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Der Pharmacia Lettre, 2015, 7 (10):273-280
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Simple and reliable gas chromatography method for the trace level determination of oxalyl chloride content in zolpidem tartrate

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ABSTRACT

A simple gas chromatography method using flame ionization detector has been developed and validated for the quantitative determination of trace level of oxalyl chloride(quantified as diethyl oxalate) in Zolpidem tartrate drug substance. Efficient chromatographic separation was achieved on DB-35 capillary gc column, 30 m long, 0.53 mm internal diameter with 1.0 µm film thickness, employing programmed temperature with split mode (1:2) and run time was 60 min. The optimized method was validated for specificity, precision, linearity, robustness and accuracy. The obtained detection and quantitation limits of oxalyl chloride were 3 µg/g and 9 µg/g respectively. The method was found to be linear in the range between 9 µg/g and 750 µg/g with correlation coefficient 0.9996. The obtained average recovery of oxalyl chloride was 99.5 %. The detailed experiment results are discussed in this research paper.

Keywords: Oxalyl chloride, Zolpidem Tartrate, GC-FID, Ethanol, Validation

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INTRODUCTION

Zolpidem tartrate [ZPT] is a prescription medication used for the short-term treatment of insomnia, and some brain disorders [1, 2]. Additionally ZPT also possesses anxiolytic and anticonvulsant properties, thus useful for the treatment of anxiety, sleep disorders and other neurological and psychiatric complaints [3, 4]. It is a rapid acting hypnotic agent of relatively short duration of action. ZPT is a widely used hypnotic agent acting at the $GABA_A$ receptor benzodiazepine site. On recombinant receptors, it displays a high affinity to α1-$GABA_A$ receptors, an intermediate affinity to α_2- and α_3-$GABA_A$ receptors and fails to bind to α_5-$GABA_A$receptors. However, it is not known which receptor subtype is essential for mediating the sedative-hypnotic action *in vivo* [5]. Its molecular formula and molecular weight are $C_{42}H_{48}N_6O_8$ and 765 respectively. The chemical structure of Zolpidem tartrate is given in Fig. 1. ZPT dosage forms are available in market as oral tablets and oral spray under trade names Ambien and Zolpimist [6]. In 2008, US Food and Drug Administration (FDA) have approved an oral spray formulation of Zolpimist for the short-term treatment of insomnia characterized by difficulty with sleep initiation [6]. It has been designed to be sprayed directly into the mouth over the tongue for fast absorption through the oral mucosa.

Oxalyl chloride (OC) is chemical reagent / catalyst and has not been listed in residual solvents category in any of the regulatory guidelines and pharmacopoeias [7, 8]. In the synthetic process of ZPT, OC was used during the preparation of Zolpidem base from the Zolpidic acid. However, the analysis of OC presents numerous distinctive

challenges due to its low molecular weight and its reactivity, (i.e) it reacts with water giving off gaseous products only: hydrogen chloride (HCl), carbon dioxide (CO_2), and carbon monoxide (CO). In this, OC is quite different from other acyl chlorides which hydrolyze with formation of hydrogen chloride and the original carboxylic acid. Moreover, excess of oxalyl chloride present in ZPT may leads to formation of N,N,N'N'-Tetramethylethanediamide. Hence, it is necessary to control the oxalyl chloride in ZPT. As it is a non genotoxic, any impurity other than active drug substance is to be controlled with appropriate limit in the drug substance irrespective of harmful nature as per International Conference on Harmonization (ICH) guidelines on impurities [9]. Till now no analytical method is available in literature for determination of low level oxalyl chloride in drug substances. However, it is very difficult to analyze in as such form by GC or by any other techniques. Hence, derivatization procedure has been chosen to quantify the analyte. From the known literature OC reacts with alcohols to give corresponding esters [10],(refer Fig. 2). Oxalyl chloride converts into diethyl oxalate in the presence of ethanol. Therefore, in this derivatized method, oxalyl chloride is determined as diethyl oxalate. 500µg/g was considered as specification level concentration for this research work. Further, in detailed experiment results are discussed.

