



# Analytical Method Development and Validation for Determination of Residual Solvent in Zopiclone Tablets by Headspace Gas Chromatography

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## ABSTRACT

A simple and sensitive headspace gas chromatographic method has been developed and validated for simultaneous determination of isopropyl alcohol and methylene chloride in zopiclone tablets. The separation was achieved on 75 m long DB-624 fused silica column, 0.53 mm inner diameter and 3  $\mu$  film thickness using nitrogen as a carrier gas at 6 ml/min flow and FID as a detector. The developed gas chromatographic method offers symmetric peak shape, good resolution and reasonable retention time for all the solvents. The limit of detection of isopropyl alcohol and methylene chloride was found to be 250  $\mu$ g/tablet and 90  $\mu$ g/tablet, respectively.

**Keywords:** DB-624 column, HSGC, residual solvents, validation, zopiclone tablets

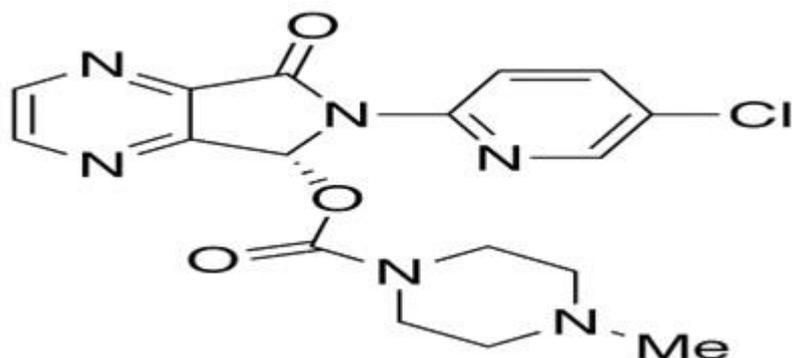
## INTRODUCTION

Residual solvents are organic volatile chemicals which are used during manufacturing of drug substances and drug products. The analysis of residual solvents in pharmaceutical preparation is an important issue in the quality control of medicines, due to potential risk for public health. In 1997 the International committee for harmonisation issued a guideline for residual solvents

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**Figure 1.** The structure of zopiclone

[1]. The limits proposed in this guideline have been adopted by United States pharmacopeia [2], the European pharmacopeia [3] and Japanese pharmacopeia [4]. Due to the importance to control residual solvents in the medicines, several methods, applications and issue concerning residual solvents were already published and recently reviewed by Grodowska and Parczewski [5].

Zopiclone is chemically designated as [6-(5-chloropyridin-2-yl)-5-oxo-7H-pyrrolo [3, 4-b] pyrazin-7-yl] 4-methylpiperazine-1-carboxylate (**Figure 1**). Zopiclone is a hypnotic agent, which rapidly initiates and sustains sleep. Zopiclone is belonging to the chemical group cyclopyrrolones and reported to have similar amnesic, anxiolytic, muscular relaxant, sedative and anticonvulsant properties to those of the benzodiazepine, although it is chemically distinct from them. In adults, the therapeutic dose is 7.5 mg of zopiclone per os [6]. Techniques for the determination of zopiclone in tablets include polarographic [7, 8], HPLC and UV [9-10] methods. As an official compound in British pharmacopeia (BP), zopiclone is assayed by non-aqueous titrimetric method with potentiometric detection of end point for pure form and HPLC method for tablets [11], Literature survey also reveals many analytical methods for determination of zopiclone [12-21]. Zopiclone tablets are a film coated tablets, where isopropyl alcohol (IPA) and methylene chloride (MDC) are used during coating. But there is no reported method for determination of residual solvents in zopiclone tablets. So, it is necessary to develop simpler, specific, highly reproducible and accurate quantitative method for residual solvents, where isopropyl alcohol and methylene chloride is quantified in zopiclone tablet by headspace gas chromatography (HSGC).

## EXPERIMENTAL

### Reagents and chemicals

Zopiclone tablets were obtained from Cipla Ltd and HPLC grade solvents as IPA, MDC and N,N-dimethylformamide (DMF) were purchased from Fisher Scientific. The remaining organic solvents were purchased from Sigma-Aldrich.

