WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 10, 864-878. Research Article ISSN 2277– 7105

GENERIC APPROACH FOR LOW LEVEL DETERMINATION OF 2-HYDROXY ETHYL HYDRAZINE IN PHARMACEUTICAL INGREDIENTS BY GC-MS USING CHEMICAL DERIVATIZATION

Pavani Jagu*, M.A. Krishna Kumar, R. Navaneeswari

Analytical Research, Neuland Pharma Research Pvt Ltd, Jinnaram, Medak Dist. 502 313, (A.P), INDIA.

Article Received on
21 September 2014,

Revised on 17 Oct 2014,
Accepted on 11 Nov 2014

***Correspondence for Author**
Pavani Jagu
Analytical Research, Neuland Pharma Research Pvt Ltd, Jinnaram, Medak Dist. 502 313, (A.P), INDIA.

ABSTRACT

2-Hydroxy Ethyl Hydrazine (2-Hydrazinyl Ethanol) is being recognized as potential genotoxic impurity (PGI). A sensitive and generic GC-MS method was developed and validated for the determination of 2-Hydroxy Ethyl Hydrazine in Pharmaceutical compounds. The method is based on the derivatisation of 2-Hydroxy Ethyl Hydrazine with Benzaldehyde to form *N*-benzylidene-2-phenyloxazolidin-3-amine. GC-MS method employing Agilent 1701 capillary column (30 m × 0.32 mm; 0.25µm), with Electron Impact Ionisation (EI) in Selected Ion Recording (SIR) mode was employed. The proposed method was specific, linear, accurate, rugged and precise. The calibration curve showed good linearity over the concentration range of 0.02 µg/mL to 0.3 µg/mL and the correlation Coefficient was greater than 0.999. Method had very low Limit of detection (LOD) and Limit of quantification (LOQ) as 0.006 µg/mL and 0.02 µg/mL respectively. Accuracy was observed within 80% to 120%. The validated method yielded good results of selectivity, linearity, precision and accuracy and this method can be further extended as a good quality control tool for low level quantitation of 2-Hydroxy Ethyl Hydrazine impurity in API's. API's or any Pharmaceutical product.

KEYWORDS: 2-Hydroxy Ethyl Hydrazine, Benzaldehyde, GC-MS, genotoxic impurity, trace analysis, Active pharmaceutical ingredient.

INTRODUCTION

According to the current regulatory guidance, it is assumed that genotoxic compounds have the potential to damage DNA at very low level of exposure. Thus, actual and potential impurities most likely to arise during synthesis, purification and storage should be identified. These potential genotoxic impurities (PGIs) are known to induce genetic mutations or chromosomal aberrations and are reported as known carcinogens in rats and mice. [1] The potential presence of these genotoxins has attracted the attention of regulatory authorities. European Medicines Agency's (EMEA) Committee for Medicinal products for Human use (CHMP) has published guidelines regarding limits of genotoxic impurities. [2] In 2008, US FDA has also come up with the draft guidelines on genotoxic and carcinogenic impurities in drug substances and products. [3] These guidelines describe ways to reduce the potential lifetime cancer risk associated with patient exposure to genotoxic and carcinogenic impurities and the ways to reduce them. Both the EMEA guidelines and a PhRMA white paper propose a maximum daily exposure target of 1.5 µg per day genotoxic impurities in pharmaceuticals [acceptable Threshold of Toxicological Concern (TTC)] is recommended in these guidelines [1, 2, 3] 2-Hydroxy Ethyl Hydrazine (2-Hydrazinyl ethanol), is a colorless, slightly viscous liquid often used as Key starting material for most of the pharmaceutical products and have been highlighted as potential genotoxic impurity (PGI). 2-Hydrazinyl Ethanol is commonly used as intermediate for organic synthesis especially heterocycles used in agrochemicals, pharmaceuticals, stabilizers and polymerizations. Hydrazine is a highly reactive molecule widely used in chemical synthesis of intermediates and active pharmaceutical ingredients a known carcinogen can have an impact on product risk assessment if present in the final drug substance and drug product as an impurity.

Hydrazines have been found at various Department of Defense hazardous waste sites. They are designated as environmental contaminants causing adverse effects to public health and have been identified at many National Priorities List (NPL) hazardous waste sites and federal facilities sites in the United States. [4, 5]

Both ammonia and amines are nitrogen nucleophiles which donate electrons (Lewis bases). But hydrazine monohydrate or 2-Hydrazinyl Ethanol (diamine) has much stronger nucleophilicity which makes them more reactive than ammonia. 2-Hydrazinyl Ethanol is dibasic and has very reactive properties. Hydrazinyl ethanol is used as a reducing agent for the recovery of precious metals. It is used as a derivative and thereof, is versatile

intermediate. It has active applications in organic synthesis for agrochemicals, pharmaceuticals, photographic, heat stabilizers, polymerization catalysts, flame-retardants, blowing agents for plastics, explosives, dyes etc.

