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Development and validation of gas chromatography method for low level detection of residual Methyl chloride, Ethyl chloride and Isopropyl chloride in Ziprasidone hydrochloride

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ABSTRACT

A simple and reliable head space gas chromatographic method has been developed for the determination of residual Methyl chloride, Ethyl chloride and Isopropyl chloride in Ziprasidone Hydrochloride. The proposed method is based on flame ionization detection technique with DB-624 as stationary phase. Linearity of detector response was established up to 13.5µg/g and the detection limit was 0.8µg/g for Methyl chloride, Ethyl chloride and 0.9µg/g for Isopropyl chloride respectively. No interference of organic solvents used in the synthesis was observed. Performance of the method was assessed by evaluating the recovery, repeatability, reproducibility, linearity and limits of detection and quantification. The proposed method has a potential for application to drug substances which may contains traces of alkyl chloride. Results prove that the validated method was suitable for determining the residual Methyl chloride, Ethyl chloride and Isopropyl chloride in Ziprasidone Hydrochloride drug substance. To widen the scope of this method, interference of 17 commonly employed solvents in the synthesis has been studied for any possible interference with Methyl chloride, Ethyl chloride and Isopropyl chloride. The potentiality of method has been studied for various drug substances containing possible alkyl chlorides residue present in their drug matrix.

Key words: Gas chromatography, Methyl chloride, Ethyl chloride, Isopropyl chloride, Ziprasidone Hydrochloride.

INTRODUCTION

Ziprasidone Hydrochloride is an effective anti-psychotic drug and serotonin dopamine antagonist that works to treat the positive, negative and depressive symptoms associated with schizophrenia [1, 2]. Schizophrenia is a chronic illness that requires lifelong treatment [3]. Therefore, it is necessary to calculate the level of genotoxic impurities in the drug substance, because the potential sources are reagents or solvents [4]. Chemically, Ziprasidone Hydrochloride is 5-[2-[4-

(1,2-benzisothiazol-3-yl) -1- piperazin -1 -yl] ethyl] -6 -chloro- 1,3 –dihydro -2*H* –indol -2-one Hydrochloride. The molecular formula is $C_{21}H_{21}ClN_4OS.HCl$ and the molecular weight is 449.42. Methyl chloride, Ethyl chloride and Isopropyl chloride may form at the salt formation step, as Ziprasidone base in alcohol solvent (Methanol, Ethanol or Isopropanol) is treated with hydrochloric acid leading to precipitate of Ziprasidone Hydrochloride. Methyl chloride, Ethyl chloride and Isopropyl chloride are reported as carcinogen [5] and methyl chloride as teratogen [6]. Therefore it is necessary that, these residual impurities should be controlled to limits permitted by threshold of toxicological concern (TTC). A TTC value was estimated to be 1.5µg/person/day intake of a genotoxic impurity is considered to be associated with an acceptable risk for most pharmaceuticals as per EMEA guideline on the limit of genotoxic impurities [CPMP/SWP/5199/02, EMEA/CHMP/QWP/251344/2006] as well as risk assessment literature [7, 8]. Ziprasidone Hydrochloride is available in the market from 20mg, 40mg and 80mg strength as "GEODON" and "ZELDOX" by Pfizer. The maximum daily dose for the drug is 160mg, based on the TTC calculation [9] the permitted value of alkyl chlorides together is 9.4µg/g.

Many analytical methods have been reported in the literature for Ziprasidone hydrochloride determination. HPLC determination has been reported by Singh et.al, [10] in 2007, video-densitometry using HPTLC technique was reported in 2010 by Robert Skibinski et.al, [11] and quantification of Ziprasidone in human plasma by LCMS was developed in 2006 by Osama Y. Al-Dirbashi et.al, [12]. Quantitative determination of alkyl chloride present in the active pharmaceutical ingredients has been studied by D.P.Elder et.al, [13], in 2008 and Noureddine Yassaa et.al, [14] in 2009 has been reported for environmental samples using GC-MS technique, but no specific gas chromatographic methods has been reported till date, for the quantitative determination of residual Methyl chloride, Ethyl chloride and Isopropyl chloride in Ziprasidone Hydrochloride drug substance.

