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Organic Volatile Impurities in Citicholine Sodium : A Robust Analytical Method Development and Validation by Head Space Gas Chromatography

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Abstract : The Active Pharmaceutical Ingredients (APIs) is an ingredient in a pharmaceutical drugs that is biologically active which are basically drug Substance and gives therapeutic benefit. These drug substances are used as bulk active in the manufacturing of medicines. There are process solvents which includes Methanol and Isopropylalcohol (IPA) which are basically Organic Volatile Impurities (OVI) used during the manufacturing process. It is very difficult to remove these solvents completely by the work-up process. It is mandatory for the pharmaceutical manufacturers to identify and qualify if any such impurities are presentin APIs.To identify and control these solvents Gas Chromatography - Head Space(GC-HS) method was developed. The method was validated as perInternational conference on harmonization guidelines (ICH) & United States Pharmacopoeia (USP).
Key Words : Citicholine Sodium, OVI, Residual solvents, GC.

Introduction

Citicholine sodium is an API. The IUPAC name is 5'-O-[hydroxy({hydroxy[2-(trimethylammonio)ethoxy]phosphoryl}oxy)phosphoryl] cytidine sodium.Molecular weight is 510.31,Molecular formula is $C_{14}H_{25}N_4NaO_{11}P_2$ (Fig 1).This drug comes under the category of nutraceuticals.Nutraceuticals are commonly defined as any substance that is considered a food, a part of a food, a vitamin, a mineral, or an herb that provides health benefits, including disease prevention and/or treatment [1].

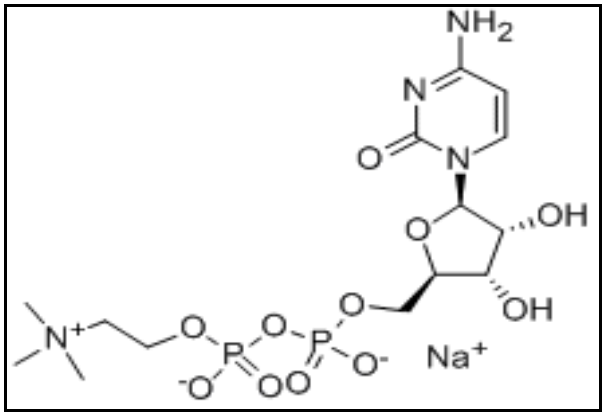

Fig 1. Structure of Citicholine sodium

Organic solvents are routinely applied during synthesis of drug substances, excipients, or during drug product formulation. They are not desirable in the final product mainly because of their toxicity, their influence on the quality of crystals of the drug substance and their odor or taste, which can be unpleasant or harmthe patients. To remove them, various manufacturing processes or techniques (usually under increased temperature or/and decreased pressure) are in use. Even after such processes, some solvents stillremain, yet in small quantities. These small quantitiesof organic solvents are commonly known as organic volatile impurities (OVIs) or residual solvents [2.]

Residual solvents in pharmaceuticals are defined as organic volatile chemicals that are used or produced in the manufacture of drug substances. The residual solvents are not completely removed by practical manufacturing techniques. The use of appropriate solvents in the manufacturing is to increase the purity of the product, yield, crystal form and solubility etc., these solvents in the products or substances do not provide therapeutic benefit, and they should be removed to the extent possible to meet the specifications [3].

The advantage of Headspace technique over neat injection offers advantage of introducing only volatile components of aliquot into the column and thereby increases the lifetime and performance of column as well as instrument [4]. Headspace sampling is essentially a separation technique in which volatile material may be extracted from a heavier sample matrix and injected into a gas chromatograph for analysis. With valve loop injection, once the equilibration time is complete, a sampling needle is inserted through vial septum and the sample vial is pressurized to provide a final pressure of 1.5 to 2.0 atmospheres.The internal standard method should compensate for variation and inaccuracies in the volume of aliquot delivered into the chromatograph and hence there is higher level of accuracy was achieved [5].