Various analytical techniques are reported for the determination of Hydrazine. [6-7] A few HPLC, GC and GC-MS analytical methods have been reported for the quantification of Hydrazine monohydrate by derivatisation methods. [8-10] But, to the best of our knowledge, no method for the quantitative estimation of 2-Hydrazinyl Ethanol in drug substances, and in pharmaceutical dosage forms has been reported. The present research work is mainly focused to develop a rapid, sensitive, and accurate method for the determination of 2-Hydrazinyl Ethanol in Pharmaceutical Products. Considering the allowable limit of 1.5 µg per day of Hydrazinyl ethanol, the concentraton limit (ppm) of Hydrazinyl ethanol varies for different Pharmaceutical products based on the daily dose of the drug substance (g/day). The developed method was validated with respect to specificity, LOD, LOQ, linearity, precision, accuracy and solution stability. All these studies were performed in accordance with established ICH guidelines.

EXPERIMENTAL

Chemicals

GC grade Dichloromethane was purchased from Merck, Darmstadt, Germany and 2-Hydrazinyl Ethanol and Benzaldehyde were purchased from Sigma Aldrich (Mumbai, India).

Instrumentation

PerkinElmer Clarus Gas chromatograph coupled with Clarus 600C single quadrupole Mass spectrometer was used for the analysis. The chromatographic data was recorded using Dell (Round Rock, TX, United States of America) computer with Turbo mass software 5.1.0 to collect and process the data. A Precisa 321; LS120A analytical balance (precision 0.1 mg, Switzerland) was used for weighing of the standard, samples etc.

GC-MS conditions

The Chromatographic separation was achieved on HP-1701 fused silica capillary column, of dimensions ; 30 m length x 0.32 mm internal diameter x 0.25 µm film thickness; J &W Scientific, Folsom ,CA, USA. The GC and MS parameters are summarized in Table 1.

Preparation of 2-Hydrazinyl Ethanol **stock solution**

Stock solution of 2-Hydrazinyl Ethanol was prepared at a concentration of 2.5 mg/mL, by adding about 25 mg of Benzaldehyde (density: 1.123 g/mL) for each mg of 2-Hydrazinyl Ethanol; heated at about 100 ℃ for 30 min in a water bath. Cooled to room temperature, dissolved in 5 mL of Dichloromethane (diluent) by cyclomix for 2 min and made up to the volume with diluent. The working solutions of 2-Hydrazinyl Ethanol were prepared by diluting at the appropriate concentrations with the diluent.

Preparation of blank solution

Prepared by the same procedure as 2-Hydrazinyl Ethanol stock solution preparation, but without 2-Hydrazinyl Ethanol.

Preparation of drug/test sample solution

The test sample was prepared at a concentration of 10 mg/mL, by adding Benzaldehyde in the ratio of 0.01 mL to each mg of test sample (considering each mg of the drug substance would contain 2-Hydrazinyl Ethanol at ppm level) ; heated at about 100 ℃ for 30 min in a water bath. Cooled to room temperature, dissolved in 5 mL of diluent by cyclomix for 2 min and made up to the mark with diluent. Filtered the solution before injecting into GC-MS.

Method Validation

The described GC-MS method has been extensively validated for the quantification of 2-Hydrazinyl Ethanol as per ICH guidelines. [11]

System Suitability

The system suitability test is an integral part of the chromatographic methods and is used to verify whether the reproducibility of the chromatographic system is adequate for the analysis to be performed. The derivatised 2-Hydrazinyl Ethanol at 100 ppm concentration was injected into GC-MS in both Scan and SIR mode and recorded the prominent fragment ion for derivatised product in SIR mode. The relative standard deviation of the peak areas and retention times at 10 ppm in SIR mode was recorded. The system suitability test results of the GC-MS method on the HP-1701 column are precised in Table 2.

Specificity

Specificity is the ability to unequivocally assess the analyte in the presence of its potential impurities. [12] Which may be expected to be present like impurities, degradants, matrix,

etc.[13] The retention times of 2-Hydrazinyl Ethanol derivative in the standard solution were compared with the ones in the sample solution. Moreover, the blank diluent solution was injected to see whether there were any interferences at the 2-Hydrazinyl Ethanol derivative retention time.

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

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

The LOD and LOQ of 2-Hydrazinyl Ethanol were determined based on the Standard Deviation of the Response and the Slope as indicated in Table 3 (ICH Q2 (R1)).