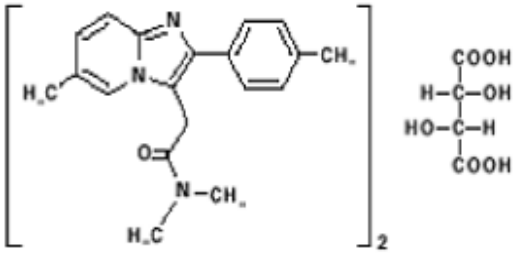
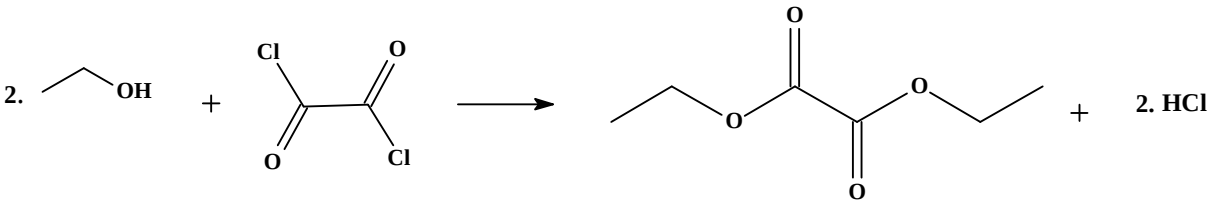


Fig. 1. Chemical Structure of Zolpidem tartrate



ethanol **oxalyl chloride** **diethyl oxalate** **hydrogen chloride**

Fig. 2. Reaction mechanism of oxalyl chloride converts in to diethyl oxalate in presence of ethanol.

MATERIALS AND METHODS

Solvents, chemicals and samples

Oxalyl chloride, dodecane, methanol, ethanol, ethyl acetate, isopropyl alcohol, methylene chloride, toluene, benzene, acetic acid and N,N-dimethylformamide were procured from Sigma Aldrich (Steinheim, Germany). The investigated sample ZPT and the synthesized impurity i.e. N,N,N'N'-Tetramethylethanediamide were gifted from APL Research Centre Laboratories (A Division of Aurobindo Pharma Ltd., Hyderabad).

Instrumentation

A gas chromatograph 6890N equipped with flame ionization detector with G1888 auto sampler (Make: Agilent Technologies, Santa Clora, CA, USA) & gas chromatograph Shimadzu 2010 equipped with flame ionization detector with AOC-5000 auto sampler (Make: Shimadzu Corporation, Kyoto, Japan) with data acquisition and processing using Empower 3 Software Build 3471 were used in research work.

Chromatographic conditions and methodology

Column : DB-35 [(35%phenyl and 65% dimethylpolysiloxane as stationary phase)(30m x 0.53mm I.D, 1.0µm film thickness)] Make: Agilent J&W
Injector temperature : 180°C

Detector temperature : 260°C
Detector : Flame ionization detector (FID)
Carrier gas : Nitrogen
Spit ratio : 1:2
Run time : 60min
Injection volume : 2µL

Column Pressure programme : 40kPa (20min) —(20 kPa/min)→ 80kPa (38min)

Column oven temperature programme:

50°C (5min) —(10° C/min)→ 150°C (5min) —(30°C/min)→ 260°C (36.33min)

Preparation of solutions

Internal standard solution (IS)

Accurately weigh and transfer about 28 mg of dodecane into a 25 ml clean, dry volumetric flask containing about 15 ml of ethanol, mix about 10 minutes and make up to volume with ethanol. Dilute 10 ml of this solution to 500 ml with ethanol.

Blank solution

Filter about 3 ml of internal standard solution through a PTFE (Polytetrafluoroethylene) filter of 0.45 µm pore size and collect the filtrate in a vial for injection.

Standard solution

Accurately weigh and transfer about 25 mg of Oxalyl chloride into a 25 ml clean, dry volumetric flask containing about 15 ml of internal standard solution, mix about 10 minutes and make up to volume with internal standard solution. Dilute 2.5 ml of this solution to 50 ml with internal standard solution.

Filter about 3 ml of this solution through a PTFE filter of 0.45 µm pore size, and collect the filtrate in a vial for injection.