In the early stage, one of the simplest methods for determining the content of volatile residuesconsists in measuring the weight loss of a sample during heating. However, this methodsuffers the great disadvantages of being totally non-specific (multicomponent solvent blendscannot be analysed and there will always be a doubt on humidity contamination) and ofneeding several grams of product to achieve a detection limit of about 0.1% [6-8].Nevertheless, when carried out by thermogravimetry, the limit can be lowered to 100 ppmusing only a few milligrams of substance20. Infrared spectroscopy (IR)[9]and FourierTransform Infrared Spectrometry (FTIR)[10]were used to determine residual Tetrahydrofuran(THF), dichloroethane and methylene chloride in polymer samples by measuring thecharacteristic solvent bands in the spectra. The most common limiting factors in thesemethods are possible interferences of solvent and matrix peaks and, in the case of IR, the highdetection limit (above 100 ppm) and a lack of accuracy at low concentrations

Headspace gas chromatography (HSGC) is a technique where the liquid or solid sample is set in a closed vessel until the volatile components reach equilibrium between the sample and the gas volume above, i.e., the so called "headspace". An aliquot of the headspace is sampled and introduced into a gas chromatographic (GC) column for analysis. Regulatory agencies and pharmacopoeias suggest headspace gas chromatography as the most suitable technique for residual solvent testing for active substances and formulations soluble in water. Residual solvent specification limits, set in accordance with the toxicity of solvents, vary from a few ppm to thousands of ppm. HSGC determination of residual solvents is nowadays a mature technique[11-16].

The methodwastakenforcompleteanalyticalmethodvalidationasperICH guidelines [17]andUSP monograph[18]**.**

Materials and Methods

Chemicals and Reagents

DMSO,GC grade purity 99.9%; 1,4-Dioxane, analytical grade purity 99.8%; Methanol, GC grade purity 99.9%; IPA, GC grade 99.8% Make: Spectrochem.

It was verified all the chemicals and the reagents were within the validity period. Description and appearance of the individual chemicals and reagents were observed and found satisfactory.

Necessary safety precautions have been taken based on Material Safety Data (MSDS)

Instrumentation

Head space Gas chromatograph, Agilent Technologies, Model.7694E with flame ionization detector, Chemstation software, Column: Supelcowax 25301-U 30 m x 0.53 mm x 1.0µm capillary column

Method development

To identify and quantify for organic solvents, the most preferred method is Gas Chromatography due to its simplicity and detection of wide variety of solvents. The choice of solvent as diluent were DMSO and Dimethyl formamide (DMF) taken during trail runs and it was observed that the re producibility and response were found to be better with DMSO when compared to DMF.The choice of internal standard were Toluene, 1,4,Dioxane were considered as part of method development and it was observed that 1,4 Dioxane has better Relative Standard Deviation (RSD)when compared with Toluene.Nitrogen as carrier gas was used. Oven temperature initially maintained at 45ºC for 3 minutes and increased to 75ºC at the rate of 4ºC per minute and held for 0.0 minutes. Again oven temperature was increased to 225ºC at the rate of 20ºC per minute and held for 15 minutes. Inlet temperature 200ºC and detector temperature is 250ºC. Carrier gas flow is 2.0 ml/minute and split ratio is 5:1, Injector port temperature is 200 ºC and transfer line loop temperature is 125ºC. Head space vial equilibration time is 45 minutes and GC cycle time is 48 minutes. Column used is Supelcowax 25301-U 30 mtr, 0.53 mm x 1.0µm capillary column. Detector is Flame Ionization Detector (FID).

The column was calibrated as per standard requirements and found satisfactory.

Results and Discussions

Preparation of solution

Diluent : Dimethyl Sulphoxide (DMSO)

Preparation of stock solution A

Weighed accurately 3.0 g of methanol and 5.0 g of isopropyl alcohol into a 100-mL volumetric flask Containing about 30.0 mL of DMSO. Dissolved and diluted to volume with DMSO. Mixed well. Label it as solution A.

Preparation of solution B

Pipetted 10.0 mL of the stock solution Ainto a 100-mL volumetric flask. Diluted to volume with DMSO. Mixed well. Label it as solution B.