A specific calibration curve was studied for 2-Hydrazinyl Ethanol in the range of detection limit. The standard deviation of y-intercepts of regression lines was used as Sigma (σ). A series of dilute solutions with known concentrations were injected to generate a calibration curve in the range of 5 ppm to 30 ppm and the LOD and LOQ concentrations were determined based on the specified formulae (Table-3.). The results of the LOD and LOQ are computed in Table-4. The precision of this method at LOQ concentration was checked by analyzing six individual preparations of 2-Hydrazinyl Ethanol at the LOQ level and calculating the percentage RSD for the area of 2-Hydrazinyl Ethanol. Accuracy at the LOQ level was also carried out by preparing three recovery solutions of test sample spiked with 2-Hydrazinyl Ethanol at the LOQ level and calculating the percentage recovery for content of 2-Hydrazinyl Ethanol.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of the analyte in the sample. The linearity evaluation was performed by diluting the standard solution of derivatised 2-Hydrazinyl Ethanol at the concentrations of LOQ, 50%, 75%, 100%, 125%, 150% and 200% levels with respect to target analyte concentration. The peak areas responses of 2-Hydrazinyl Ethanol derivative were plotted against the corresponding concentration and the linear

regression equation was computed. The correlation coefficient of regression line, slope, and intercept and % y-intercept of the calibration curve were computed in Table-4.

Accuracy

Standard addition and recovery experiments were conducted to determine the accuracy of the method for the quantitation study for 2-Hydrazinyl Ethanol in the developed analytical method The accuracy was evaluated in triplicate by recovery study of impurity spiked at LOQ level and at 50%, 100% and 150% of target analyte concentration with respect to the specification level. The recovery experiments were conducted by adding underivatised 2-Hydrazinyl Ethanol and Benzaldehyde at different levels and maintained at specified conditions. The percentage recovery for 2-Hydrazinyl Ethanol was calculated, considering the amount of impurity spiked, amount of impurity available in un-spiked sample and amount of impurity recovered.

Solution stability

Stability of sample solution spiked with 2-Hydrazinyl Ethanol at specification level (18 ppm) was studied by keeping the solution in tightly capped volumetric flask at room temperature on a laboratory bench for 24 h. Content 2-Hydrazinyl Ethanol was checked at initial and at every 6 h interval up to the study period. The 2-Hydrazinyl Ethanol content and % variation of content at 6h, 12h, 18h and 24h with respect to the initial content of 2-Hydrazinyl Ethanol was calculated.

Precision

The precision of an analytical procedure expresses the closeness of an agreement among series of sample measurements obtained from multiple samplings of the same homogeneous sample under prescribed conditions. Method precision was determined by measuring the repeatability (intra-day precision) and intermediate precision was determined by measuring the reproducibility (inter-day precision) of retention times and peak areas for 2-Hydrazinyl Ethanol derivative in the test sample. The intra-day variability was performed by injecting six separate preparations of 2-Hydrazinyl Ethanol by same analyst over a day, while inter-day precision (rugeddness) was carried out similarly, by another independent analyst on a column from different lot over 3 days. The relative standard deviation of intra-day and inter-day repeatability experiments for 2-Hydrazinyl Ethanol content in the spiked test sample is evaluated to ascertain the ruggedness of the method.

RESULTS AND DISCUSSION

Method development and optimization

If an impurity that is present at levels below the ICH qualification thresholds is identified, the impurity should be evaluated for genotoxicity and carcinogenicity based on structural activity relationship (SAR) assessments (i.e., whether there is a structural alert). This evaluation can be conducted via a review of the available literature or through a computational toxicology or by Ames test etc. [14] If a (potentially) genotoxic impurity is formed or introduced in a step before the final synthesis step, it is considered possible to not include this impurity in the drug substance specification if it is controlled by a suitable limit in a synthesis intermediate and if it is unambiguously demonstrated by analysis results (use of spiking experiments are encouraged) that presence of this impurity does not exceed 30 % of the limit, derived either from TTC or otherwise defined acceptable limit etc, in the drug substance. [15]

The objective of this work was to evaluate the 2-Hydrazinyl Ethanol content for accurate quantification at low level (ppm) in the drug substance or any release test samples. Many methods have been reported in the literature for the low level quantification of hydrazine monohydrate using derivatisation techniques. Initially HPLC and Ion chromatographic methods were tried using derivatisation using 4-dimethylaminobenzaldehyde. However, the detection at low level was very difficult and no reproducible results were achieved. Published GC and GC-MS methods are available for the quantification of Hydrazine monohydrate, where acetone is generally used as a derivatisation technique.

2-Hydrazinyl Ethanol is a small, polar, basic molecule with no chromophore. Due to its high reactive nature, derivatisation to produce a stable product is the appropriate method for the analysis and quantification of 2-Hydrazinyl Ethanol; which leads to a stable behaviour in GC column. As the polarity of 2-Hydrazinyl Ethanol is reduced by derivatisation, a non-polar HP-5 MS column was initially chosen. The preliminary trails were carried out with the similar approach of using acetone as derivatising reagent for 2-Hydrazinyl Ethanol, where reproducible results were not achieved. Then, based on the literature, as hydrazinyl ethanol found to react with aldehydes to produce stable products; Benzaldehyde was chosen as the derivatising agent. The typical derivatisation reaction is represented in the Fig. 1.

