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# Residual Solvents Quantitation Method for Lasmiditan Hemisuccinate API by HSGC-FID

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## ABSTRACT

Lasmiditan Hemisuccinate (LDT) is an innovative medication designed for the treatment of acute migraines, operating by activating 5-HT1F receptors found within the central nervous system. The synthesis of LDT necessitates the use of various solvents. According to regulatory standards, assessing the levels of residual solvents in drug substances is imperative in API (Active Pharmaceutical Ingredients). Present study concentrates on the evaluation and validation of a method for quantifying residual solvents like methanol, acetone, isopropyl alcohol, dichloromethane, methyl ter. butyl ether, n-hexane, ethyl acetate, n-heptane in LDT. Analysis was conducted using a DB-1 capillary column, measuring 60 meters in length with an internal diameter of 0.32 mm and a film thickness of 5 µ. The oven temperature was set to 65°C for 12 minutes, followed by a ramping rate of 15°C per minute to reach 85°C, where it was held for 5 minutes. Subsequently, a second ramping phase increased the temperature at a rate of 10°C per minute until it reached a final temperature of 220°C, which was maintained for 5 minutes. The injector temperature was maintained at 200°C and nitrogen was utilized as the carrier gas with 1-methyl-2-pyrrolidinone (NMP) diluent serving as the sample solvent. A suitable sensitive and robust HSGC-FID method is developed for quantitation of residual solvents with flame ionization (FID) detector. The evaluated method can applied to analyse of solvents present in a various range of APIs, intermediates.

**Keywords**: Residual solvents, Lasmiditan Hemisuccinate, Method validation

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## INTRODUCTION

Residual solvents, which lack therapeutic value, present risks of toxicity and undermine the quality and stability of active pharmaceutical ingredients (APIs) or drug products [1-4]. Despite their insignificance, completely removing solvents via conventional manufacturing methods like elevated process temperatures or reduced pressure proves challenging. Therefore, minimizing their presence is crucial. The International Conference on Harmonization (ICH) Q3C has set permissible daily exposure limits (PDE) for various solvents, categorizing them into four classes based on toxicity and environmental hazards. Class I solvents include 5 residual solvents, Class II comprises 29 solvents, and Class III covers 26 solvents. Although Head Space Gas Chromatography (HSGC) is frequently employed for residual solvent quantification, it effectively mitigates interference from degradation and decomposition products. However, it necessitates larger sample loads and extended analysis times due to prolonged equilibration periods and solvent evaporation. Nonetheless, the headspace technique provides the advantage of bypassing direct liquid or solid injections.[5-11]

Lasmiditan hemisuccinate ( LDT ) is a new acute migraine treatment that works by activating 5-HT1F receptors, which are serotonin receptors observed in the central nervous system.[12-15] It is highly selective for these receptors and does not affect other types of receptors and it will reduce to cardiovascular risk factors.[16-18] It works by activating serotonin receptors in the brain, which helps to reduce the inflammation and pain associated with migraine. This important API synthesis involves the eight solvents i.e. acetone, Isopropyl alcohol, dichloromethane, n-hexane, isopropyl acetate, n-heptane and they need to control. The detailed literature search does not shown the any specific methods related to the analysis of residual solvents by HSGC for LDT. In this research article, we discussed a detailed method development and validation of an HSGC robust analytical method for determination of residual solvents. The chemical structure of LDT is shown in Figure 1.

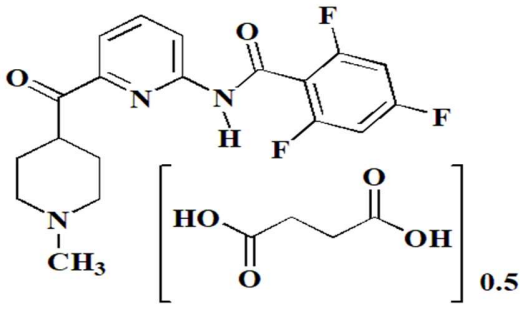


**Figure 1: Chemical structure of LDT**

# MATERIALS AND METHOD

## Chemical, material, and reagents

LDT was synthesized in Uquifa India Private Limited. (Hyderabad, Telangana, India). Acetone, isopropyl alcohol, dichloromethane, n-hexane, ethyl acetate, isopropyl acetate, and n-heptane were acquired from Merck (India), while 1-Methyl-2-Pyrrolidinone (NMP) was obtained from Spectrochem (Mumbai, India).