Preparation of solution C

Weighed accurately 1.0 g of 1,4- dioxane into a 100-mL volumetric flask containing about 30.0 mL of DMSO. Dissolved and diluted to volume with DMSO. Mixed well. Pipetted 10.0 mL of stock solution C into a 100-mL volumetric flask. Diluted to volume with DMSO. Mixed well. Labelled it as solution C.

Preparation of standard solution

Pipetted 1.0 mL of solution B and 1.0 mL of solution C into a 20-mL headspace vial. Added 8.0 mL of DMSO. Crimped and kept in headspace carousel. Six such preparations of standard solution were prepared for establishing system suitability.

Samplesolution

Weighed 1.0 g of sample into a 20-mL headspace vial. Added 1.0 mL solution C and 9.0 mL of DMSO. Crimped and kept in GC headspace carousel. Prepared the sample in duplicate.

The following parameters have been considered for the analytical method validation for the estimation of residual solvents in Citicoline sodium by GC.System suitability, Specificity, Linearity, LOD/LOQ, Accuracy, Precision, and Robustness.

The resolution between each solvent peaks should be NLT 1.0. The RSD for area ratio of the individual solvent peak areas to internal standard peak areas obtained from six replicate injection of standard solution should be NMT 15.0 %.Pipette 10.0 mL of DMSO into a 20-mL headspace vial. Crimp and keep in GC headspace carousel

System Suitability

To verify that the analytical system is working properly and can give accurate and precise results, the system suitability parameters have to be set.Injected blank, Standard solution of 6 injections into gas chromatograph and recorded the chromatograms. Calculated resolution between each solvent peaks. Calculated the area ratio of solvent peak area to internal standard peak area obtained from six replicate injections. Acceptance criteria for The resolution between each solvent peaks should be NLT 1.0 and The RSD for area ratio of each solvent peak area to internal standard peak area obtained from six replicate injections of standard solution should be NMT 15.0 %.

