 3,17 for ethanol, 3.59 for acetonitrile, and 3.69 for IPA. By observing the results in Table 6 it clearly understood that the %RSD values were within the limits and below 5%; method and system were said to be precise.

Ruggedness
Ruggedness is a criterion for determining the constancy of the results when the external factors such as analyst, instrument, and lab are varied. In this developed method, ruggedness has been carried out by different analysts, and the results were found to be satisfactory for which the %RSD values were below 5% (Table 7).

Robustness
It is a statistic that indicates a method's capacity to remain unaffected by deliberate parameter changes. Robustness was determined in this method by varying the temperature of the oven (Table 8).

Table 4: Specificity and system suitability parameters

Solvent	Retention time (min)	Tailing factor	Theoretical Plates	Resolution
Acetone	2.86	1.2	5184	-
Methanol	5.28	1.0	7744	18.46
Ethanol	7.34	1.1	8464	10.42
Acetonitrile	9.55	1.4	9525.8	8.46
IPA	13.00	1.0	11664	17.6
Acceptance Criteria (For information)	-	NMT 2	NLT 5000	NLT 1.5

*NMT: Not more than, NLT: Not less than.

Table 5: Linearity, LOD, and LOQ values

Solvent	Linearity (γ^2)	Slope	LOD (µg/ml)	LOQ (µg/ml)
Acetone	0.991	149.22	5.72	17.33
Methanol	0.994	222.736	7.25	21.9
Ethanol	0.999	104.044	9.64	29.21
Acetonitrile	0.99	1186.96	8.10	24.55
IPA	0.997	209.832	9.77	29.60

*Where LOD and LOQ are limits of detection and limit of quantification, respectively measured in µg/ml; γ^2 indicates correlation coefficient values.

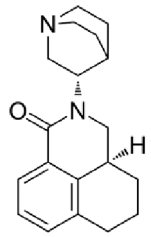

Fig. 1: Structure of palonosetron

Accuracy
Accuracy is determined by calculating the percentage recovery at three different levels in triplicates by the formula:

$$\%Recovery = [(A_{sp} - A_s)/A_{std}]*100$$

Where A_{sp} denotes the solvent area in a spiked sample, A_s denotes the solvent area in sample and A_{std} denotes the solvent area in standard solution.

The acceptance limit of %recovery for accuracy is 80–120%. The values for the accuracy data were given in Table 9 and all of them were found to be within the prescribed limits (Table 10).

DISCUSSION

From the literature, its understood that very few analytical methods have been reported for palonosetron [18-20] which includes the methods viz., UV, single-dose, and fixed-dose method development and validation of palonosetron using HPLC, whereas the HS-GC method has not been reported.

The developed method has passed the system suitability (Table 4) and was found to be specific as there is no interference at the retention times of the targeted residual solution from each other and solvent peaks or unknown peaks (Fig. 2). The retention times of acetone, methanol, ethanol, acetonitrile, and IPA were found to be 2.860, 5.286, 7.346, 9.563, 13.007 min respectively.

Table 6: Results for precision

Solvent	Acetone		Methanol		Ethanol		Acetonitrile		IPA	
Parameters	**Area**	**RT**	**Area**	**RT**	**Area**	**RT**	**Area**	**RT**	**Area**	**RT**
Standard 1	10056.6	2.86	14245.7	5.27	9310.8	7.34	80974.6	9.53	26059.0	13.0
Standard 2	10266.3	2.86	14285.2	5.28	9314.3	7.34	80489.9	9.56	16438.1	13.0
Standard 3	10175.4	2.86	14000.3	5.28	9585.0	7.34	78787.7	9.55	16202.3	13.0
Standard 4	10806.8	2.86	15354.7	5.28	10141.8	7.34	86837.4	9.55	17788.2	13.0
Standard 5	10250.9	2.85	14096.0	5.28	9653.4	7.34	79121.7	9.55	16383.9	13.0
Standard 6	10626.8	2.86	14947.9	5.28	9963.8	7.34	84120.6	9.55	17408.9	13.0
Mean (SD); n=6	10311 (258.7)	-	14396 (490.0)	-	9601.1 (303.9)	-	81242 (2914.3)	-	16855 (621.2)	-
%RSD	2.51	-	3.40	-	3.17	-	3.59	-	3.69	-

*Where RT is retention time in mins, SD is standard deviation, %RSD is percentage relative standard deviation and n is number of observations.