## Instrumentation

The method development and validation studies utilized an Agilent Technologies 6890N HSGC system equipped with a Flame Ionization Detector and a headspace sampler (Agilent Technologies G1888). Data acquisition and chromatographic data integration were performed using EZ-Chrome software. Sample weighing was conducted on a Mettler Toledo XP205 model balance, and micro-pipettes ranging from 100 to 1000 µL from Borosil were employed.

## Gas chromatographic parameters

60 m x 320 mm, 5.0 µ film thickness DB-1 (100% dimethylpolysiloxane) capillary GC column to achieve proper separation. DB-1 column was manufactured by J&W Scientifc (Agilent Scientifc Technologies, Wilmington, DE, USA). The detailed method parameters mentioned in Table 1.

**Table 1: HSGC Chromatographic conditions of LDT**

| | | |
|---|---|---|
| Instrument | : | A gas chromatograph equipped with headspace and FID |
| Ionization detector | : | Flame Ionisation Detector |
| Column type | : | DB-1 60 m x 320 mm, 5.0 µ, (Part No.:123-1065) |
| Initial temperature (°C) | : | 65 |
| Initial hold time (min) | : | 12 |
| Ramp rate 1 (°C) | : | 15 |
| Temperature 1 (°C) | : | 85 |
| Hold time 2 (min) | : | 5 |
| Ramp rate 2 (°C) | : | 10 |
| Temperature 2 (°C) | | 220 |
| Hold time 3 (min) | : | 5 |
| Run time (min) | : | 34.83 |
| Injector mode | : | Spilt |
| Split ratio | : | 1:20 |
| Carrier flow (mL/min) | : | 1.5 |
| Injector temperature | : | 200 |
| Detector temperature (°C) | : | 260 |
| Carrier Gas | : | Nitrogen |
| $H_2$ flow (mL/min) | : | 30 |
| Air flow (mL/min) | : | 300 |
| Makeup flow (mL/min) | : | 30 |
| Head space Operating conditions | | |
| Oven temperature (°C) | : | 90 |
| Loop temperature (°C) | : | 100 |

| | | |
|---|---|---|
| Transfer line temperature (°C) | : | 110 |
| GC cycle time (min) | : | 40 |
| Vial equilibration time (min) | : | 20 |
| Injection time (min) | : | 1.0 |
| Vail fill volume | : | 2 mL |
| Shake vials | : | Level-3 |

**Preparation of standard and sample solution**

A composite standard stock solution of all the specified solvents prepared in periodic dilutions to attain a final concentration of 3000 µg $mL^{-1}$ methanol, 5000 µg $mL^{-1}$ acetone, 5000 µg $mL^{-1}$ isopropyl alcohol, 690 µg $mL^{-1}$ dichloromethane, 5000 µg $mL^{-1}$ methyl ter. butyl ether, 290 µg $mL^{-1}$ n-hexane, 5000 µg $mL^{-1}$ ethyl acetate, 5000 µg $mL^{-1}$ n-heptane in 1-methyl-2-pyrrolidinone (NMP). The blank and standard solution vails was prepared with 2.0 mL of NMP, and the sample vials were prepared with 200 mg per 1.0 mL NMP.

**Method validation**

Validation of the method involved assessing key parameters such as specificity, limit of detection, limit of quantitation, linearity, accuracy, intermediate precision, system suitability, and method precision for residual solvents, in accordance with the ICH harmonized tripartite guideline (2005).

## RESULTS AND DISCUSSION

**HSGC method development**

In the development of methods using HSGC, essential considerations include selecting the sample solvent, detector, column, carrier gas, and optimizing headspace and chromatographic conditions. The developed method should exhibit specificity, sensitivity, robustness, and suitability for quality control (QC) purposes. Below, we discuss these critical parameters in detail.

**Detector and Carrier gas selection**

The method utilized a flame ionization detector (FID) due to its excellent sensitivity. Nitrogen was chosen as the carrier gas for its cost-effectiveness compared to helium.