MATERIALS AND METHODS

Chemicals and Reagents

Methanol, Tetrahydrofuran, Benzene, Isopropyl alcohol, Dimethylsulfoxide (DMSO), Ethanol, Acetonitrile, 2-Methyl pentane, 3-Methyl pentane, *n*-Hexane, Methyl cyclopentane Ethyl acetate, Cyclohexane, Toluene, 1,4-Dioxan, Methyl isobutyl ketone, and *n*-Butanol procured from Fluka Ltd., Reference standard of Ethyl chloride (1000 µg mL^{-1} in Methanol), Methyl chloride (200 µg mL^{-1} in in Methanol) was purchased from Supelco Analytical Inc., (Belleforte, USA). Isopropyl chloride obtained from Sigma Aldrich. Co., USA. All reagents used were of analytical GC reagent grade. HPLC water was obtained from Merck Limited., Mumbai. Sample under investigation was obtained by the Aurobindo Pharma Ltd, Hyderabad, India.

Instrumental

Gas chromatographic systems of two different makes i.e., Agilent 6890N GC with Gerstel Multipurpose sampler and HPCHEM station software with FID and Shimadzu 2010GC system with FID, with combipal auto sampler with GC solution and Waters Empower software's was used. For the separation of alkyl chlorides, high purity helium was used as carrier gas and purge gas was Nitrogen. The separation was performed on DB-624 60m long, 0.53mm I.D. and 3µm film thickness capillary column. The carrier gas flow was adjusted at 41.4 kPa using pressure mode and the sample was introduced with split ratio 1:2. The capillary injector temperature set at 180°C and FID-detector set at 260°C. The column oven temperature was 35°Cfor 10 minute, further the temperature ramp set at 30°C/min to attain 220°C, and then hold for 9 minutes, the chromatograph stop time was set to 25 minutes. The head space model AOC5000 was used with 2.5 ml-HS syringe and the sample volume set at 1.0mL. The sample in sealed head space vials

was incubated at 60°C for 20 minutes using auto sampler and the agitation speed set at 500rpm for 10 seconds switch over on/off time. The syringe temperature set at 70°C and the injection speed was 1mL/second with 500 milliseconds for pull up delay and the syringe flushed time set at 5 minutes with optimum head space run time set at 40 minutes, detailed in Table-1.

Table 1. Gas chromatography conditions

Components	Parameters	Requirement
Autosampler	Injector volume	1 ml
	Syringe	2.5 ml HS
	Incubation temperature	60°C
	Incubation time	20 min
	Agitation speed	10 seconds
	Syringe flushing	5 min
	Syringe temperature	70°C
	Fill speed	1mL/sec
	Pull up delay	500 m sec
	Inject speed	1 mL/sec
Injector	Injector temperature	180°C
	Carrier gas	Helium
	Gas flow	41.4 kPa (Constant flow)
	Injection mode	Split 1:2
	Liner	Split Glass
	Stop time	25 min
Column	DB-624 [60m x 0.53 x 3µm) Cyanopropylphenyl 6% and Dimethylpolysiloxane copolymer 94%	
	Oven temperature program	35°C (10 min) → 30°C/min → 220°C (9min)
	Run time	25 min
	Injection delay	15 min
Detector	Type	FID
	Temperature	260°C

Diluent solution

Accurately measured and transferred 2.0 mL of Methanol into a 100 ml clean, dry volumetric flask containing about 25 mL of Dimethylsulfoxide, mixed and make up to volume with Dimethylsulfoxide.

Standard solution A

Accurately weighed and transferred about 0.0225 g of Isopropyl chloride into a 50 mL clean, dry volumetric flask containing about 25 mL of Dimethylsulfoxide, mixed and make up to volume with Dimethylsulfoxide.

Standard solution B

Accurately measured and transferred 1.0 ml of Ethyl chloride reference standard into a 5 mL clean, dry volumetric flask containing about 2 mL of Dimethylsulfoxide, mixed and make up to volume with Dimethylsulfoxide.

Standard stock solution
Accurately measured and transferred 1.1 mL of Methyl chloride reference standards into a 50 mL clean, dry volumetric flask containing about 25 mL of Dimethylsulfoxide, further the resulting solution was added with 1.1mL of Standard solution B and 0.5 mL of Standard solution A and make up to volume with Dimethylsulfoxide.

Standard solution
Transferred accurately 0.2 mL of the Standard stock solution to the headspace vial containing 0.125 mL of water and 0.675 mL of Dimethylsulfoxide, sealed the vial immediately.