**Table 1.** Experimental condition of the determination of residual solvents in zopiclone tablets

Chromatographic conditions		Headspace conditions	
<b>Make up gas</b>	Nitrogen	<b>Headspace vial size</b>	20 ml
<b>Injection mode</b>	Split	<b>Cycle</b>	HS-inj
<b>Split Ratio</b>	1:1	<b>Injection volume</b>	1.0 ml
<b>Injector port temperature</b>	180°C	<b>Incubation temperature</b>	85 °C
<b>Detector port temperature</b>	260°C	<b>Vial incubation time</b>	20 min.
<b>Column oven temperature</b>	Hold at 40°C for 10 minutes, then increase at rate of 10°C/min to 150°C and hold for 5 minutes and then Increase at rate of 40°C/min to 230°C and hold for 3 minutes.	<b>Agitator speed</b>	500 rpm
		<b>Syringe temperature</b>	115°C
		<b>Flush time</b>	10 min.
<b>Diluent</b>	Dimethylformamide	<b>GC run time</b>	

### Instrumentation/Chromatographic conditions

An Agilent 6890A GC equipped with an FID is used for the experiments. The HSGC system, data acquisition and processing were controlled using a computer program (Chromeleon 6.80 SR13 Build). The GC column was an Agilent DB-624 (6% cyanopropylphenyl/94% dimethyl polysiloxane) fused silica capillary column with dimensions 75 m x 0.53 mm ID, 3 µ film thickness. Nitrogen is used as mobile phase (carrier gas) with detection at 260 °C. The Optimized HSGC parameters are listed in **Table 1**.

### Preparation of Standard solution

Accurately weighed 0.8 g of IPA and 0.1 g of MDC in a 100 ml volumetric flask containing 70 ml of diluent (DMF) and diluted up to the mark. Further, 2 ml of this solution is diluted to 100 ml with diluent.

### Preparation of Sample solution

Two accurately weighed tablets are transferred into 20 ml headspace vial and 5 ml diluent is added, and the vial is immediately equipped with septum, metallic cap and crimped properly. The sample is analysed by HSGC with FID and the quantification is performed.

### Procedure

Using a gas tight syringe, equal volumes of solutions are separately injected as per sequence of injection into the chromatograph and peak area responses for the major peaks are recorded and checked for the system suitability requirements. If blank interference is observed, blank solutions are injected three times.

## Validation of Method

Analytical method validation was carried out as per ICH guideline [22]. The parameters evaluated are specificity, system suitability, limit of detection and limit of quantification, linearity and range, precision, trueness and robustness.

Specificity of the analytical method was performed by injecting both solvents IPA (5000 µg/tablet) and MDC (600 µg/tablet), individually and blank (i.e. DMF) under the same chromatographic condition. System suitability of method was performed by injecting standard solution. The system suitability is confirmed by resolution and tailing factor.

The limit of detection (LOD) and limit of quantification (LOQ) for IPA and MDC was achieved by injecting a series of dilute solutions by using standard deviation slope method (ICH Q2 (R1)).

The LOQ level of the developed residual solvent method for IPA and MDC was checked by analyzing six solution prepared at LOQ levels and calculating the percentage relative standard deviation of area.

Detector response linearity was assessed by preparing seven calibration solutions of IPA (625 to 7500 µg/tablet) and MDC (210 to 900 µg/tablet) in diluent. The regression curve was obtained by plotting peak area versus concentration, using the least square method. The slope, Y intercept, correlation coefficient, F significance, percentage relative standard deviation of the slope and y-intercept of the calibration curve was calculated.