**Column selection**

The choice of GC column plays a crucial role in the development of HSGC methods. Typically, a stationary phase containing 6% cyanopropylphenyl and 94% dimethylpolysiloxane (DB-624) is suitable for the majority of residual solvents. The DB-624 column is mid-polar nature and high temperature limit as compare to other polar columns like DB-Wax. Moreover, it helps the polar solvent to elute at longer retention times as compare to non-polar column (DB-1). As we targeted to develop sensitive, rugged HSGC method, a DB-1 column (60 m×0.32 mm, 5.0 µ film thickness) was selected. The specimen chromatogram shown in Figure 2. During method development, we

encountered challenges in separating acetone and isopropyl alcohol. Ultimately, the resolution of these solvents in the evaluated method was approximately 2.5. We also tried the separation in DB-624 (94% dimethyl polysiloxane) column to understand the effect of the stationary phase. In DB-624 column the resolution is decreased from 2.5 to 1.2. Hence, the DB-1 column is selected for the separation residual solvents in LDT.

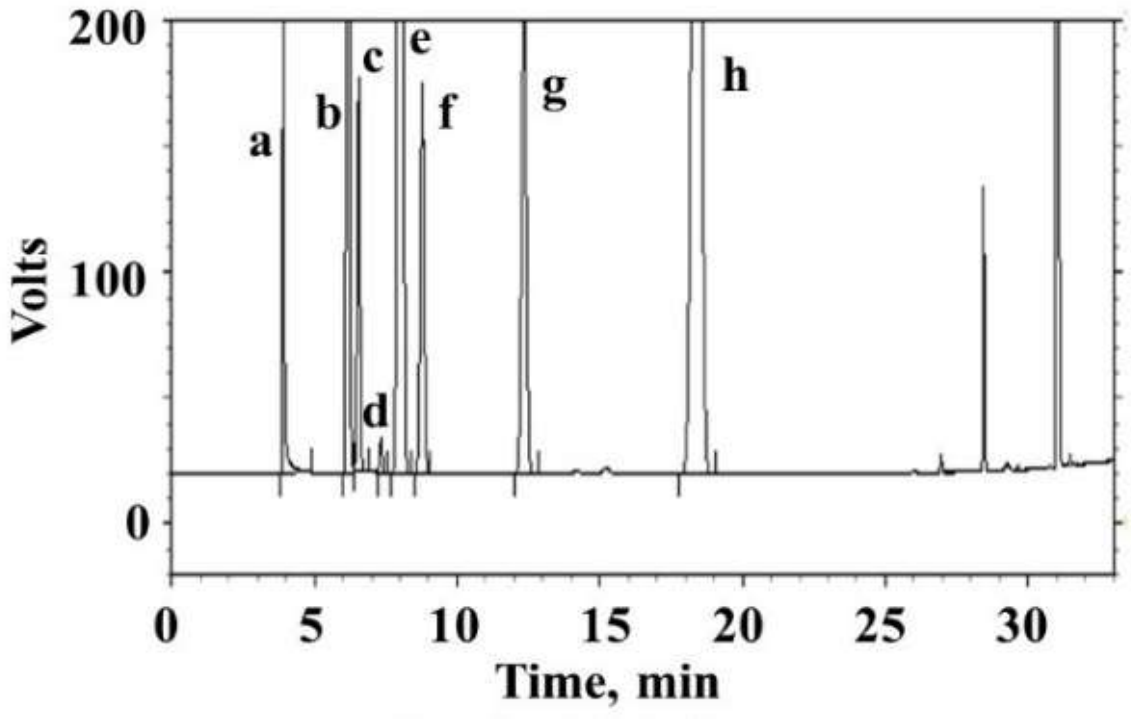


**Figure 2: Specificity chromatogram of LDT residual solvents Solvent Names: a) 3000 µg mL$^{-1}$ of methanol b) 5000 µg mL$^{-1}$ of acetone c) 5000 µg mL$^{-1}$ of isopropyl alcohol d) 690 µg mL$^{-1}$ of dichloromethane e) 5000 µg mL$^{-1}$ of methyl ter. butyl ether f) 290 µg mL$^{-1}$ of n-hexane h) 5000 µg mL$^{-1}$ of ethyl acetate i) 5000 µg mL$^{-1}$ of n-heptane )**

**Selection of sample solvent**

To verify the sample solubility and to select the sample solvent, a series of solvents like tried like Dimethyl formamide (DMF), Dimethyl sulfoxide (DMSO) and 1-methyl-2-pyrrolidone (NMP). As there was minimal interference at the retention times of the targeted solvents and high boiling point, NMP considered as sample solvent.