Sample solution
Accurately weighed and transferred about 0.1 g of sample to the headspace vial containing 0.125 mL of water and 0.675 mL of Dimethylsulfoxide. Further 0.20 mL of diluent solution was added and sealed the vial immediately.

Blank solution
Taken 0.125 ml of water, 0.675 ml of Dimethylsulfoxide and 0.2 ml of diluent solution into the headspace vial and sealed the vial immediately.

RESULTS AND DISCUSSION

Method development and optimization summary
Preliminary experiments were carried out based on the retention of Methyl chloride, Ethyl chloride and Isopropyl chloride, which were discussed in many Agilent gas chromatographic applications [15]. By using non polar stationary phase [16] like DB-1, DB-5, DB VRX and mid polar stationary phase [17-18] like DB-624 and Rtx-624 capillary column, elution of alkyl chloride including other solvents used in the synthesis was investigated using different composition of hydrogen and nitrogen gases. The separation was achieved on DB-624 column (containing 6% Cyanopropylphenyl and 94% Dimethylpolysiloxane copolymer) of 60 m length, 0.53 mm internal diameter and film thickness of 3µm with Helium as carrier gas. In the diluent composition Methanol and DMSO was used, slight low response and some base line disturbance for alkyl chlorides was observed. Therefore, a slight amount of water was added during the sample preparation. The peak shape and response of residual Methyl chloride, Ethyl chloride and Isopropyl chloride were improved. Finally, satisfactory result was achieved in reasonable time with flow of Helium gas set to constant pressure mode.

Validation of the method
In order to judge the suitability of method for determining the Methyl chloride, Ethyl chloride and Isopropyl chloride traces in Ziprasidone Hydrochloride drug substance, the method was validated as per the ICH guideline [19], for specificity, limit of detection, limit of quantification, linearity, accuracy, precision and robustness.

Specificity
To assess the ability of the method, individual solutions were prepared with known amount of Methyl chloride, Ethyl chloride and Isopropyl chloride with respect to Ziprasidone hydrochloride drug substance concentration and injected in to the gas chromatograph and the chromatograms were recorded. The sample solution was prepared as per the methodology and injected into the chromatograph (Control sample). The drug substance shows no peaks either due to Methyl chloride, Ethyl chloride and Isopropyl chloride in the sample solution. So it reveals that the drug substance highly pure and free from alkyl chlorides residue under investigation.

Therefore the sample solution was spiked with known amount of Methyl chloride, Ethyl chloride and Isopropyl chloride reference standard at target level, and injected into the chromatograph (Spiked sample). The relative retention time for Methyl chloride, Methanol, Ethyl chloride, Isopropyl chloride and Dimethylsulfoxide was found 0.22, 0.28, 0.32, 0.43 and 1.00 respectively. The resolution between Methyl chloride and methanol was found to be 11.1 and between Methanol and Ethyl chloride was found to be 1.59 respectively. No interference of blank was observed corresponding to Methyl chloride, Ethyl chloride and Isopropyl chloride peaks and the analyte peaks were well resolved. Further the GC/MS study was conducted to confirm that the injected alkyl chlorides peaks are homogenous and pure.