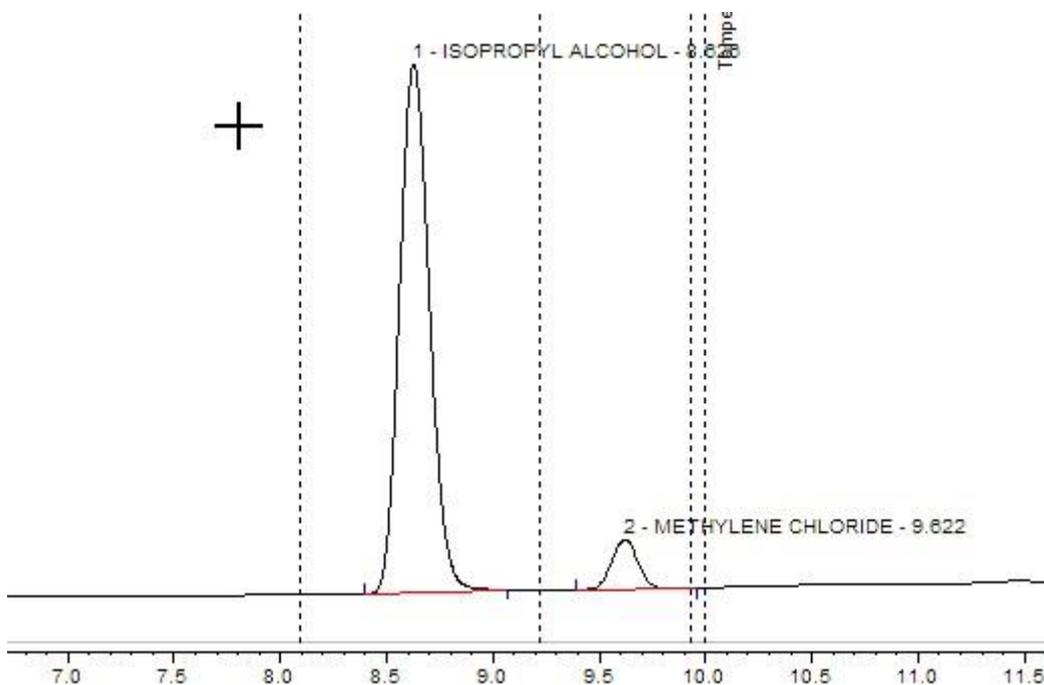
Method reproducibility was determined by measuring repeatability and intermediate precision of peak area for IPA and MDC. The repeatability of method was determined by analyzing six replicate injections containing two zopiclone tablets spiking with IPA (5000 µg/tablet) and MDC (600 µg/tablet). The intermediate precision was determined by performing six successive injections (n=6) on different day, different system and analyst. The trueness was calculated in term of recovery (%). The study was carried out in triplicate for IPA (625 to 7500 µg/tablet) and MDC (210 to 900 µg/tablet) in diluent.

Robustness of the method was assessed by deliberately altering the experimental conditions such as flow rate ( $\pm 0.5$  mL/min), column temperature ( $\pm 1$  °C), and injector port temperature ( $\pm 5$  °C) by keeping all the other chromatographic conditions constant as described above.

## RESULTS AND DISCUSSION

### Optimization of HS conditions

The HS sampler has a number of parameters affecting the method sensitivity, precision, and efficiency, including: temperature (oven and loop), time (vial equilibration and pressurization, loop fill, and injection), pressure (vial and carrier gas) and phase ratio (vial size and sample volume). Selecting a proper sample diluent for HSGC analysis is very critical for



**Figure 2.** Chromatographic separations of IPA and MDC in standard solution

method sensitivity, precision and sample equilibration temperature and time, and it will affect the final optimized HS conditions. We have tried water and DMF as diluent. We found consistent results with DMF, as it has high boiling point and tablets are completely soluble in it. So, we have decided to use DMF as diluent. We evaluated HS equilibration temperature at 85, 90 and 100 °C with equilibration times of 10, 20, 30 and 40 min, when the equilibration time at 85 °C was extended from 20 to 40 min, the recoveries of both the solvents remained constant. Therefore, we determined that equilibrating at an oven temperature of 85 °C for 20 min was optimal. There was no significant change in solvent recoveries, when temperature of the injection port and detector port were kept higher than 180 °C and 260 °C, respectively.

### Optimization of GC conditions

The choice of GC column is crucial for establishing an efficient and robust HSGC method. The Agilent DB-624 column (75 m × 0.53 mm ID, 3 µ) is a commonly used column for residual solvents determination, because of its medium polarity. Most of the ICH Q3C solvents can be resolved by the Agilent DB-624 column. To obtain efficient separation and sample sensitivity, a number of GC parameters were evaluated when developing this method, such as the GC oven temperature gradient, carrier gas flow rate and sample split ratio: initial temperature 40 °C at different holding time (5, 10, 15, and 20 min), temperature ramping rate (5, 8, 10, 20 °C/min up to 150 °C), carrier flow rate (5, 6 and 7 mL/min) and split ratio (splitless or 1 to 1–5 ratio). Our data indicated that the GC parameters listed in Section 2.2 were the most efficient combination for separation and sensitivity of this method. Under these optimized

**Table 2.** Linearity parameter of IPA and MDC

	IPA	MDC
<b>Slope ± Standard Error</b>	0.1603 ± 0.0038	0.1001 ± 0.0011
<b>Y intercept ± Standard Error</b>	-0.7871 ± 21.4132	0.6339 ± 0.7960
<b>Correlation Coefficient</b>	1.000	1.000
<b>Significance F</b>	1.50431E-07	2.82723E-09
<b>% y intercept</b>	-0.1	0.8
<b>% RSD of peak area response for level 1</b>	3.4	2.5
<b>% RSD of peak area response for level 7</b>	5.4	1.9
<b>% RSD of retention Time for level 1</b>	0.0	0.0
<b>% RSD of retention Time for level 7</b>	0.0	0.0
<b>% RSD for response factor</b>	2.3	1.2

conditions, IPA and MDC were analyzed. Advantage of our generic HSGC method is its capability to separate solvents in a considerably shorter time (total running time is 31 min, including 20 min for HS vial equilibration and 11 min for GC separation).