Sample solution

Accurately weigh and transfer about 300 mg of sample into a clean, dry glass centrifuge tube. Add 3 ml of internal standard solution and shake vigorously for 10 minutes. Immediately, filter this solution through a PTFE filter of 0.45 µm pore size, and collect the filtrate in a vial for injection.

RESULTS AND DISCUSSION

Method development and optimization

The objective of this work is to determine trace level of OC in ZPT by using GC. Development trails were initiated on GC head-space technique using stationary phase 100% dimethylpolsiloxane (DB-1, Make: Agilent). About 500µg/g concentration of OC solution was prepared in N, N-Dimethylformamide and was transferred into headspace vial and sealed the vial with help of screw cap. The vial was incubated at 80°C for 20 min and injected through AOC-5000 auto injector into GC. In this trail, oxalyl chloride was eluted with very low response it may due to low volatile nature (boiling point-61°C).

Further, different trails were performed by changing the injection technique (Head space to direct injection). In direct injection technique, the same standard solution (500µg/g solution) and sample spiked with standard solution were injected (2.0µl) through AOC-5000 auto sampler. In this trail, lot of interference was observed at retention time of OC and also observed low response of OC. Further, derivatization technique has chosen for control of interference and good response. Methanol, ethanol and isopropyl alcohol have been chosen as derivatization solvents as OC reacts with alcohols to give corresponding esters. Lot of peaks were observed when methanol and isopropyl alcohol were used as derivatization solvents. But, OC exhibited as a single peak i.e. Diethyl oxalate when the ethanol was used as derivatization solvent. The obtained peak was also confirmed by GCMS as Diethyl oxalate peak.

OC standard solution (500µg/g) was prepared in ethanol and injected into GC in DB-1 column and kept the run time as 30min. But in the sample preparation, filtration technique was adopted as ZPT was insoluble in ethanol solvent. In DB-1 column, after every 4 sample injections, one unknown peak was observed at diethyl oxalate peak retention time when the runtime kept as 30min. This was may be due to some carryover / interference from sample matrix. After that, many trails were performed to avoid the problem by changing different column like DB-Waxetr, DB-FFAP and DB-624. In all above trails, carryover / interference was not resolved. Satisfactory separation and peak shape was achieved on DB-35 column,(30 m x 0.53 mm x 1.0µm) using nitrogen as carrier gas in constant column pressure mode and varying column oven temperature analytes. Dodecane was chosen as internal standard as this solvent was not utilized in the synthetic process of ZPT. In this column also, carryover peak was observed from sample matrix that was also identified by GCMS i.e. Zolpidem peak. Hence, by increasing the run time to 60 minutes, the carryover problem was closed. In this derivatization process, OC was completely converted to diethyl oxalate. This was confirmed by injecting OC as such. No peak was observed at diethyl oxalate retention time. After number of trails, finally, satisfactory separation with better peak shapes and desired trace level determination of OC as diethyl oxalate was achieved. The final chromatographic conditions are given in methodology section.

Method validation

The optimized method was validated as per the ICH guidelines [11], individually in terms of specificity, limit of detection (LOD), limit of quantification (LOQ), linearity, accuracy, robustness and precision (system precision, method precision and intermediate precision).

Specificity

Specificity is the ability of the method to measure the analyte response in presence of other analytes. For specificity determination, blank solution, all residual solvents which are used in synthetic process of ZPT (methanol, ethanol, methylene chloride, ethyl acetate, toluene, benzene, acetic acid and N,N-dimethylformamide) including OC and dodecane solutions were prepared individually and injected into GC to confirm the retention times. After that, solution of ZPT (control sample), ZPT spiked with at 500µg/g of OC (spiked sample) and ZPT spiked with all residual solvents including 500µg/g of OC (all spiked sample) were prepared and injected in to GC. Based on obtained data, diethyl oxalate peak was well separated from all residual solvents indicating that the test method is selective and specific for the determination of OC in ZPT. All solvents individual retention times are given Table 1, and all spiked sample data is given in Table 2. Typical GC chromatograms of blank solution, standard solution, as such sample, spiked sample and all spiked sample solution are shown in Figure 3.

