WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 11, 1127-1139. Research Article ISSN 2277– 7105

DEVELOPMENT AND VALIDATION OF A HEAD SPACE GAS CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF ETHYLENE OXIDE CONTENT IN DIPYRIDAMOLE API

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Article Received on
26 Aug 2015,

Revised on 15 Sep 2015,
Accepted on 05 Oct 2015

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ABSTRACT

To provide quality control over the manufacture of any API, it is essential to develop highly selective analytical methods. Gas chromatography with Head space (HSGC) is widely used for the determination of residual impurities and solvents in API's. In the current article we are reporting the development and validation of a rapid and specific Head space gas chromatographic (HSGC) method for the determination of Ethylene oxide in Dipyridamole API. The developed method was validated in terms of Specificity, Linearity, Precision, Accuracy, Limit of detection (LOD) and Limit of quantitation (LOQ) and Precision and Accuracy at LOQ. The developed method was utilized for the investigation of Ethylene oxide content in bulk drug.

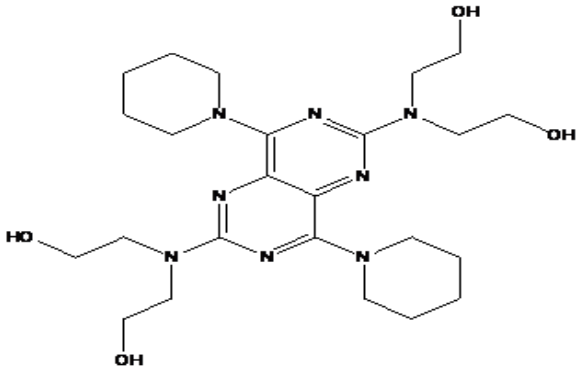
KEYWORDS: Ethylene oxide, Dipyridamol, Head Space Gas Chromatography, Method development, Method validation.

1. INTRODUCTION

Dipyridamole [2, 2', 2'', 2'''- (4, 8-di (piperidin-1-yl) pyrimido [5, 4-d] pyrimidine-2, 6-diyl) Bis (azanetriyl) tetra ethanol]. It acts by inhibition of platelet cAMP-phosphodiesterase by potentiation of adenosine inhibition of platelet function by blocking reuptake of vascular and Blood cells and subsequent degradation of adenosine.

Literature survey revealed that very few methods have been reported for the analysis of Ethylene oxide in Dipyridamole API which includes UV spectroscopy, Reverse Phase High

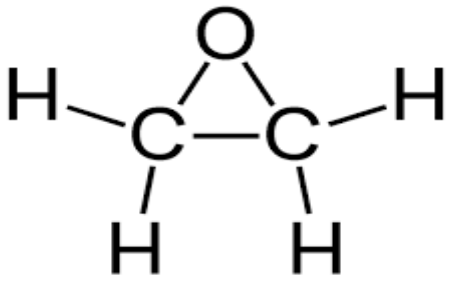
Performance Liquid Chromatography, Ultra Pressure Liquid Chromatography, LCMS, GC-MS methods. The present studies illustrate development and validation of simple, economical, selective, accurate, precise GC-HS method for the determination of Ethylene oxide in Dipyridamole API as per ICH guidelines. The limit of Ethylene oxide in Dipyridamle API is 2.5ppm. In the present work a successful attempt had been made to develop a method for the determination of Ethylene oxide in Dipyridamole API and validate it. The method would help in estimation of the Ethylene oxide in single run which reduces the time of analysis and does not require separate method for the drug.



Molecular Formula=C24H40N8O4.

Molecular Weight = 504.6256 g/mol.

Chemical Structure of Dipyridamole



Molecular Formula= C_2H_4O.

Molecular Weight = 44.05256 g/mol.

Chemical Structure of Ethylene oxide

Ethylene oxide is a flammable, colorless gas at temperatures above 51.3 ºF (10.7 ºC) that smells like ether at toxic levels. Ethylene oxide is found in the production of solvents, antifreeze, textiles, detergents, adhesives, polyurethane foam, and pharmaceuticals. Smaller

amounts are present in fumigants, sterilants for spices and cosmetics, as well as during hospital sterilization of surgical equipment.