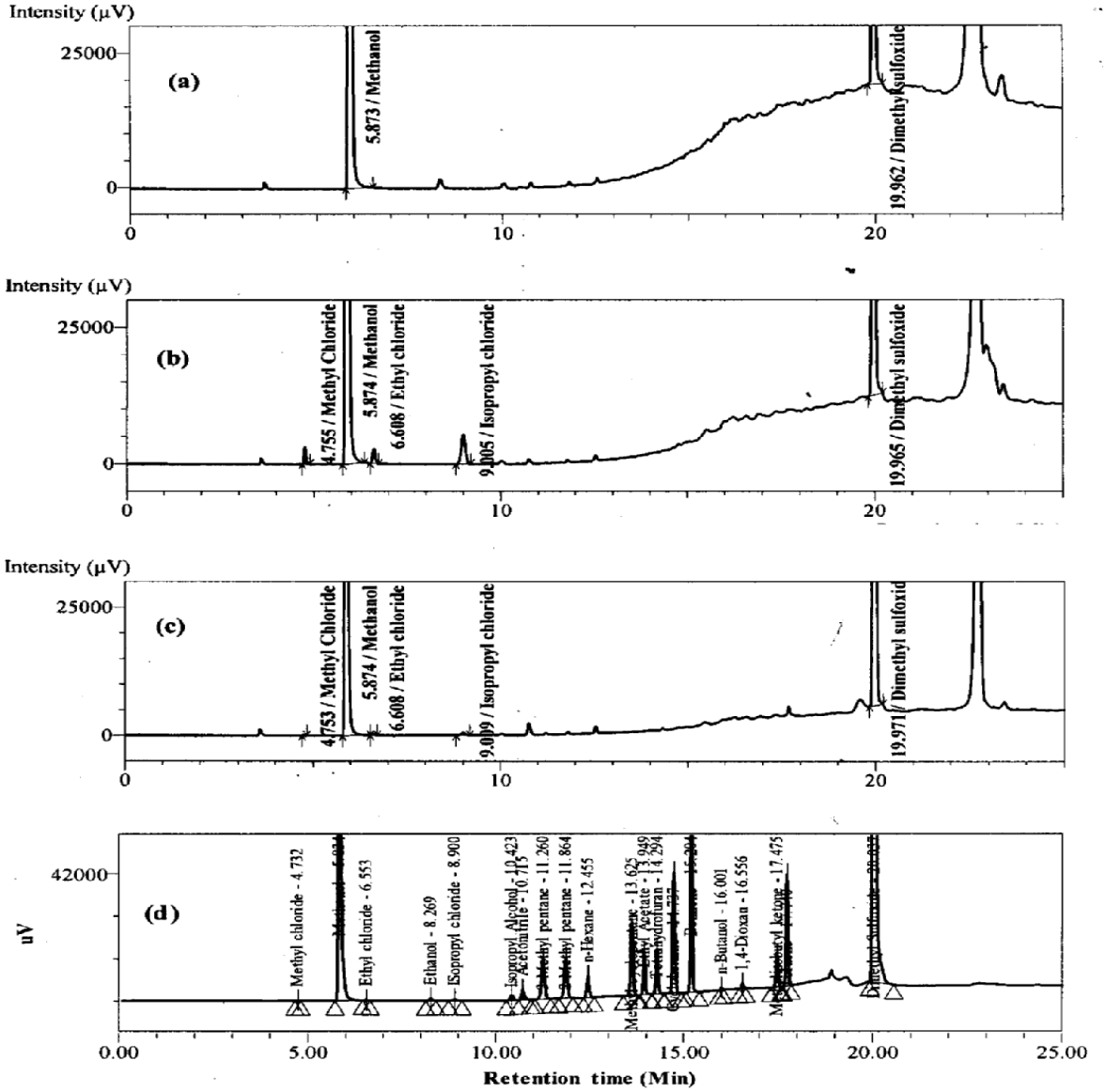

Figure 1. Representative chromatogram obtained from (a).Blank, (b).Standard, (c) Spiked sample, and (d). Spiked sample with additional solvents including standard.

To broaden the scope of the method, series of possible commercially employed solvents which may be used during the synthesis was prepared individually and injected to chromatograph at known concentration level. Methanol, Ethanol, Isopropyl alcohol, Acetonitrile, 3-Methyl pentane, 2-Methyl pentane, *n*-Hexane, Methyl cyclopentane, Ethyl acetate, Tetrahydrofuran, Cyclohexane, Benzene, *n*-Butanol, 1,4-Dioxan, Methyl isobutyl ketone, Toluene and, Dimethyl

sulfoxide are the probable solvents and their retention time was confirmed by respective individual chromatogram.

Each of the above individual solvents was spiked with Methyl chloride, Ethyl chloride and Isopropyl chloride reference standard at about 9 µg/g levels to the sample solution (Spiked sample with additional solvents). The resolution between Methyl chloride and methanol was found to be 10.6 and between Methanol and Ethyl chloride was found to be 1.63 respectively. There is no co-eluting peak due to additional solvents with alkyl chlorides peaks under investigation. Therefore a system suitability criterion has been established from the specificity experiment that, the resolution between Methyl chloride and methanol should not be less than 5.0 and between Methanol and Ethyl chloride should not be less than 1.5. From the above experiment, Figure 1 shows the chromatogram obtained from blank, Standard, Spiked sample with standard at LOQ level, and Spiked sample with additional solvents including standard.