Column selection and separation

Different GC stationary phases were employed during the method development namely HP-5, DB-WAX, DB-624 etc and HP-1701 of dimensions (30 m x 0.320 mm x 0.25 µm) column

was found to be give good separation between 2-Hydrazinyl Ethanol, residual Benzaldehyde and derivatised product peak.

Optimization of Sample preparation

Benzaldehyde is used as the derivatising reagent, instead of acetone and different mole ratios or volumes have been tried to attain 100% conversion of Benzaldehyde to *N*-benzylidene-2-Phenyloxazolidin-3-amine. Sample preparation is an important part of the GTI (Genotoxic Impurity) analysis, because matrix effects in trace analysis are magnified, causing loss of sensitivity, abnormal recovery and analyte instability. Different diluents were evaluated with respect to extraction efficiency and chromatography. Solubility of analyte and proper extraction was good in Dichloromethane. The analyte peak in the final method has a quite symmetric peak shape with optimum retention time. The detailed derivatisation reaction is illustrated in Fig 1. Benzaldehyde reacts with 2-hydrzinyl ethanol, in the ratio of 2:1, to produce *N*-benzylidene-2-phenyloxazolidin-3-amine under hot condition. However, about five times excess Benzaldehyde is used to avoid the formation of monomer as by-product and to ensure the 100% conversion to dimer product. No medium was employed for the dissolution of 2-Hydrazinyl Ethanol, where Benzaldehyde is added to the test sample directly, to avoid the formation of any other by products. The time required for complete derivatisation and the stability of the product formed were determined by measuring the GLC response due to product as a function of the time during which the derivatisation was allowed to proceed. The derivatisation was found to complete within 30 min at about 100 °C and the product is found to be stable for 24 h. Neither residual benzaldehyde nor diluent peak interfered with the derivatised during monitoring.

Selection of mass conditions

The predominant fragment ions identified from full scan of 2-Hydrazinyl Ethanol (derivative) are m/z = 51.0, 77.0, 90.0, 118.0, 175.0 and 252.1. For the quantification of 2-Hydrazinyl Ethanol 90.0, 175.0 and 252.1 were selected. The abundance ratios of the three fragment ions in SIR mode were utilized for the quantification of 2-Hydrazinyl Ethanol. The full scan Total Ion Chromatogram is represented in Fig. 2.

Optimized Chromatographic Conditions

Choosing a detection method is the most important part of pharmaceutical analysis. From the Instrument simplicity, stability and accessibility point of view HPLC-UV, Ion chromatography and GC-FID techniques by derivatisation procedure were first evaluated.

However, on these techniques sufficient sensitivity for the trace level analysis of 2-Hyadrazinyl ethanol was not achieved. In view of this, sensitive and specific mass spectrometric detection of Electron Impact ionisation (GC-MS-EI) was evaluated in SIR mode. The Chromatographic separation was achieved on HP-1701 of dimensions (30 m x 0.320 mm x 0.25 µm), maintained at oven program, 130 °C (0 min hold) to 280 °C (3 min hold) @ 50°C/min. Flow rate was 1.2 mL/min and injection volume was 2µL and split ratio:5:1.

METHOD VALIDATION

Precision: The precision of an analytical procedure expresses the closeness of agreement among a series of measurements obtained from multiple samplings of the same homogenous sample under prescribed conditions. The %RSD of the area of 2-Hydrazinyl Ethanol in method precision study was 1.13% conforming good precision of the method. Also, the individual values fall well within the range of the confidence interval of the average, confirming the precision of the method. The %RSD values are reported in Table 4.

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

The limit of detection of 2-Hydrazinyl Ethanol was achieved as 0.7 ppm (with respect to the target analyte concentration) for the 2µL injection volume (Fig. 3). The limit of quantification of 2-Hydrazinyl Ethanol was achieved as 2.7 ppm (with respect to the target analyte concentration) for the 2µL injection volume (Fig. 3). The %RSD of the impurity content at the LOQ level was less than 7.0 % and the recovery values at the LOQ level were from 96.1 to 100.3. The LOD & LOQ values of 2-hydraziyl ethanol, the precision at the LOQ level and the accuracy results at the LOQ level are tabulated in Table 4.

Linearity

Linear calibration plot for the method was obtained over the calibration ranges tested i.e. LOQ to 200% for 2- Hydrazinyl ethanol. The correlation coefficient was greater than 0.999. The values of the slope, intercept and %Y-intercept of the calibration curves were determined and the results showed that an excellent correlation existed between the peak areas and the concentrations of 2-Hydrazinyl Ethanol. The results are computed in Table 4.