**Chromatographic conditions**

HSGC method can developed in two ways for selecting oven programs. One approach is kept the low oven temperature followed by gradient oven temperature and in second approach is isothermal elution. At initiation of method, development has thrown a challenge where most of the solvents are eluting early. As we are interested in extending the retention time of the solvents, we

selected the first strategy. In first strategy, we started the development and finalized 65 °C temperature as initial oven temp and hold time for 15 minutes. After that a gradient was applied i.e. 15 °C/min to 85 °C and hold for the 5 minutes, to improve the resolution of acetone and isopropyl alcohol. The oven temperature 10 °C/min increment to 220 °C with 5 min hold time to elute other unknown impurities and 1-methyl-2-pyrrolidone (NMP). The nitrogen flow rate was finalized at 1.5 mL/min with definite run time of about 35 minutes.

**Headspace parameters optimization**

Generally, the Headspace oven temperature similar to the boiling point of specified residual solvents. The loop temperature and transfer line temperature were maintained 10–15 °C higher than the oven temperature to mitigate carryover issues. The headspace oven temperature was kept at 90 °C, because of all residual solvents have close to that boiling point. The loop line and transfer line temperature were kept to 100 ºC and 110 ºC, where the evaporated solvent need to reach the injector. The equilibration time of vail was set to 20 min. Table 2 outlines the specific headspace parameters.

**Table 2: Specificity data of residual solvents in LDT**

| | Retention time (minutes) | |
|---|---|---|
| **Solvent name** | **Spiked sample** | **Un-spiked sample** |
| Methanol | 3.98 | 3.96 |
| Acetone | 6.30 | 6.26 |
| Isopropyl alcohol | 6.68 | 6.64 |
| Dichloromethane | 7.49 | 7.47 |
| Methyl *tert* butyl ether | 8.17 | 8.16 |
| n-Hexane | 8.96 | 8.95 |
| Ethyl acetate | 12.60 | 12.59 |
| n-Heptane | 18.77 | 18.78 |

**Method validation**

The final method underwent validation as per the requirements outlined in the ICH guidelines

**Specificity**

Specificity was assessed by injecting a blank, individual standard solutions of residual solvents, and spiked solvent sample solutions. It, does not have any shakles at the retention time of targeted residual solvents or any other unknown peaks. Hence, the method is specific. The retention time (in minutes) of methanol, acetone, isopropyl alcohol, dichloromethane, methyl tert butyl ether, n-hexane, ethyl acetate and is n-heptane is 3.96, 6.26, 6.64, 7.47, 8.16, 8.95, 12.59, 18.78 respectively. The retention time of diluent (NMP) was 33.81 minutes. A specimen chromatogram of each individual solvent chromatogram at specification level is shown in Figure 2 and retention times for spiked and un-spiked sample were shown in Table 3.

**Table 3: Linearity data of residual solvents in LDT**

| Solvent name | Correlation coefficient | Slope | Intercept | % Y-Intercept at |
|---|---|---|---|---|
| Methanol | 1.000 | 0.00043 | -286.02 | -0.0037 |
| Acetone | 0.999 | 0.00016 | -183.51 | -0.0006 |
| 2-Propanol | 0.998 | 0.00061 | -312.69 | -0.0036 |
| Dichloromethane | 0.999 | 0.00092 | -36.61 | -0.0049 |
| Methyl *tert* butyl ether | 0.998 | 0.00007 | -322.14 | -0.0036 |
| n-Hexane | 0.996 | 0.00003 | -17.85 | -0.0001 |
| Ethyl acetate | 0.999 | 0.00029 | -341.90 | -0.0004 |
| n-Heptane | 0.997 | 0.00005 | -415.85 | -0.0004 |

**Method sensitivity**

The LOQ limit found as 911 µg $mL^{-1}$ for methanol, 1513 µg $mL^{-1}$ for acetone, 1498 µg $mL^{-1}$ for isopropyl alcohol, 192 µg $mL^{-1}$ for dichloromethane, 1503 µg $mL^{-1}$ for methyl tert butyl ether, 91 µg $mL^{-1}$ for n-hexane, 1517 µg $mL^{-1}$ for ethyl acetate and 1499 µg $mL^{-1}$ for n-heptane. The LOQ chromatograms were shown in Figure 3.