LOD and LOQ

For determining the limit of detection (LOD) and limit of quantification (LOQ) values of each residual alkyl chlorides the standard solution at targeted level injected in to the chromatographic system, the LOD and LOQ values predicted from the signal to noise (S/N) ratio data. The LOD/LOQ values were predicted by [3.3 x Concentration of Standard /(S/N)] for LOD and [10 x Concentration of Standard /(S/N)] for LOQ. The solutions of Methyl chloride, Ethyl chloride and Isopropyl chloride for LOD and LOQ evaluation were prepared at predicted concentration levels and précised by analyzing six times, detailed in Table 2.

Table 2. Evaluation of LOD and LOQ, Linearity data

Components	Methyl Chloride	Ethyl Chloride	Isopropyl Chloride
Concentration (µg/g)	8.8	8.8	9.1
S/N ratio	35.0	36.8	32.2
Limit of detection (µg/g)	0.83	0.79	0.93
Limit of quantification (µg/g)	2.51	2.39	2.83
Limit of detection precision (%RSD)[a]	12.9	13.4	10.9
Limit of quantification precision(%RSD) [a]	2.4	3.2	2.7
Slope	1105.1	1623.5	5112.7
STEY X	146.0	116.1	886.4
Correlation Co-efficient	0.9996	0.9999	0.9993
RSQ(r^2)	0.9992	0.9998	0.9986

[a] *Average of n=6 determinations*

Linearity

The linearity of the method was determined by preparing a series of solutions by using Methyl chloride, Ethyl chloride and Isopropyl chloride at concentration levels from LOQ level to 150% of the target level (9µg/g) using GC-FID detector response. The concentration studied ranges between 2.40µg/g to 13.49µg/g for Methyl chloride, 2.35µg/g to 13.37µg/g for Ethyl chloride and 2.53µg/g to 13.59µg/g for Isopropyl chloride respectively. The statistical parameters slope, intercept, residual standard on deviation response and correlation co-efficient values were calculated in Table 1. The area and concentration were treated by least square linear regression analysis plot [Area count in terms of Intensity (mV) at Y-axis Vs Concentration (µg/g) at X-axis] as shown in Figure 2.

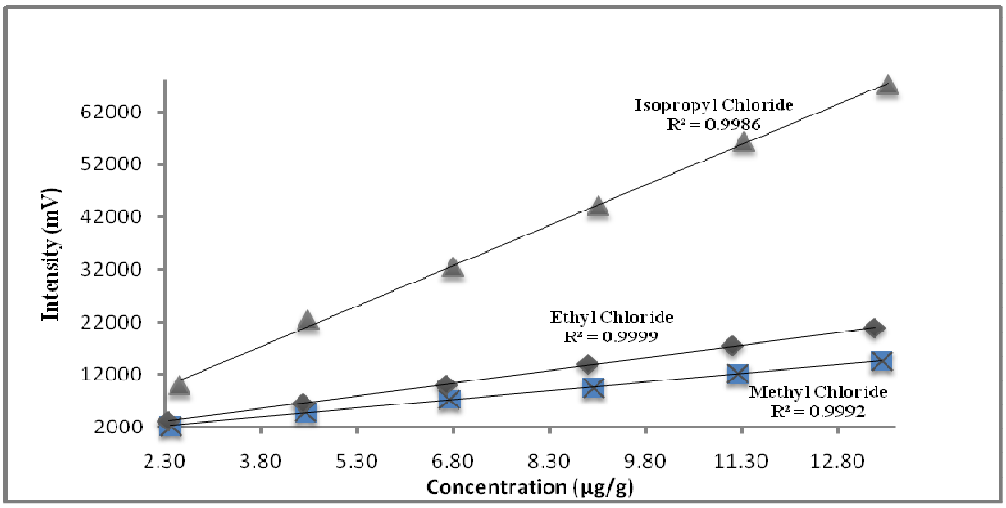

Figure 2. Regression plot for Methyl chloride and Ethyl chloride, Isopropyl chloride

Precision

The precision was the study of the method using repeatability and reproducibility (ruggedness). The method performance was evaluated with replicate injections of the standard and sample solutions. Standard solution was analyzed six times for checking the performance of the gas chromatography system under the chromatographic conditions on the day tested (System precision), the %RSD was found to be 3.0, 2.6 and 3.3 for Methyl chloride, Ethyl chloride and Isopropyl chloride respectively.