Accuracy

The percentage recovery of 2-Hydrazinyl Ethanol in the tested bulk drug samples at LOQ,50,100 and 150 % levels ranged from 97.2 – 102.87 %. (Table 4, Fig 4). All the

individual recoveries were well within the confidence interval of the mean values. Good recovery values were obtained indicating excellent capability of the method's accuracy.

Solution stability

No significant changes were experienced in the content of the impurity during the solution stability experiments. The accuracy of the content of impurity after 24h against the initial value is 98.3%.The solution stability experimental data confirmed that the sample solution used was stable up to 24 h.

Table. 1. GC and MS chromatographic conditions.

GC Chromatographic conditions		
Column	:	HP-1701 (30 m x 0.320 mm x 0.25 µm)
Temperature program	:	130 °C (0 min hold) to 280 °C (3 min hold) @ 50 °C
Injector temperature	:	230°C
Detector temperature	:	280°C
Diluent	:	Dichloromethane
Carrier gas	:	1.2 mL/min (Helium) ;constant flow
Split ratio	:	5:1
Injection Volume	:	2.0 µL
Run time	:	6.0 min
Typical Mass Parameters		
Ion Source	:	Electron impact ionization (EI) mode
Ionization energy	:	70 ev
Tune file	:	Atune
Acquisition mode	:	Selected Ion Recording (SIR)
Ion Source temperature	:	230°C
Quadrupole temperature	:	150°C
Solvent delay	:	2.2 min
Full scan range	:	25 to 500 m/z
Selected Ion Recording (SIR) Parameters		
Resolution/ Ions/dwell time	:	Low/ 252.1 , 175.0, 90.0 (200)

Table. 2. Results of system suitability test.

Scan mode fragments (m/z)	**Ions selected (m/z)**	**Retention time**	**Area**
		%RSD	
252.1		0.03	0.2
175.0	90.0		
118.0	175.0		
90.0	252.1		
77.0			

Table 3. LOD and LOQ calculation formulae.

Limit of detection	=	$\frac{3.3 \times \text{Sigma } (\sigma)}{\text{Slope of the calibration curve}}$
Limit of quantitation	=	$\frac{10 \times \text{Sigma } (\sigma)}{\text{Slope of the calibration curve}}$

Sigma (σ): The standard deviation of y-intercepts of regression lines

Table.4. Method validations Results of Hydroxy ethyl Hydrazine (or) 2-Hydrazinyl Ethanol.

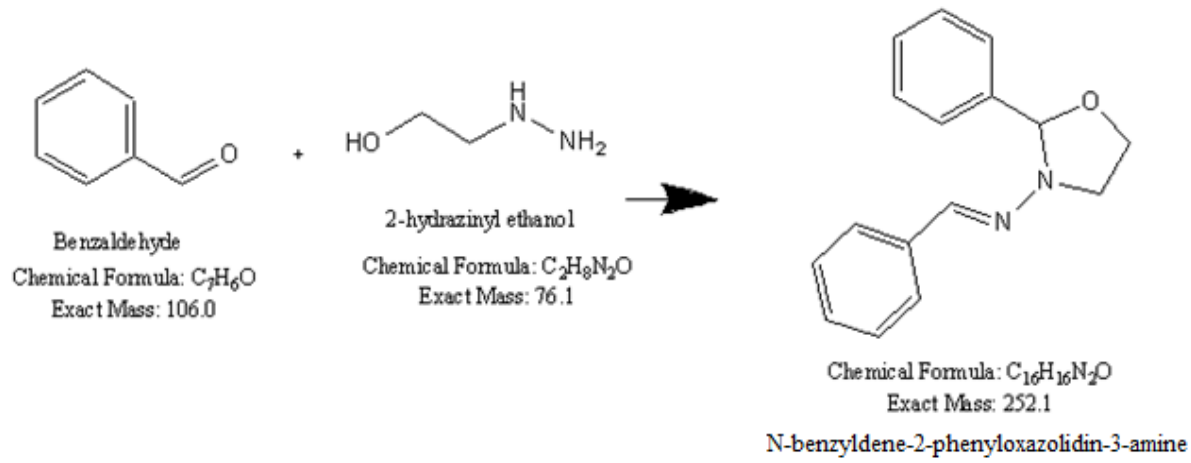
Parameter	**2-Hydrazinyl Ethanol**
LOD (µg/mL)	0.006
LOQ (µg/mL)	0.02
Precision (%RSD)[b]	1.3
Precision at LOQ (%RSD)[b]	6.3
Accuracy (%recovery)[c]	
LOQ	97.2
50%	98.02
100%	99.47
150%	102.87
Linearity[a]	
Correlation coefficient (R)	0.9997
Slope (m)	2187.6
Intercept (C)	1486.35351
% Y-Intercept	-3.93

a = Linearity range was from LOQ to 200 % of 2-Hydrazinyl Ethanol

b (n=6)

c (n=3)

Fig 1. Derivatisation reaction of 2-Hydrazinyl Ethanol.