Tab. 1 Individual injections of all residual solvents

Solvent Name	RT(min)
Oxalyl chloride as Diethyl oxalate	11.052
Dodecane	12.219
Methanol	0.855
Ethanol	1.141
Toluene	3.531
Methylene chloride	Not Detected
Acetic acid	1.788
N,N-Dimethylformamide	6.482
Ethyl acetate	1.520
Benzene	1.913
N,N,N',N'-Tetramethylethanediamide	18.782

Tab. 2. All spiked sample (Zolpidem tartrate drug substance spiked with oxalyl chloride including all residual solvents)

Solvent Name	RT(min)	RRT	Resolution
Methanol	0.862	0.07	-
Ethanol	1.120	0.09	-
Ethyl acetate	1.531	0.13	1.8
Toluene	3.586	0.29	11.4
N,N-Dimethylformamide	6.451	0.53	13.4
Diethyl oxalate	11.074	0.91	27.8
Dodecane	12.221	1.00	8.2
N,N,N',N'-Tetramethylethanediamide	18.700	1.53	35.9

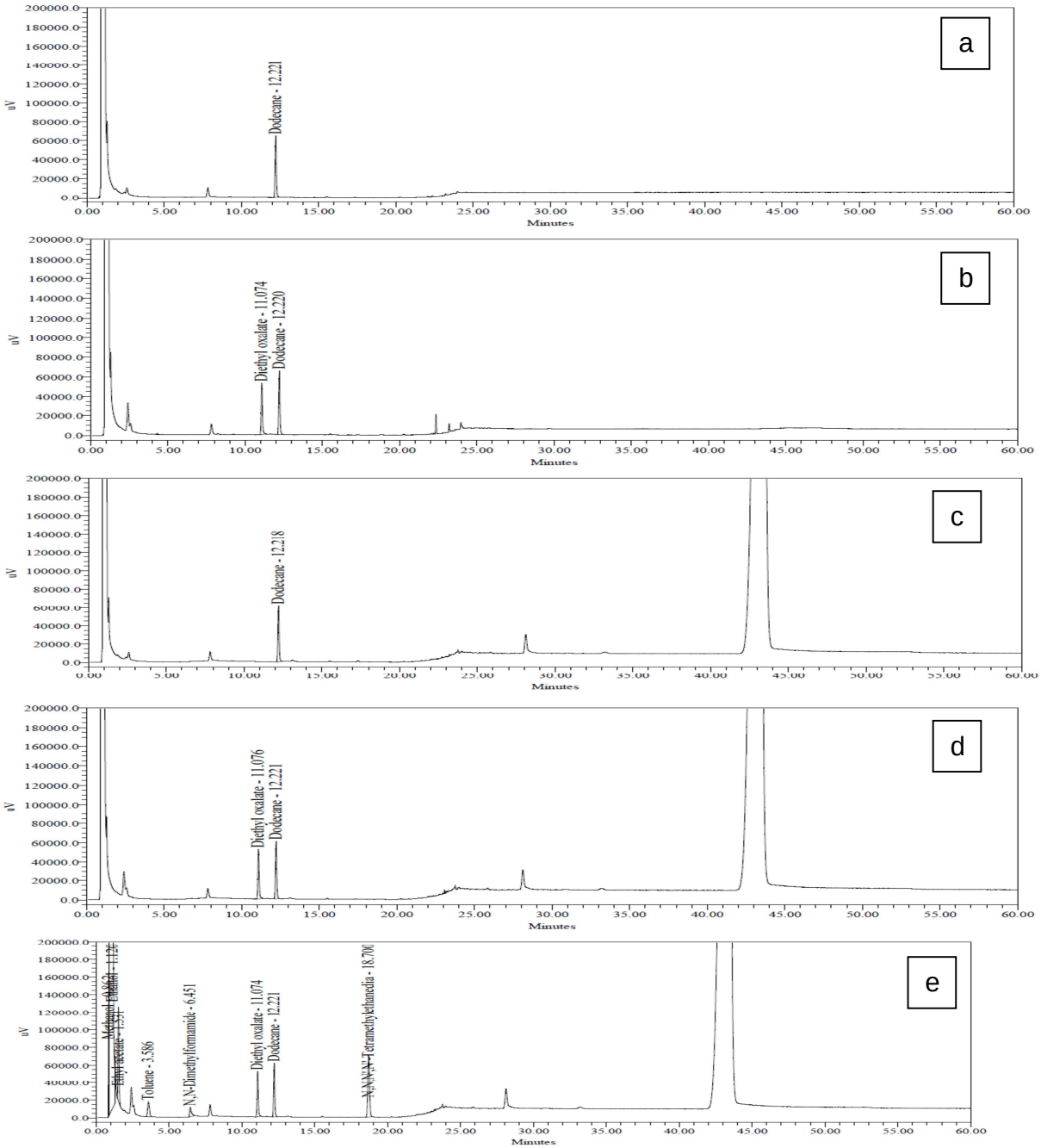


Fig. 3. Typical GC chromatograms of (a) Blank solution, (b) Standard solution, (c) Zolpidem tartrate drug substance (as such sample), (d) Zolpidem tartrate spiked with Oxalyl chloride and (e) Zolpidem tartrate spiked with Oxalyl chloride and all residual solvents

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

Standard solution (500µg/g concentration) of OC was prepared and injected into gas chromatograph. Based on signal to noise (s/n) ratio method, LOD and LOQ concentrations were predicted by using standard solution concentration(C) and s/n ratio value with the formula [3.3 x C/(s/n)] for LOD, [10 x C/(s/n)] for LOQ. The predicted LOD and LOQ values obtained for OC were 3 µg/g and 9 µg/g respectively with respect to sample concentration. LOD and LOQ solutions were prepared at predicted concentration levels and précised by analyzing six times. The achieved précised values are given in Table 3.

Linearity

The linearity was evaluated by measuring area ratio for OC with respective internal standard (dodecane) over concentration range of 9 µg/g to 750µg/g (LOQ to 150% of specification level) and the obtained data was subjected to statistical analysis using a linear regression model. The statistical results like correlation coefficient, slope, intercept, STEYX are given in Table 3.