Table 7: Results for ruggedness

Solvent	Acetone		Methanol		Ethanol		Acetonitrile		IPA	
Parameters	**Area**	**RT**	**Area**	**RT**	**Area**	**RT**	**Area**	**RT**	**Area**	**RT**
Standard 1	10068.7	2.86	14246.1	5.29	9301.4	7.35	80983.9	9.56	16261.3	13.0
Standard 2	10278.3	2.86	14276.1	5.28	9307.1	7.33	80493.9	9.56	16437.3	13.0
Standard 3	10183.5	2.86	14001.2	5.28	9582.2	7.34	78775.0	9.55	16205	13.0
Standard 4	10817.1	2.86	15356.6	5.28	10143.2	7.34	86817.0	9.55	17786.4	13.0
Standard 5	10262.7	2.85	14099.3	5.28	9662.5	7.33	79111.5	9.55	16382.4	13.0
Standard 6	10639.7	2.87	14950.9	5.28	9961.0	7.34	84108.1	9.55	17408.0	13.0
Mean (SD); n=6	10375 (263.9)	-	14488.4 (493.3)	-	9659.6 (311.8)	-	81714.9 (2907)	-	16746.7 (615.9)	-
%RSD	2.54	-	3.41	-	3.23	-	3.56	-	3.68	-

*Where RT is Retention Time in mins., SD is standard deviation, %RSD is relative standard deviation and n is number of observations.

Table 8: Results for robustness

Solvent	Acetone	Ethanol	Methanol	Acetonitrile	IPA
Low					
Standard 1	10818.32	15354.49	10151.08	86830.64	17788.78
Standard 2	10262.27	14099.81	9670.86	79124.4	16382.7
Standard 3	10639.72	14952.33	9973.55	84125.62	17408.53
Mean (SD); n = 3	10513.57 (283.67)	14802.21 (640.67)	9931.83 (242.82)	83366.12 (3909.88)	17193 (727.32)
%RSD	2.67	4.28	2.43	4.64	4.18
Control					
Standard 1	10175.38	14000.26	9585.00	78787.69	16202.29
Standard 2	10806.84	15354.70	10141.78	86837.42	17788.30
Standard 3	10250.91	14096.93	9963.76	79121.67	17408.94
Mean (SD); n = 3	10311 (258.7)	14396.89 (490.0)	9601.1 (303.9)	81242 (2914.3)	16855 (621.22)
%RSD	2.51	3.40	3.12	3.59	3.69
High					
Standard 1	10068.73	14246.12	9318.37	80986.47	16261.27
Standard 2	10278.34	14286.18	9320.74	80505.34	16439.48
Standard 3	10183.43	14002.31	9593.0	78789.68	16205.94
Mean (SD); n = 3	10176.83 (104.96)	14178.2 (153.64)	9410.7 (157.88)	80093.8 (1154.76)	16302.23 (122.04)
%RSD	1.03	1.20	1.64	1.47	0.75

*Where SD is standard deviation, %RSD is percentage relative standard deviation and n is number of observations.