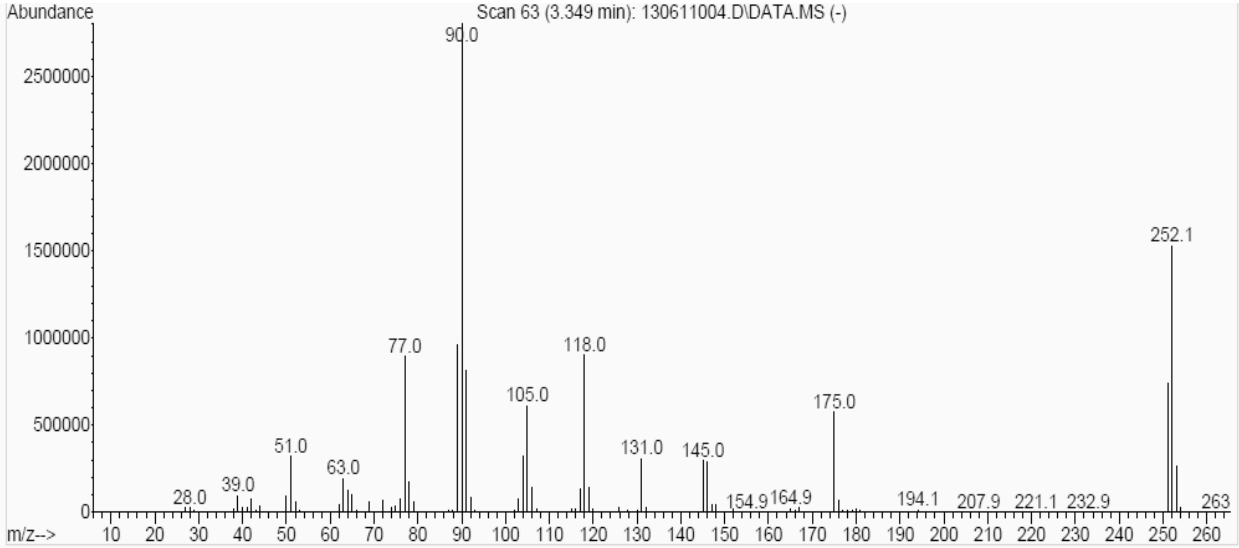


Fig 2. Typical fragmentation pattern of 2-Hydrazinyl Ethanol derivative in Scan mode.

a) LOD

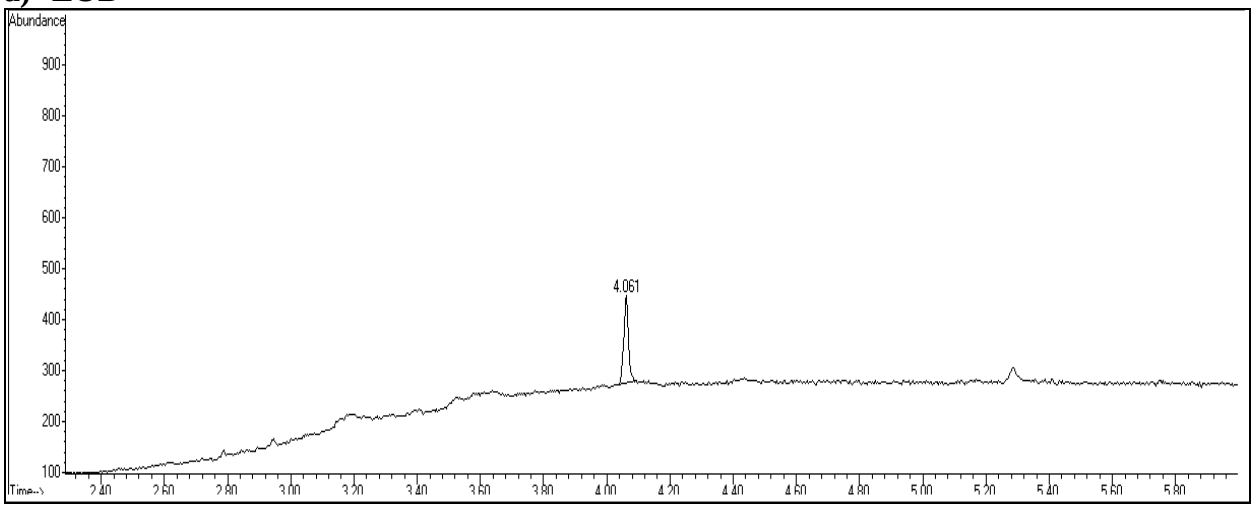
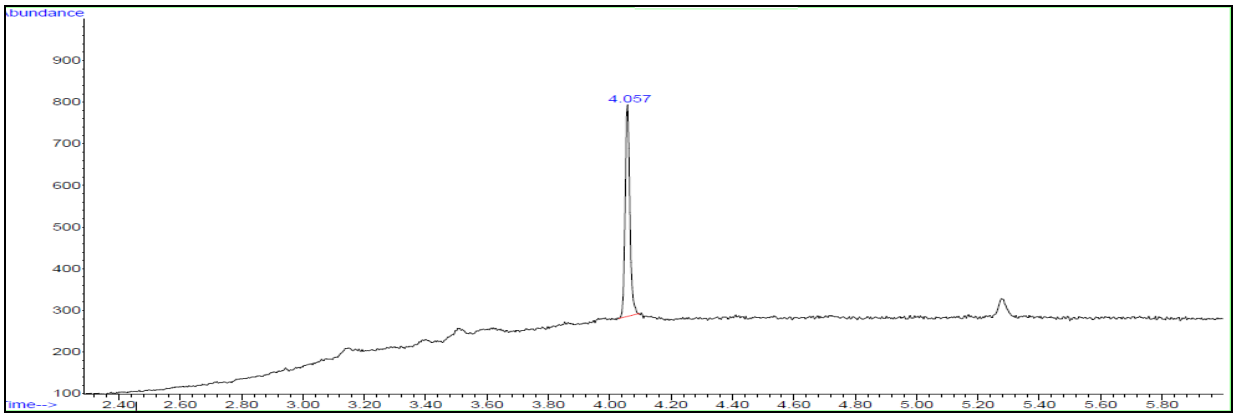