Tab. 3. Statistical data of LOD, LOQ and Linearity experiments

Statistical Parameters	Experimental Results
LOD and LOQ Experiment:	
Limit of Detection (LOD) (µg/g)	3
Limit of Quantification (LOQ) (µg/g)	9
Precision for LOD (RSD%) (n=6)	5.7
Precision for LOQ (RSD%) (n=6)	4.5
Linearity Experiment:	
Correlation coefficient	0.9996
Concentration range (µg/g)	9 – 750
Intercept	-0.0240
Slope	0.0017
STEYX	0.0138
No. of points covered	7

Accuracy

Accuracy experiment was performed using standard addition technique. The recoveries were determined by spiking known amount of OC at four levels i.e. LOQ level, 50%, 100% and 150% of specification level (i.e. 500µg/g) into ZPT. These samples were prepared and analyzed in triplicate. The obtained recovery results are tabulated in Table 4.

Tab. 4. Accuracy data of oxalyl chloride as diethyl oxalate

Accuracy (Average of 3 replicates)	Level-I (at LOQ)	Level-II (at 50%)	Level-III (at 100%)	Level-IV (at 150%)
Added(µg/g)	9.0	250	502	751
Found(µg/g)	7.8	249	520	812
Recovery(%)	86.6	99.7	103.7	108.0
RSD(%)	4.5	0.2	0.1	0.1
Overall Recovery (%) (Average of 12 replicates)			99.5	

Precision

The precision was the study of the method using repeatability and reproducibility (ruggedness). The performance of the method was evaluated with replicate injections of standard and sample solutions. Standard solution was analyzed by injecting six times for checking the performance of the gas chromatograph under the chromatographic conditions on the day tested (system precision) and calculated the area ratios of OC(as diethyl oxalate) and dodecane (IS)from obtained areas.

Repeatability and reproducibility of the method was studied by analyzing six sample solutions separately. Repeatability was the intra-day variation(method precision) demonstrated by preparing six sample solutions individually using a single batch of ZPT spiked with OC at about 500µg/g concentration level and content was determined.