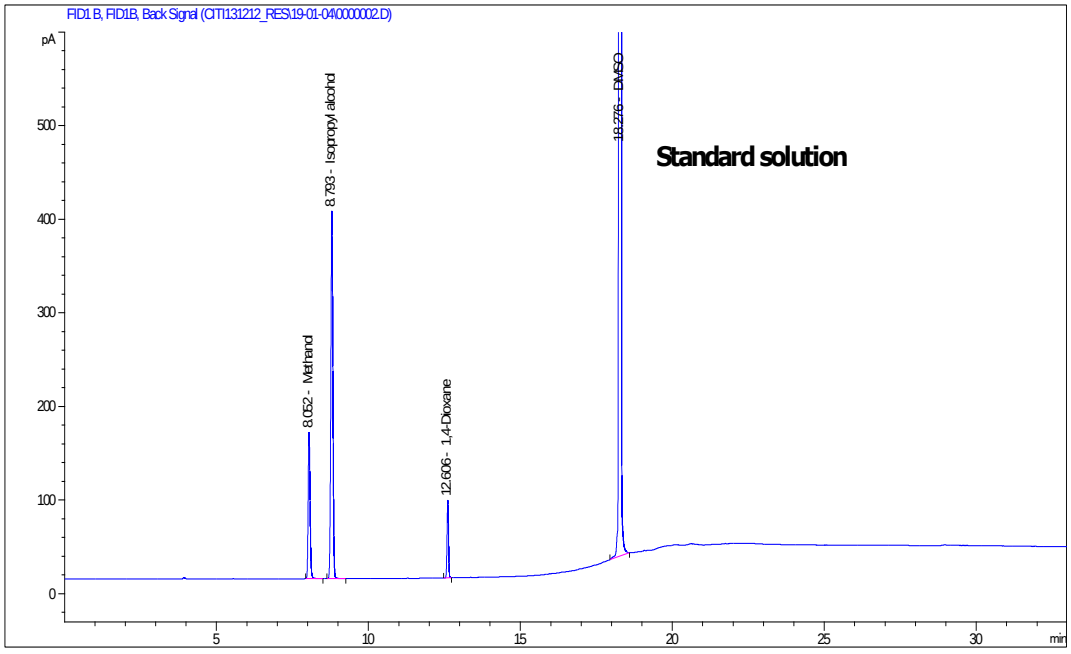

Fig 2 .System Suitability Chromatogram

Table 1. System suitability of Methanol and Isopropyl alcohol

Injection number	**The RSD for area ratio of each solvent from six replicate injection**	
	Methanol	**Isopropyl alcohol**
1	2.490	6.816
2	2.479	6.770
3	2.462	6.735
4	2.481	6.772
5	2.473	6.726
6	2.487	6.784
Mean	2.479	6.767
SD	0.0101	0.0330
RSD	0.41	0.49
System suitability parameters		**Resolution**
Resolution between methanol and isopropyl alcohol		6.073
Resolution between isopropyl alcohol and 1,4-Dioxane		35.107
Resolution between 1,4-Dioxane and DMSO		63.580

It was observed from the data tabulated as above, that the system suitability parameters were passed (Table 1), Hence, it can be concluded that the system suitability parameter meets the requirement of method validation. i.e., individual peaks were separated well (Fig 2)

Specificity

Specificity is the ability of analytical method to assess unequivocally the analyte in the presence of specified impurities that may be expected to be present.Performed the specificity parameter of the method. Separately injected blank, methanol solution, Isopropyl alcohol solution, 1, 4-dioxane solution, sample solution and solution as a mixture into GC system. Recorded the retention time of blank and individual solutions. It was observed from the all chromatograms that all peaks were well separated from blank and also it was observed from the RT of DMSO was 18.280 min where there was no other peak interference (Fig 3).

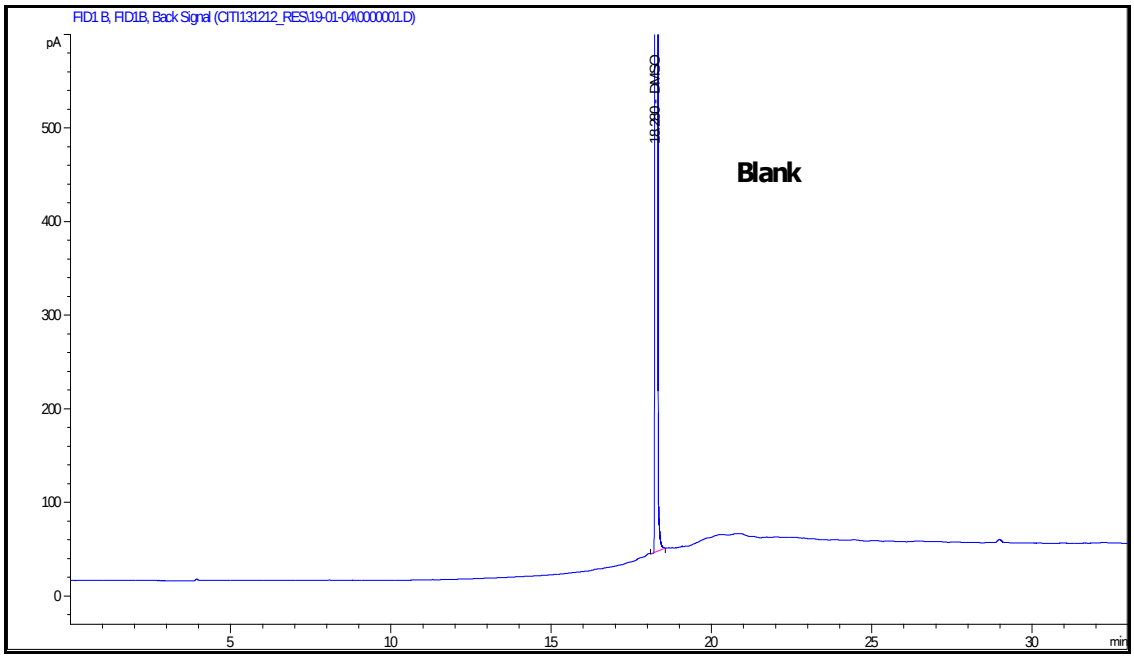

Fig 3. Blank Chromatogram –DMSO

Linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range.Prepared linearity solutions containing methanol, isopropyl alcohol at a concentration, which is equivalent to 1%, 2%, 5% 10%, 30%, 50%, 80%, 100%, 130% and 150% of the specified limits for each solvent.