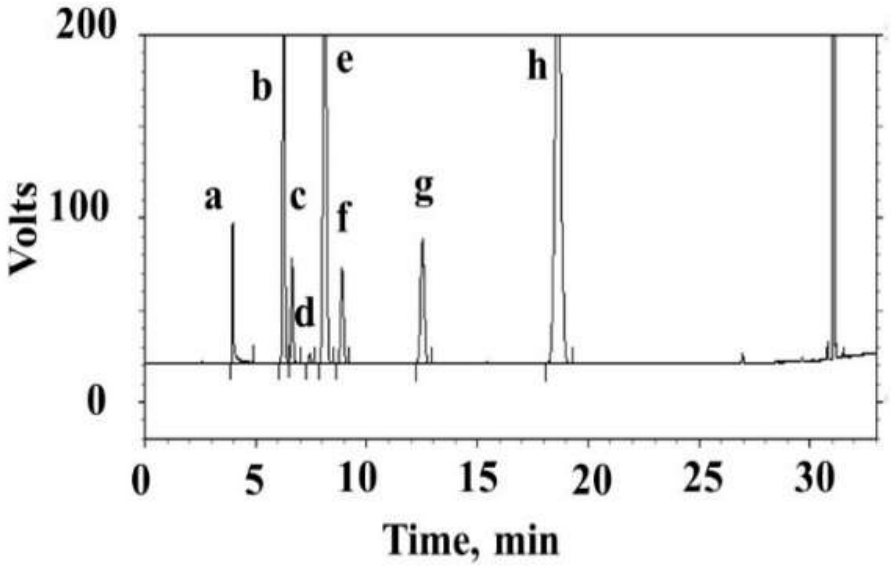


**Figure 3: LOQ solution chromatogram of LDT residual solvents Solvent Names: a) 911 µg $mL^{-1}$ of methanol b) 1513 µg $mL^{-1}$ of acetone c) 1498 µg $mL^{-1}$ of isopropyl alcohol d) 192 µg $mL^{-1}$ of dichloromethane e) 1503 µg $mL^{-1}$ of methyl ter. butyl ether f) 91 µg $mL^{-1}$ of n-hexane h) 1517 µg $mL^{-1}$ of ethyl acetate i) 1499 µg $mL^{-1}$ of n-heptane**

**Linearity and range**

The calibration curve plotted from LOQ level to 150 % level w.r.t to specification limit. The method exhibited linearity across the specified range, with correlation coefficient (R2) values exceeding 0.99 for all specified solvents.. The linearity graph provided in Figure 4 and Table 3. Therefore, the range of the developed method was 911-4556 µg $mL^{-1}$ for methanol, 1513-7563 µg

$mL^{-1}$ for acetone, 1498-7489 µg $mL^{-1}$ for isopropyl alcohol, 192-959 µg $mL^{-1}$ for dichloromethane, 1503-7517 µg $mL^{-1}$ for methyl tert butyl ether, 91-456 µg $mL^{-1}$ for n-hexane, 1517-7584 µg $mL^{-1}$ for ethyl acetate and 1499-7495 µg $mL^{-1}$ for n-heptane.