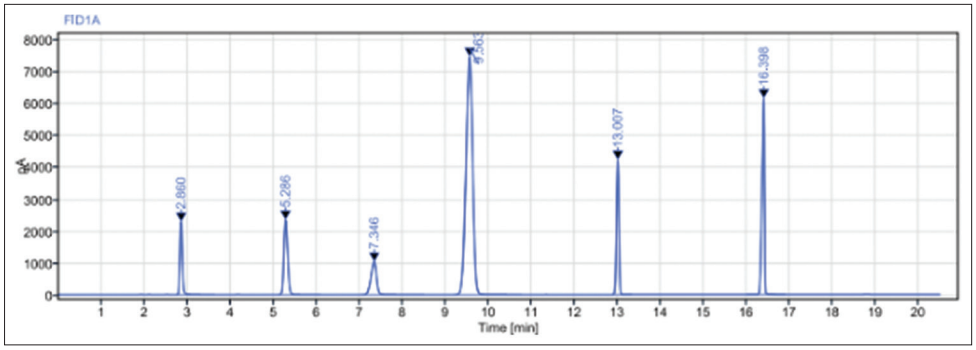

Fig. 2: A typical chromatogram of standard solution

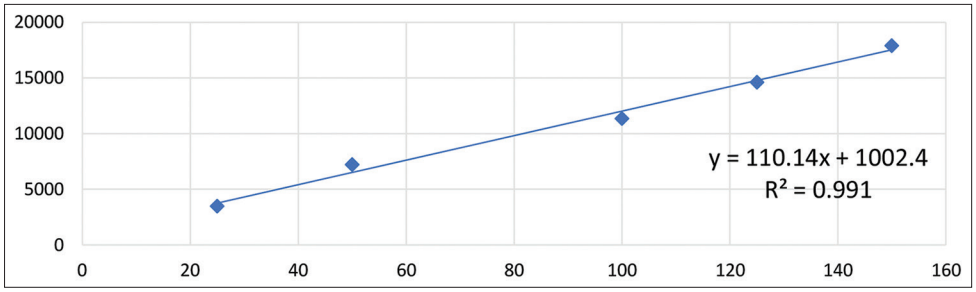

Fig. 3: Linearity graph for acetone

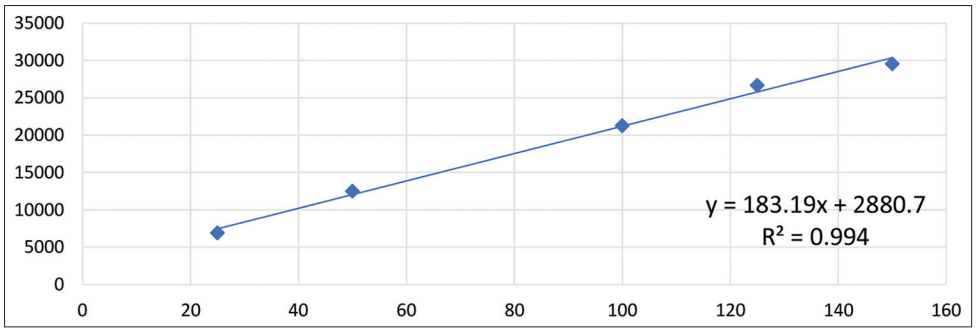

Fig. 4: Linearity graph for methanol

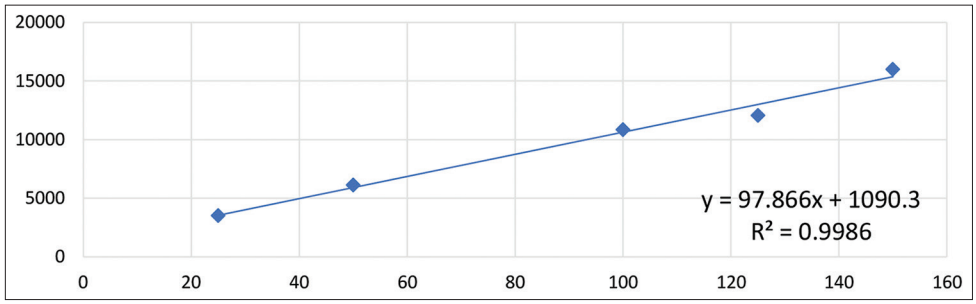

Fig. 5: Linearity graph for ethanol

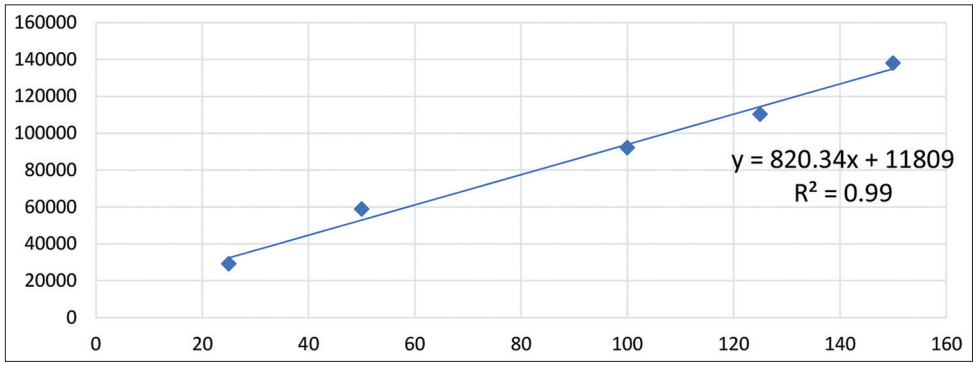

Fig. 6: Linearity graph for acetonitrile

The linearity was evaluated within the concentration range 25–150 µg/ml for the residual solvents- acetone, methanol, ethanol, acetonitrile, and IPA, for which γ^2 values were found to be >0.99 (Figs. 3-7 and Table 5), hence, the method was linear. LOD values were found to be 5.72 µg/ml for acetone, 7.25 µg/ml for methanol, 9.64 µg/ml for ethanol, 8.10 µg/ml for acetonitrile, and 9.77 µg/ml for IPA. LOQ values were found to be 17.33 µg/ml for acetone, 21.9 µg/ml for methanol, 29.21 µg/ml for ethanol, 24.55 µg/ml for acetonitrile, and 29.60 µg/ml for IPA. These values were found to be satisfactory(Table 5). %RSD values for precision, ruggedness, and robustness were found to be NMT 10% which signifies the reliability and reproducibility of the study (Tables 6-8). The values obtained for percentage recoveries of the solvents prove the accuracy of this method (Table 9). Excellent results were achieved with a faster run time of 20 min.