Table 2. Linearity data of Methanol

Level in % w.r.to specification level	ppm of solvent	Trial 1			Trial 2			Mean area ratio
		Area of methanol (S)	Area of internal standard (I)	Area ratio (S/I)	Area of methanol (S)	Area of internal standard (I)	Area ratio (S/I)	
1	30.0	8.43277	272.35855	0.0310	8.20521	268.34433	0.0306	0.0308
2	60.0	14.65062	271.61954	0.0539	14.49613	273.79083	0.0529	0.0534
5	150.0	33.64177	269.33167	0.1249	32.47020	268.68512	0.1208	0.1229
10	300.0	70.05444	266.36819	0.2630	68.65221	267.93597	0.2562	0.2596
30	900.1	203.39034	268.55640	0.7573	201.74187	274.30679	0.7355	0.7464
50	1500.1	326.61472	265.88312	1.2284	334.64603	272.78406	1.2268	1.2276
80	2400.2	525.93799	271.04999	1.9404	516.63495	262.45743	1.9685	1.9545
100	3000.2	627.11249	246.68465	2.5422	617.12390	244.76625	2.5213	2.5318
130	3900.3	796.26929	245.87325	3.2385	792.91461	237.35899	3.3406	3.2896
150	4500.3	905.47046	239.90445	3.7743	895.60254	236.12703	3.7929	3.7836
					Slope			0.0008
					Intercept			-0.0065
					Correlation coefficient			0.9999
					Regression coefficient			0.9997

Fig 4. Linearity graph of Methanol

Table 3. Linearity table of Isopropyl alcohol

Linearity of isopropyl alcohol								
Level in % w.r.to specification level	ppm of solvent	Trial 1			Trial 2			Mean area ratio
		Area of Isopropyl alcohol (S)	Area of internal standard (I)	Area ratio (S/I)	Area of Isopropyl alcohol (S)	Area of internal standard (I)	Area ratio (S/I)	
1	50.0	19.00286	272.35855	0.0698	18.61801	268.34433	0.0694	0.0696
2	100.0	36.66585	271.61954	0.1350	36.92945	273.79083	0.1349	0.1350
5	250.1	91.07571	269.33167	0.3382	89.55715	268.68512	0.3333	0.3358
10	500.2	192.13466	266.36819	0.7213	184.00923	267.93597	0.6868	0.7041
30	1500.6	558.82153	268.55640	2.0808	554.18359	274.30679	2.0203	2.0506
50	2501.0	891.31683	265.88312	3.3523	915.59827	272.78406	3.3565	3.3544
80	4001.7	1438.94092	271.04999	5.3088	1404.12573	262.45743	5.3499	5.3294
100	5002.1	1693.37988	246.68465	6.8646	1677.46240	244.76625	6.8533	6.8590
130	6502.7	2152.46411	245.87325	8.7544	2131.30835	237.35899	8.9793	8.8669
150	7503.1	2446.16772	239.90445	10.1964	2420.86743	236.12703	10.2524	10.2244
					Slope			0.0014
					Intercept			-0.0080
					Correlation coefficient			0.9999
					Regression coefficient			0.9999