Fig. 3. : Typical chromatograms of 2-Hydrazinyl ethanol (derivative).

b) LOQ



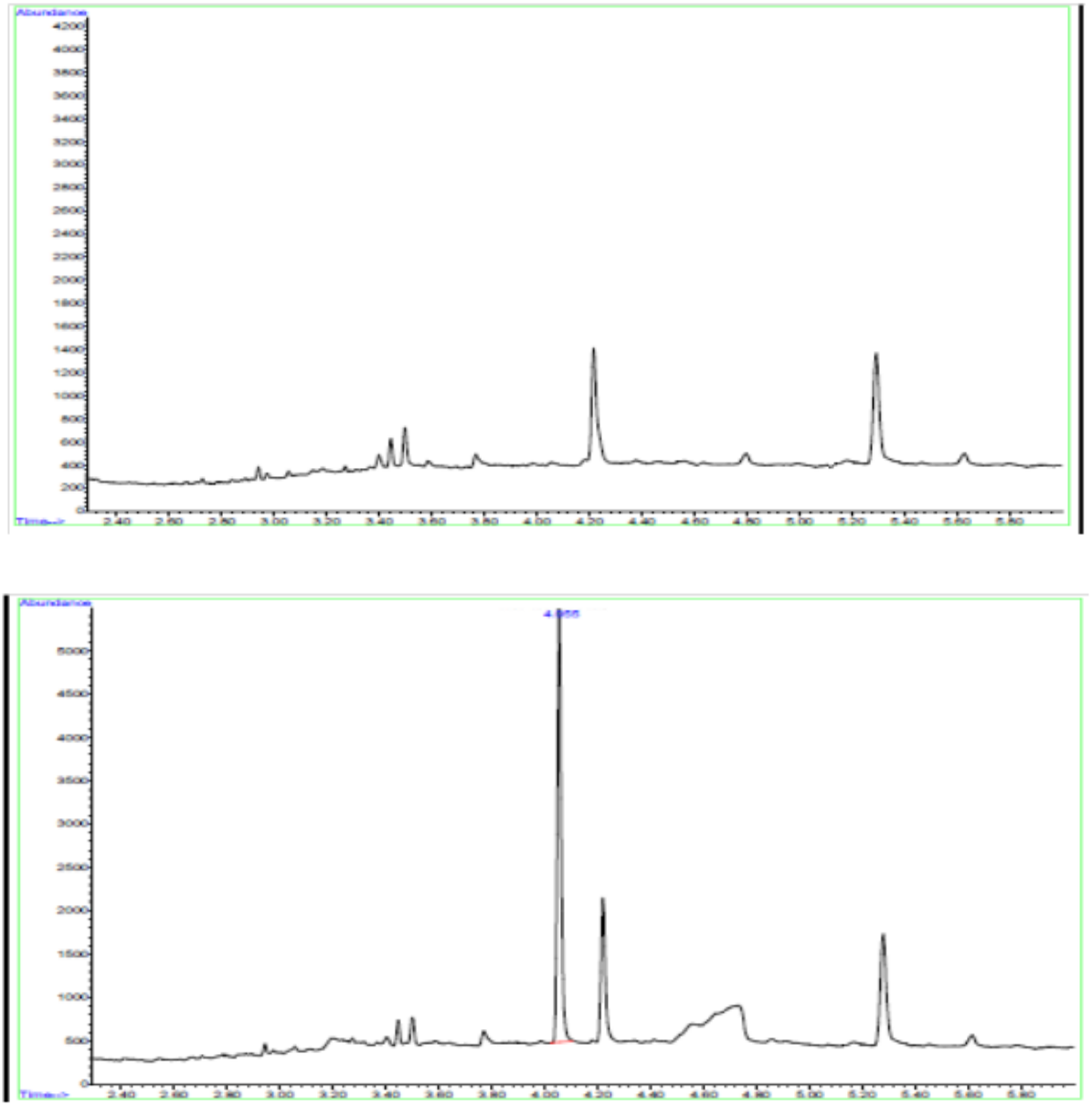


Fig.4. Typical chromatograms of test sample and 2-Hydrazinyl Ethanol (derivative) recovery.