The intermediate precision was the inter-day variation (ruggedness) was defined as the degree of reproducibility obtained by following the same procedure as mentioned for method precision experiment. Ruggedness of the method was evaluated by preparing six individual sample preparations (same sample which was used in method precision experiment) by spiking OC to ZPT and injected into different column, different instrument and different analyst on different days. The achieved precision experiment results are given in Table 5.

Tab. 5. Statistical data of precision experiments

Injection ID	System Precision Ratios of area counts [Diethyl oxalate/Dodecane]	Method Precision Oxalyl chloride content(as Diethyl oxalate), µg/g	Ruggedness Oxalyl chloride content(as Diethyl oxalate), µg/g
1	0.8007	503	484
2	0.8019	508	507
3	0.8025	512	510
4	0.8030	514	515
5	0.8012	516	517
6	0.8045	515	514
Mean	0.8023	511	508
SD	0.0014	5.0	12.2
RSD(%)	0.2	1.0	2.4
95%Cl(±)	0.0015	5.0	13.0
Overall statistical dat(n=12)	**Mean**	510	
	SD	9.1	
	RSD(%)	1.8	
	95%Cl(±)	6.0	

Robustness

To assess the robustness of the method, experimental conditions were deliberately altered. The study was carried out with respect to flow pressure variation of carrier gas initial pressure and ramp temperature ±10% and column oven initial temperature and ramp temperature ± 2°C as follow.

Conditions: - In each robustness conditions remaining gas chromatography conditions are same as per test method.

(i) Column flow/Pressure programme (-10%): 36kPa (20min) $\xrightarrow{18\ kPa/min}$ 80kPa (38min)

(ii) Column flow/Pressure programme (+10%): 44kPa (20min) $\xrightarrow{22\ kPa/min}$ 80kPa (38min)

(iii) Column oven temperature (-2°C): 48°C (5min) $\xrightarrow{8°\ C/min}$ 150°C (5min) $\xrightarrow{28°C/min}$ 260°C (36.33min)

(iv) Column oven temperature (+2°C): 52°C (5min) $\xrightarrow{12°\ C/min}$ 150°C (5min) $\xrightarrow{32°C/min}$ 260°C (36.33min)

In each robustness condition, solutions of Blank, Standard and ZPT spiked with OC at about 500µg/g concentration level were prepared per methodology and injected in to GC to confirm the retention times. There is no much variation in the relative retention time (RRT) of diethyl oxalate of different deliberately varied robustness conditions from the developed methodology. Hence the test method is robust for all varied conditions. All experiments system suitability results (resolution between Diethyl oxalate and Dodecane) are given in Table 6.

Tab. 6. Robustness data of Oxalyl chloride

Robustness condition	Variation	Diethyl oxalate		Dodecane		Resolution
		RT, min	**RRT**	**RT, min**	**RRT**	
Methodology (As per test method)	-	11.063	0.91	12.219	1.00	8.6
Flow Pressure variation – Initial Pressure and Ramp	-10% & -10%/min	11.396	0.91	12.555	1.00	9.0
	+10% & +10%/min	10.765	0.90	11.916	1.00	8.1
Temperature variation – Initial Oven and Ramps	-2°C & -2°C/min	12.143	0.90	13.521	1.00	8.6
	+2°C & +2°C/min	10.264	0.91	11.27	1.00	8.4

CONCLUSION

A simple and reliable gas chromatography method was developed and validated for the determination of oxalyl chloride as Diethyl oxalate in Zolpidem tartrate drug substance. The results of various validation parameters demonstrated that the method is specific, sensitive, linear, precise, robust and accurate. Hence, the validated method is simple, sensitive and can be used for the determination of oxalyl chloride content in Zolpidem tartrate drug substance.

Acknowledgements
The authors gratefully acknowledge the management of APL Research Centre-II (A Division of Aurobindo Pharma Ltd.) for giving us the opportunity to carry out the present research work. The authors are also thankful to the colleagues of Analytical Research Department and Chemical Research Department for their co-operation.

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