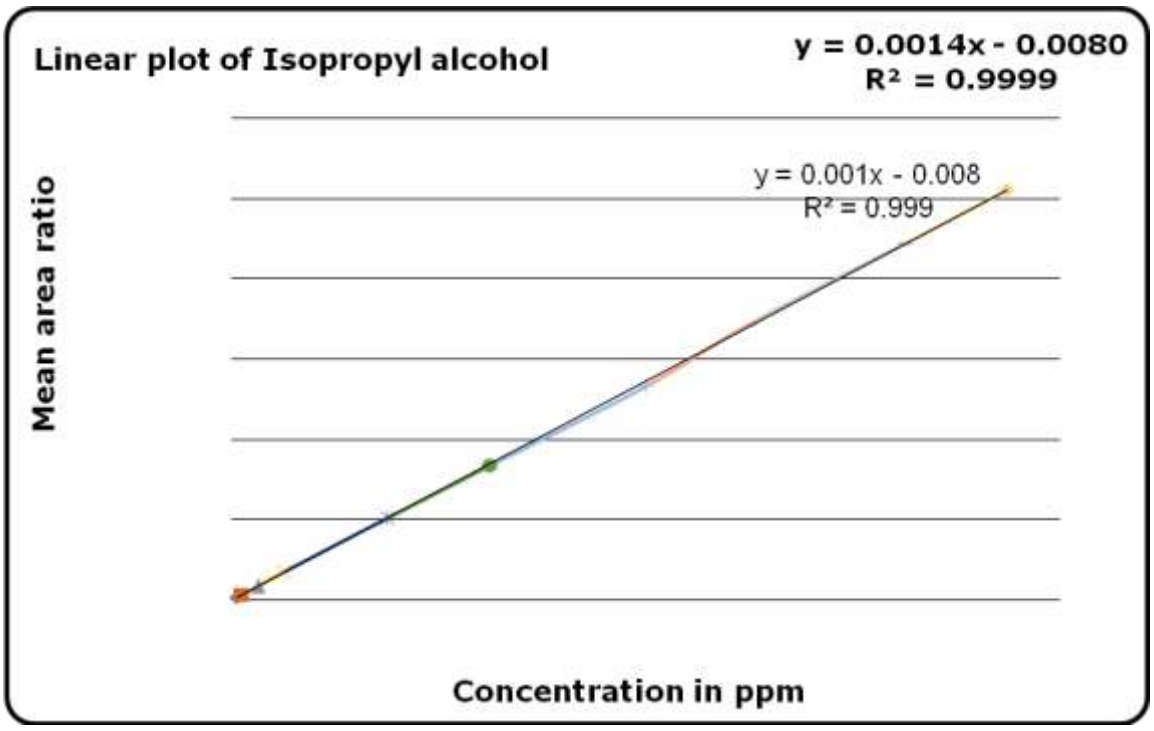

Fig 5.Linearity graph of Isopropyl alcohol.

The correlation coefficient and the regression coefficient between concentration and mean area ratio of each solvent is greater than acceptance criteria of not less than 0.995 in the linear range (Fig 4 & 5).The RSD for area ratio of each solvent obtained from six replicate injections at 100% of the specification level is less than 15.0%. This confirms that the method is linear (Table 2 & 3).

LOD/LOQ.

Limit of detection is the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions.Limit of quantitation is the lowest amount of analyte in a sample that can be quantitated with acceptable precision, under the stated experimental conditions.Calculated the slope and residual standard deviation from the linearity data and calculated the limit of detection and quantitation from the intercept, slope and residual standard deviation.To confirm the LOQ calculated theoretically, prepared standard solution at theoretical LOQ level concentration and injected into the chromatograph in six replicates. Calculated the RSD for the area ratio of each solvent peak obtained from six replicate injections of LOQ solution (Table 4).

Table 4. RSD data at LOQ level

Injection number	**% RSD for standard at LOQ level**	
	Methanol	**Isopropyl alcohol**
1	0.085	0.153
2	0.082	0.149
3	0.091	0.158
4	0.083	0.148
5	0.085	0.153
6	0.084	0.154
Mean	0.085	0.153
SD	0.0032	0.0036
RSD	3.72	2.37
LOQ in ppm	104.9	125.1
LOD in ppm	22.3	45.2

Accuracy

The accuracy of an analytical method is the closeness of test results obtained by the method to the true value. Analyzed the sample as per the specified method. The solvent contents are as follows.