CONCLUSION

This study describes a trace-level method for the determination of 2-Hydrazinyl Ethanol, a potential genotoxic impurity; in bulk drug samples. The described analytical method is cost effective, direct, accurate and convenient quality control tool for determination of 2-Hydrazinyl Ethanol in bulk drug samples. The advantage of this method lies in its improved sensitivity, shorter runtime and simpler sample preparation technique. The method was validated to the requirements of ICH guidelines and based on the experimental data on the various method validation parameters; it is proved that this method which was designed to determine 2-Hydrazinyl Ethanol content by GC-EI-MS in SIR mode is specific, linear,

precise and accurate. This method can be further studied for its application to various drug substances.

ACKNOWLEDGMENT

The authors wish to thank the management of Neuland Pharma Research Pvt Ltd. for supporting to carry out this work. The authors also wish to acknowledge the support from the process research group, especially Dr. Kashyap Wadekar and Mr. N. Nageshwara Rao and thank the colleagues in the separation science division of Analytical Research for their cooperation in carrying out this work.

REFERENCES

1. Guideline on the Limits of Genotoxic Impurities, Committee for Medicinal Products for Human Use (CHMP). European Medicines Agency (EMEA), London, 2006; 28: (CPMP/SWP/5199/02, EMEA/CHMP/QWP/251344/2006).
2. Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches; U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER). Silver Spring, MD, U.S.A, December 2008.
3. R. Kroes, A.G. Renwick, M. Cheeseman, J. Kleiner, I. Mangelsdorf, A. Piersma, B. Schilter J, Sclatter, F. Van Schothorst, J.G. Vos, G. Wurtzen, Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet. Food Chem. Toxicol, 2004; 42: 65-83.
4. Gangadhar Choudhary, Hugh Hansen. Human health perspective of environmental exposure to hydrazines: A review Chemosphere, August 1998; 37(5): 801–843. http://dx.doi.org/10.1016/S0045-6535(98)00088-5
5. N Wald, J Boreham, R Doll, J Bonsall Occupational exposure to hydrazine and subsequent risk of cancer. Br J Ind Med, 1984; 41:31-34 DOI:10.1136/oem.41.1.31
6. G. W. Watt , J. D. Chrisp Spectrophotometric Method for Determination of Hydrazine Anal. Chem, 1952; 24(12): 2006–2008 DOI: 10.1021/ac60072a044
7. I. M. Kolthoff, J. Am. The Volumetric Analysis of Hydrazine by the Iodine, Bromate, Iodate And Permanganate Methods. *Chem. Soc*, 1924; 46(9): 2009–2016. DOI: 10.1021/ja01674a006
8. James R. Holtzclaw , Susan L. Rose , Jeffrey R. Wyatt , David P. Rounbehler , David H. Fine Simultaneous determination of Hydrazine, Methyl Hydrazine and1, 1-Dimethyl

Hydrazine in air by Derivatisation/Gas chromatography. Anal. Chem, 1984; 56(14): 2952–2956. DOI: 10.1021/ac00278a074

9. Alexander D. Smolenkov, Alla V. Chernobrovkina, Roman S. Smirnov, Mikhail G. Chernobrovkin & Oleg A. Shpigun. A sensitive chromatographic determination of hydrazines by naphthalene-2,3-dialdehyde derivatisation. International Journal of Environmental Analytical Chemistry, 2013; 93(12): 1286-1295.
10. Mingjiang Sun, Lin Bai, David Q. Liu A generic approach for the determination of trace hydrazine in drug substances using in situ derivatization-headspace GC–MS Journal of Pharmaceutical and Biomedical Analysis, 2009; 49(2): 529–533. DOI.org/10.1016/j.jpba.2008.11.009
11. ICH Q2 (R1), Validation of Analytical Procedures: Text and Methodology, November 2005.
12. ICH Q1A (R2), Stability Testing of New Drug Substances and Products, 2000.
13. ICH Q3A (R2) Current *Step 4* Version, impurities in new drug substances, October 2006.
14. Guidance for Industry: Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), December 2008, Pharmacology and Toxicology.
15. European Medicines Agency; London, 28 June 2006 CPMP/SWP/5199/02, EMEA/CHMP/QWP/251344/2006.