Repeatability was intra-day variation (method precision) and inter-day variation (ruggedness). The repeatability of the method was studied by analyzing six sample solutions separately by the addition of residual alkyl chlorides at known concentration levels was found to be 8.1µg/g, 8.6µg/g and 8.3µg/g and %RSD values were 2.7, 3.0 & 0.7 for Methyl chloride, Ethyl chloride and Isopropyl chloride respectively.

The degree of reproducibility is known as ruggedness, obtained by the analysis of the same sample concentration (which is used in the method precision) under a variety of conditions using different series of column, with different user on different day by using new standard also found to be 8.7µg/g, 8.5µg/g and 8.6µg/g and %RSD values are 1.5, 3.4 & 3.2 for Methyl chloride, Ethyl chloride and Isopropyl chloride respectively.

Table 3. Accuracy experimental data

Components	Methyl Chloride				Ethyl chloride				Isopropyl chloride			
Target Level (%)	25	50	100	150	25	50	100	150	25	50	100	150
Spike Conc. (µg/g) [b]	2.37	4.5	8.9	13.3	2.54	4.5	9.1	13.5	2.61	4.4	8.9	13.2
Determined Conc.(µg/g) [b]	2.46	4.2	8.0	12.7	2.56	4.4	8.9	13.3	2.52	4.3	8.5	13.0
%RSD [b]	1.9	2.4	1.9	4.0	2.0	1.8	1.1	2.6	1.8	0.6	1.6	1.3
Percent Recovery [b]	103.8	93.3	89.9	95.5	100.8	97.8	97.8	98.5	96.6	97.7	95.5	98.5
Average % Recovery	95.6				98.7				97.1			

[b] *Average of n=3 determinations*

Accuracy

The accuracy of the method was evaluated by recovery experiment using standard addition technique. The recoveries were determined by spiking the respective residual solvent at three different levels ranging from 50% to 150% (with respect to 9µg/g) into Ziprasidone

hydrochloride drug substance. The samples were prepared as per the methodology, analyzed in triplicate and percentage recoveries were calculated. The average recovery values were summarized in Table 3.

Robustness

To assess the robustness of the method, experimental conditions were deliberately altered. The study was carried out with respect to flow rate of mobile phase ± 10% and initial temperature of the column oven 35°C± 2°C and ramp temperature 30°C/min ± 2°C. The system suitability results met the acceptance criteria at each of the deliberately varied conditions. There is no much variation in the RRT's of alkyl chlorides obtained at different deliberately varied conditions from the developed methodology. Hence the test method is robust for all varied conditions.

Potential application of the method

The potential application of the method has been conducted for the drug substances containing possible alkyl chloride moiety in their drug matrix. Most of the selected drug substance has shown no alkyl chloride residue during the analysis even though precipitation step uses hydrochloric acid treatment for their respective alcoholic drug base. The absence of alkyl chloride residue in the drug confirms the complete precipitation step, leads to no un-reacted alcoholic moiety in the drug matrix. Therefore, the method has been applied to various drug substances based on the precipitation treatment. But it need slight modification for sampling preparation to obtain accuracy result with suitable spiking level of alkyl chloride. Table 4, describes the experimental result for respective drug substances confirm the potentiality of the method.

Table 4. Recovery result for the variable drug substance showing

Drug	Alkyl chloride determined	Amount Added (µg/g)	Amount Found (µg/g)	%Recovery [c]	%RSD [c]	± 95%CI [d]
Risperidone	Isopropyl chloride	2.87	2.75	95.8	0.1	0.1
Alfuzosin Hydrochloride	Ethyl chloride	2.98	2.89	97.1	2.5	6.2
Valganciclovir Hydrochloride	Methyl chloride	0.75	0.76	101.3	1.5	3.8
	Ethyl chloride	0.75	0.74	98.7	0.8	1.9
	Isopropyl chloride	0.75	0.77	102.7	1.6	4.0
Fluoxetine Hydrochloride	Methyl chloride	2.99	2.91	97.3	1.8	5.0
	Ethyl chloride	2.98	2.96	99.3	3.0	4.1
	Isopropyl chloride	2.99	2.92	97.7	1.4	0.8