### Validation of the method

The Analytical method was validated according to International Conference on Harmonization (ICH) Guideline [1].

#### *Specificity and System Suitability*

As for specificity, no interference was seen from blank and placebo solution corresponding to the peaks of IPA and MDC. Retention times of IPA and MDC in their identification solution is found comparable with their standard solutions. Resolution between IPA and MDC is around 4.3. Chromatographic separations of IPA and MDC in standard solution is shown in **Figure 2**. Tailing factor for IPA and MDC were observed around 1.1 and 1.0, respectively. Similarity factor for IPA and MDC were observed around 1.01 and 1.00, respectively.

#### *Limit of detection (LOD), Limit of quantification (LOQ)*

LOD and LOQ were calculated for IPA and MDC based on signal-to-noise ratio (S/N). The LOD and LOQ were found for IPA at 250 µg/tablet (S/N=10) and 625 µg/tablet (S/N=24, % RSD of peak area response=11.1, % RSD of retention time= 0.0), respectively. The LOD and LOQ were found for MDC at 90 µg/tablet (S/N=21) and 210 µg/tablet (S/N=49, % RSD of peak area response=0.8; % RSD of retention time= 0.0), respectively.

#### *Linearity*

The calibration curves were created by plotting the peak area of the given analyte against its concentration of IPA (625 to 7500 µg/tablet) and of MDC (210 to 900 µg/tablet). The calibration equations were calculated using linear regression analysis. The results show a satisfactory linear correlation of IPA and MDC (**Table 2**).

**Table 3.** Recovery details of IPA & MDC

IPA				MDC			
Accuracy Level	Concentration $\mu\text{g}/\text{Tablet}$	Recovery	% RSD	Accuracy Level	Concentration $\mu\text{g}/\text{Tablet}$	Recovery	% RSD
1	625	104 to 112	3.6	1	210	98 to 100	0.7
2	2500	103 to 112	4.4	2	300	89 to 94	3.0
3	5000	97 to 111	4.9	3	600	85 to 92	2.8
4	7500	101 to 108	3.5	4	900	89 to 91	1.2

### *Repeatability, Intermediate precision and accuracy determination*

The repeatability and intermediate precision shows good results, with % RSD 6.6 and 7.4 for IPA, and 3.3 and 1.2 for MDC, respectively. Accuracy of the IPA and MDC was evaluated in triplicate at four concentration levels for IPA (625, 2500, 5000 and 7500  $\mu\text{g}/\text{tablet}$ ) and for MDC (210, 300, 600 and 900  $\mu\text{g}/\text{tablet}$ ). The average total recoveries of IPA and MDC are summarized in **Table 3**. It can be concluded that the developed analytical method gives true and precise results for determination of IPA and MDC.

### *Robustness*

To assess robustness of the method, the experimental conditions were deliberately altered and results were evaluated. To study the effect of flow rate on the resolution and peak area, the same was altered by 0.5 units that is 5.5 and 6.5 mL/min. The effect of column temperature was studied at 44 and 46 °C instead of 45 °C. The effect of changing the injector port temperature by  $\pm 5$  °C (175 °C and 185 °C instead of 180 °C) on resolution and peak area was also studied. All the other chromatographic conditions were held constant as described above. In all the deliberate varied chromatographic conditions, the results were found within the limits (resolution between two analyte peak  $> 4$ , % RSD for peak area of IPA 5.5 and for MDC 4.5), illustrating the robustness of the method.

## CONCLUSION

In this study, a generic HSGC method is successfully developed and validated for the determination of IPA and MDC residual in drug substances. The method is specific, accurate, precise, linear, sensitive and efficient. DMF was selected as the sample diluent due to its high capacity for dissolving organic drug substances, stability and high boiling point. The conditions of HS sampler and GC were optimized to make the HSGC method more sensitive, efficient and reproducible. This method has better separation with resolution 4.3, and a higher sensitivity. This method meets ICH guideline requirements, and may be suitable for quantitative determination residual solvents in zopiclone tablets.

## CONFLICT OF INTEREST

All the authors declare that there are no conflicts of interest.

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