Spiked the sample with each solvents applicable upto 50%, 80%, 100% and 130% of the specification limits. Analyzed each sample in triplicate. Calculated each solvent in the spiked sample. Calculated the %recovery of each solvent in the spiked samples. Calculated the RSD for % recovery of each solvent from 12 determinations (4 levels X 3). Performed the accuracy at LOQ level also (Table 5 & 6).

Table 5. Method Accuracy-Methanol

Method accuracy -Methanol [4 levels X 3 = 12 determinations]					
Sl. No.	**Level in % w.r.to specification level**	**% Recovery**	**Mean% Recovery**	**SD**	**% RSD**
1	50	94.60	94.27	0.8769	0.93
2		94.94			
3		93.28			
4	80	94.71	94.00	0.6160	0.66
5		93.72			
6		93.58			
7	100	87.38	87.63	0.5714	0.65
8		87.22			
9		88.28			
10	130	87.66	88.34	0.6219	0.70
11		88.88			
12		88.48			
Mean		91.06			
Standard deviation		3.2787			
%RSD		3.60			

Table 6. Method Accuracy- Iso Propyl Alcohol

Method accuracy –Isopropyl alcohol [4 levels X 3 = 12 determinations]					
Sl.No.	**Level in % w.r.to specification level**	**% Recovery**	**Mean% Recovery**	**SD**	**% RSD**
1	50	99.30	98.39	1.0696	1.09
2		98.65			
3		97.21			
4	80	100.18	99.86	0.3530	0.35
5		99.48			
6		99.91			
7	100	99.85	99.93	0.3372	0.34
8		100.30			
9		99.64			
10	130	100.55	100.57	0.2706	0.27
11		100.31			
12		100.85			
Mean		99.69			
Standard deviation		0.9810			
%RSD		0.98			

Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample. The precision of analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of series of measurement.The system precision is checked by using standard chemical substance i.e. solvent to ensure that the analytical system is working properly. Prepared standard solution at a concentration equivalent 100% with respect to specification limit for each solvent. Injected each solution in six replicates. Recorded the chromatograms. Calculated the RSD for retention time and area ratio of the each solvent peak area to internal standard peak area at each concentration.The RSD for retention time at 100 of the specification limit for each solvent peak should be NMT 1.0%.The RSD for area ratio of each solvent peak area to internal standard peak area at 100% of the specification limit should be NMT 15.0%.

Table 7.System Precision – Methanol & Isopropyl alcohol

Injection number	**System precision at 100% specification level**			
	Methanol		**Isopropyl alcohol**	
	Retention time (in minutes)	Area ratio	Retention time (in minutes)	Area ratio
1	8.032	2.542	8.766	6.865
2	8.033	2.521	8.767	6.853
3	8.032	2.541	8.767	6.889
4	8.033	2.533	8.767	6.861
5	8.032	2.512	8.766	6.812
6	8.032	2.523	8.766	6.854
Mean	8.032	2.529	8.767	6.856
SD	0.0005	0.0120	0.0005	0.0251
%RSD	0.01	0.47	0.01	0.37

It is observed from the data tabulated above, that the retention time and area ratio of the individual solvent peak area to internal standard peak area of each solvent is consistent. Hence, it is concluded that the system precision parameter meets the requirements of method validation (Table 7).

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Spiked the sample with each solvent upto 100% of specification limit. Analyzed the sample by changing different method parameters. Reported the system suitability criteria and the % recovery of each solvent peak in sample solution. Flow rate was changed ±0.2 mL/minute of the specified method and Change of temperature±5°C of the specified method.It was observed that System suitability criteriacomplies for all parameters and the % recovery of each solvent were observed to be 80 - 120.0% in all conditions.

Conclusion

The analytical method validation for the estimation of OVI in Citicoline sodium was carried out as per analytical method validation protocol which is in line with ICH guidelines. The method was found to be specific for the estimation of (OVI) in Citicoline sodium. The method was found to be linear in the specified range for all (OVI). Accuracy of the method was established. The method was found to be precise and robust. A system suitability test was established and recorded. Hence, this method stands validated and can be used for routine sample analysis.

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Conflict of Interest. There are no conflict of interest

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