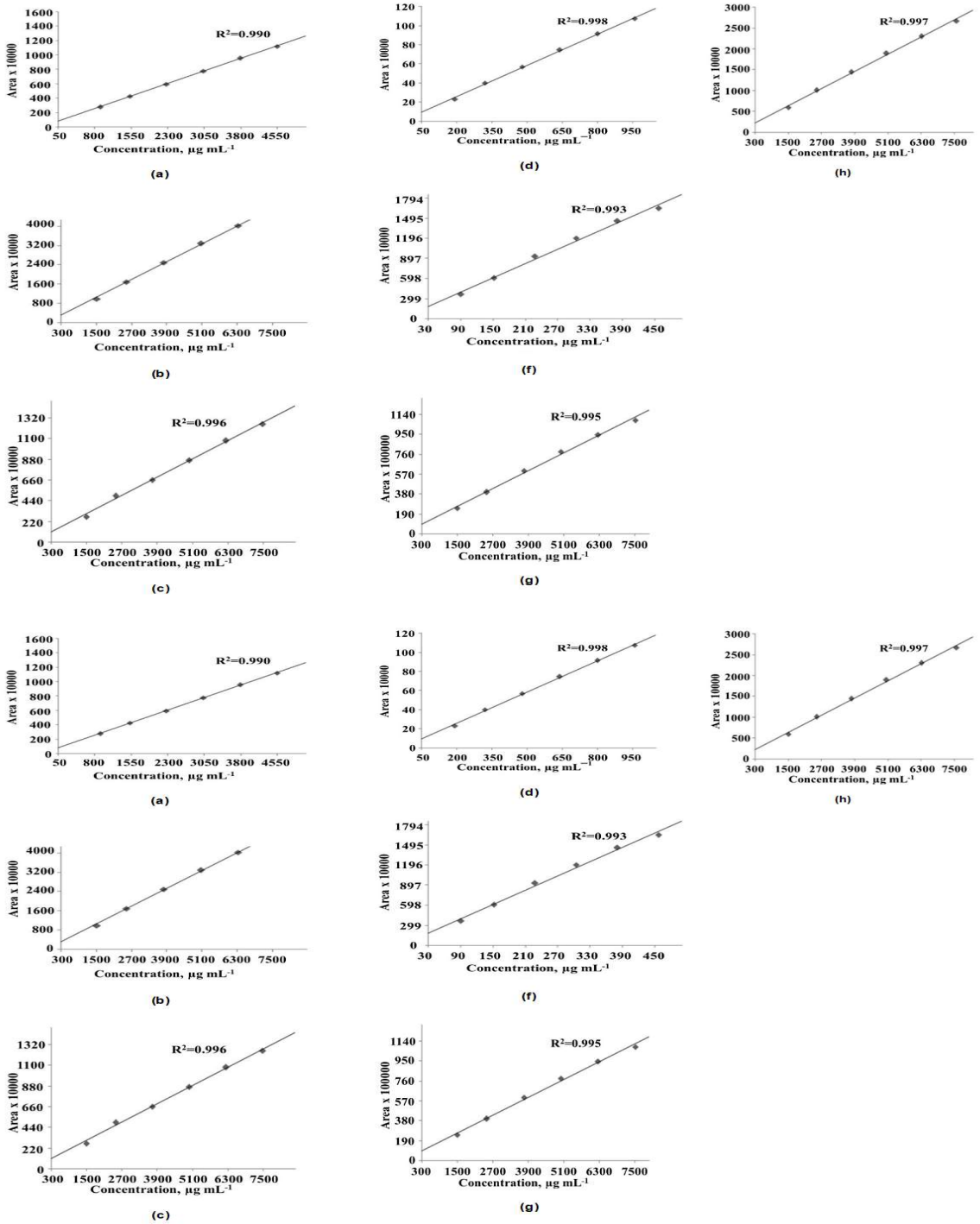


**Figure 4: Linearity chromatograms of LDT residual solvents Solvent Names: a) methanol b) acetone c) isopropyl alcohol d) dichloromethane e) methyl ter. butyl ether f) n-hexane g) ethyl acetate h) n-heptane**

**Accuracy (recovery)**

The method's accuracy was determined by spiking all solvents at various levels, including the LOQ level, 100% level, and 150% level relative to the test concentration. Accuracy at the LOQ level was verified by injecting three separate preparations of LDT spiked with each residual solvent at the LOQ level and calculating the percentage recoveries of each solvent. All LOQ level specimen chromatograms were shown in Figure 3. Recovery for all solvents was found between 80–120%. Recovery study and method precision results indicate that the method was accurate. The accuracy data is presented in Table 4.

**Table 4: Accuracy data of residual solvents in LDT**

| | % Recovery at | | | |
|---|---|---|---|---|
| **Solvent Name** | ***LOQ level** | **50 % level** | **100 % level** | **150 % level** |
| Methanol | 105.9 | 103.1 | 92.6 | 102.4 |
| Acetone | 90.8 | 91.2 | 85.5 | 94.8 |
| 2-Propanol | 103.2 | 92.9 | 92.3 | 108.8 |
| Dichloromethane | 108.6 | 106.5 | 94.4 | 106.6 |
| Methyl *tert* butyl ether | 97.3 | 97.5 | 90.2 | 98.9 |
| n-Hexane | 89.7 | 94.2 | 84.0 | 94.2 |
| Ethyl acetate | 94.1 | 92.4 | 86.2 | 97.4 |
| n-Heptane | 108.2 | 107.3 | 98.5 | 110.0 |

* LOQ: Limit of quantitation

**Precision**

The precision of the method verified by system precision and method precision. System precision is analyzed by six imitate injections of solvent standard solution and method precision performed by six varied preparation of spike solution. In both studies % RSD found as less than 10.0%. The precision data is presented in Table 5.