CONCLUSION

For the determination of residual solvents in palonosetron API, a simple HS-GC method has been developed using flame ionization detection and quantitatively proven to be both accurate and precise.

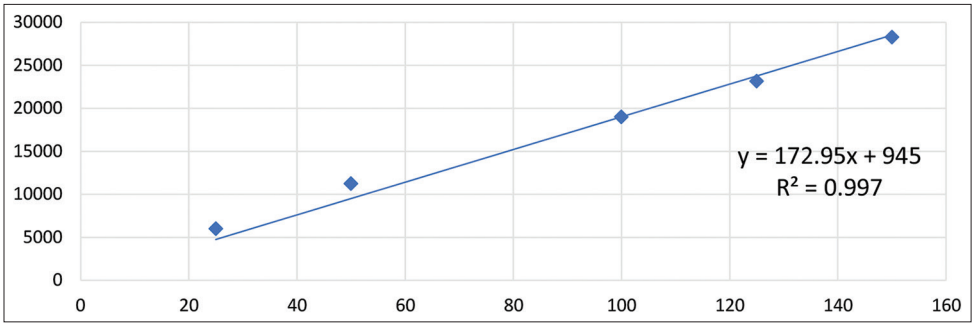

Fig. 7: Linearity graph for IPA

Table 9: Results for recovery studies

Spiking level (% of QL)	Acetone	Methanol	Ethanol	Acetonitrile	IPA
50%-R-1	84.75	118.31	91.24	87.68	87.57
R-2	85.23	116.20	94.13	88.56	89.43
R-3	88.61	115.15	92.60	88.13	86.43
100%-R-1	100.70	98.26	104.06	96.35	99.72
R-2	102.87	99.01	105.32	98.87	98.63
R-3	102.65	98.40	103.11	99.54	98.04
150%-R-1	98.47	110.59	95.12	95.40	94.44
R-2	99.14	113.76	96.71	96.18	95.64
R-3	99.01	111.13	96.54	96.65	96.32

*QL is the quantification limit and R is recovery percentage.

Table 10: Summary of validation parameters

Parameter	Result	Acceptance Criteria
Specificity	No interference of solvent peaks w.r.t. diluent or API peaks.	Good peak resolution
System Suitability	1. Tailing factor - 1.0–1.4 2. Theoretical plates - 5184–11686 3. Resolution- >1.5	NMT 2 NLT 5000 NLT 1.5
Linearity	γ^2-0.990–0.997	γ^2>0.99
LOD	5.72–9.77 µg/ml	Satisfactory
LOQ	17.33–29.60 µg/ml	Satisfactory
Precision (n=6)	%RSD - 2.51–3.69	NMT 10%
Ruggedness (n=6)	%RSD - 2.54–3.68	NMT 10%
Robustness (n=3)	%RSD - 0.75–4.64	NMT 10%
Accuracy	R1 - 84.75–118.31% R2 - 96.35–105.32% R-3 - 94.44–113.76%	R - 80–120%

*w.r.t: With respect to; API: Active pharmaceutical ingredient; NMT: Not more than; NLT: Not less than; γ^2: Correlation coefficient; LOD: Limit of detection; LOQ: Limit of quantification; %RSD: Percentage relative standard deviation; R: Recovery percentage; R1, R2 and R3 are recovery percentages at 50%, 100% and 150% respectively.

In universities and small-scale companies, this method can be used for routine testing.

ACKNOWLEDGEMENT

Our sincere gratitude to the Centre for Scientific and Industrial Research(CSIR), New Delhi and University College of Technology, Osmania University, Hyderabad.

AUTHORS CONTRIBUTION

Research work, analysis of data, preparation, and drafting of a manuscript was done by G. Sunny Grace; and thorough review and revision of manuscript was done by G. Vijaya Lakshmi.

CONFLICT OF INTEREST

None.

AUTHORS FUNDING

Financial assistance was provided by the Centre for Scientific and Industrial Research(CSIR), New Delhi.

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