[c] n=3, determination, [d] CI: stands for Confidence interval

Table 5. Sample preparation optimization summary

Drug	Sample preparation technique
Risperidone	Sample 0.4g diluted to 5.0mL DMSO with 3µg/g spiking level
Alfuzosin Hydrochloride	Sample 0.25g, 0.25mL DMSO, 0.25mL Water &0.5mL (2% MeOH in DMSO) with 3µg/g spiking level
Valganciclovir Hydrochloride	Sample 0.25g, 0.25mL DMSO, 0.25mL Water &0.5mL (0.4% MeOH in DMSO) with 0.75µg/g spiking level
Fluoxetine Hydrochloride	Sample 0.25g, 0.25mL DMSO, 0.25mL Water &0.5mL (4% MeOH in DMSO) with 3µg/g spiking level

The Accuracy of all these alkyl chlorides was found to be in between the predefined acceptance criterion of 96.0-103% and the data is given Table 4 showing the potentiality of the method.

The sample preparation was optimized and the summary also describes in Table 5 with definite spiking level of alkyl chlorides based on the calculations.

CONCLUSION

The developed gas chromatographic method has to evaluate reliable and economical result for the simultaneous determination of Methyl chloride, Ethyl chloride and Isopropyl chloride residue present in the Ziprasidone Hydrochloride. The results of various validation parameters confirmed that the method is specific, robust, linear, precise and accurate. The method has been applied to various drug substances containing possible alkyl chloride moiety in the drug matrix. The experimental data shows that the method has potential application for the quantitative determination of alkyl chloride moiety present in the drug substances.

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REFERENCES

[1]. Chandra Prakash, Amin Kamel, Judith Gummerus & Keith Wilner, *Drug Metabolism and Disposition*, **1997**, 25, 7, 863.
[2]. TF Seeger, PA Seymour, AW Schmidt, SH Zorn, DW Schulz, LA Lebel, S McLean, VGuanowsky, HR Howard, JA Lowe, *J Pharmacol Exp Ther*, **1995**, 275, 101.
[3]. GD David, FC Laura, *Expert opinion on investigational drugs*, **2000**, 9, 4, 819-828.
[4]. International conference on harmonization Q3A (R2) Draft revised Guidance onimpurities in new drug substances, **2006**.
[5]. HM Bolt, B Gansewendt, *Crit. Rev. Toxicol*, **1993**, 23, 3, 237.
[6]. R Wolkowski-Tyl , M Phelps , JK Davis, *Teratology*, **1983**, 27, 2, 181.
[7]. R Kroes , J Kleiner and A Renwick, *Toxicological sciences*, **2005**, 86, 2, 226.
[8]. R Kroes, AG Renwick, M Cheeseman, J Kleiner, I Mangelsdorf , A Piersma, B Schilter, J Schalatter, F Van Schothorst, JG Vog, *Food Chem Toxicol*, **2004**, 42, 1, 65.
[9]. International conference on harmonization Q3C (R5) Impurities: Guideline for residual solvents, **2011**.
[10]. A Singh, BM Rao, GR Deshpande, S Sangaraju, MK Srinivasu, M Lalitha Devi, PVV Satyanarayana and K Chandrasekhar, *Chromatographia.*, **2007**, 65, 3-4, 191.
[11]. Robert Skibinski and Lukasz Komsta, *Journal of Planar Chromatography.*, **2010**,23, 1, 23.
[12]. OY Al-Dirbashi, HY Aboul-Enein, Ahmed Al-Odaib, Minnie Jacob, Mohammed SR, *Biomedical Chromatography*, **2006**, 20, 4, 365.
[13]. DP Elder, A.M. Lipczynski, A Teasdale, *Journal of Pharmaceutical and Biomedical Analysis*, **2008**, 48, 3, 497.
[14]. Noureddine Yassaa, Asher Wishkerman, Frank Keppler, Jonathan Williams, *Environmental Chemistry*, **2009**, 6, 4, 311.
[15]. Introduction to Basic Gas Chromatography, *Agilent J&W GC column selection guide*, **2007**.
[16]. Technically advanced solution for optimized laboratory performance, Agilentseparation times, **2004**, 17, 1, 2.
[17]. Robert Lee Grob and FB Eugene, Modern practice in gas chromatography, John Wiley & Sons Inc., New Jersey, **2004**.
[18]. Robert Lee Grob and FB Eugene, Column for gas chromatograph, Performance andselection, John Wiley & Sons Inc, New Jersey, **2007**.

[19]. ICH harmonized tripartite guideline, Q2 (R1), Validation of analytical procedure: Text and methodology, **2005**.