**Table 5: Precision data of residual solvents in LDT**

| | | % ^RSD at | |
|---|---|---|---|
| **Solvent Name** | ***LOQ level** | **100 % level** | **150 % level** |
| Methanol | 2.22 | 2.2 | 4.0 |
| Acetone | 3.25 | 3.25 | 4.7 |
| 2-Propanol | 5.90 | 5.90 | 9.7 |
| Dichloromethane | 3.91 | 3.91 | 6.0 |
| Methyl *tert* butyl ether | 4.50 | 4.50 | 7.1 |
| n-Hexane | 4.98 | 4.98 | 7.6 |
| Ethyl acetate | 4.03 | 4.03 | 7.0 |
| n-Heptane | 4.86 | 4.86 | 8.7 |

* LOQ: Limit of quantitation

^ RSD: Related standard deviation

**System suitability**

The system suitability criterion considered for the resolution of closely eluting peaks like acetone

and isopropyl alcohol is not less than 1.0. The system suitability verified before starting the each validation parameter and resolution found as 2.5. The specimen chromatogram of shown in Figure 1. and other system suitability parameters were shown in Table 6.

**Table 6: System suitability data of residual solvents in LDT**

| Solvent Name | Resolution | Tailing factor |
|---|---|---|
| Methanol | 0.00 | 1.79 |
| Acetone | 19.18 | 1.04 |
| 2-Propanol | 2.33 | 1.01 |
| Dichloromethane | 4.60 | 1.05 |
| Methyl *tert* butyl ether | 3.11 | 1.02 |
| n-Hexane | 3.00 | 1.00 |
| Ethyl acetate | 12.77 | 1.00 |
| n-Heptane | 14.95 | 0.96 |

**Robustness**

Robustness study performed by slightly changing HSGC parameters like, carrier gas flow rate and oven temperature. The oven temperature ±3 °C from the initial conditions and the flow rate ±5% from the initial conditions. The absence of notable variances in % RSD, resolution, and elution order indicates the robustness of the method.

**Batch analysis**

The batch analysis was conducted using the validated method for two lab verification batches of LDT. During the batch analysis, the system suitability complies as per the validation parameters and other batch analysis results were reported in Table 7.

**Table 7: Batch Analysis data of residual solvents in LDT**

| Batch Analysis | | | |
|---|---|---|---|
| **Solvent name** | **Specification** | **Results (LDT-DS/U-066/001)** | **Results (LDT-DS/U-049/173)** |
| Methanol | 3000 ppm | Below #LOD | 2222 ppm |
| Acetone | 5000 ppm | Below #LOD | 3351 ppm |
| Isopropyl alcohol | 5000 ppm | 4402 ppm | 17.8 ppm |
| Dichloromethane | 600 ppm | Below #LOD | 21.8 ppm |
| Methyl *tert* butyl ether | 5000 ppm | Below #LOD | Below #LOD |
| n-Hexane | 290 ppm | 1 ppm | Below #LOD |
| Ethyl acetate | 5000 ppm | 140 ppm | 140 ppm |
| n-Heptane | 5000 ppm | Below #LOD | Below #LOD |

#LOD : Limit of detection

## CONCLUSION

An expedient and discriminating static Headspace Gas Chromatography (HSGC) methodology has been assessed for the precise quantification of solvents including acetone, isopropyl alcohol, dichloromethane, n-hexane, ethyl acetate, isopropyl acetate, and n-heptane in LDT. This method,

developed with careful consideration of synthesis routes and solvent properties, exhibits specificity, resilience, linearity, and accuracy. Notably, its utility extends beyond LDT to encompass the analysis of residual solvents in diverse settings. It is well-suited for routine and in-process quality control analyses across different manufacturing contexts. We are confident in the precision, affordability, and commercial viability of this demonstrated quantitative technique in LDT, which also holds promise for large-scale industrial manufacturing applications.

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## DECLARATION

The authors declare that there is no conflict of interest. This article does not contain any studies with human participants or animals performed by any of the authors.

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