Alkyl halides are categorized as genotoxic impurities (GTI) based on structure-activity relationship. During the manufacturing process of Dipyridamole, formation of Ethylene oxide is possible due to residual methanol available in the manufacturing process and may also be formed due to thermal interaction in presence of methanol. This paper discusses analytical methodology for a specific class of GTI, the alkyl halides. GTI's are unusually toxic material which could potentially impact genetic material by mutation. These changes to the genetic material, caused by exposure to very low levels of a genotoxin, can lead to cancer. While ICH published adequate controls for general process-related impurities (Q3A, Q3B), Pharmaceutical Genotoxic impurities (GTI) gained global prominence when regulatory bodies like European Medicines Agency (EMEA) and United States Food and Drug Administration (USFDA). Genesis of these impurities could be from variety of sources, namely but not limited to starting materials, reagents, intermediates, solvents or unwanted side reactions of the Active Pharmaceutical Ingredient (API) in synthetic process get carried over into the final product. In addition, the API itself can decompose to form genotoxic impurities or they can form in the drug product by reaction between excipients or containers and the API. In some cases, trace level of solvents could react with intermediates and form potential genotoxic impurities. Determination of Ethylene oxide at trace levels requires highly sensitive analytical methodology.

2. EXPERIMENTAL

2.1 Materials

Dipyridamole and Ethylene oxide was obtained from Local Research Laboratories. Milli Q water was obtained from in house Milli Q water plant. N, N-Dimethyl Acetamide was purchased from Fluka Chemical Co., Inc. (Milwaukee, WI, USA).

2.2 GC Operating Conditions

A Gas chromatograph (Shimadzu, GC 2010) equipped with a flame ionization detector, a Headspace sampler (Teledyne tekmar H) was used to load the sample. An analytical balance (XS 205 from Mettler Toledo) and autopippette (100 – 1000µL from Eppendorf) were used.

The Column used for the analysis was ZB-624, 30m length, 0.53 mm internal diameter, and film thickness 3.0µm .The column flow is 3.0 ml/min. with 22.7cm/sec linear velocity. A

volume of 1ml standard and sample solution was injected into the GC injection port. The temperature of the injection port was maintained at 225°C at a split ratio of 10:1, with nitrogen as a carrier gas. The pressure was maintained at 2.1 psi with flow of 3 mL min-1. The temperature of the detector was set at 250 °C. Temperature gradient was maintained at 40 °C for 5 min and then increased at a rate of 20 °C min-1 up to 200 °C to a final temperature of 200 °C and maintained for 10 min.

2.3 HEAD SPACE Operating Conditions

Vial temperature: 90^{o}C

Incubation time: 30 min

Transfer line temperature: 110^{o}C

Needle temperature: 100^{o}C

Pressurization time: 2min

Loop fills time: 0.5min

Injection volume: 1ml

Injectiontime: 1.0µl

2.4 Preparation of Solutions for Analysis

2.4.1 Diluent

N, N-Dimethyl Acetamide was selected as the standard and sample diluent because of its ability to dissolve a wide variety of substance. It has a high boiling point that does not interfere with more volatile solvents, analyzed by GC.

2.4.2 Preparation of Standard Stock Solution (20ppm)

Weighed accurately 100 mg of Ethylene oxide in 50 ml of volumetric flask dissolve and make up with diluent. Transfer 1 ml of above solution into a 100 ml volumetric flask make up with diluent.

2.4.3 Preparation of Standard solution (1.25ppm)

Transfer 3.125ml of above Standard stock solution into a 50 ml volumetric flask and make up to the mark with the same diluents to get a standard solution. Then the standard vials were prepared with 2 ml of the Standard solution and seal the vial with aluminum closure. Heat the sealed vial at 90^{o}C for 30min. (With respect to sample concentration).

2.4.4 Preparation of Sample solution

Weighed accurately 1.0 gm of the Dipyridamole API into Head Space vial, and add 2 ml of DMA solvent and seal the vial with aluminum closure. Heat the sealed vial at 90^{o}C for 30min.

3. RESULTS AND DISCUSSION

3.1 Method Optimization

An understanding of the nature the various Genotoxic Impurities present in API is the foremost prerequisite for successful method development in HSGC. In addition, successful method development should result in a fast, simple and time efficient method that is capable of being utilized in a manufacturing setting. Following were the stepwise strategies for the method development in our case.

3.1.1 Column selection

The primary goal of column selection was to resolve a Genotoxic Impurity which is formed during the synthesis and manufacturing of Dipyridamole API. Several columns were initially investigated to finalize a single method for the separation and quantitation of solvent. Wall-coated capillary columns of various brands with a variety of phases and dimensions have been investigated, e.g., Column A is VF-1 ms (30 m length, 0.32 mm i.d. with a stationary phase of 100% dimethyl polysiloxane film of 1.0 µm) and Column B is ZB-624 (30 m length, 0.53 mm i.d. with a stationary phase of 6% cynopropyl phenyl and 94% dimethyl polysiloxane film of 3.0 µm). In the above two columns, the response was found to be comparatively lower and peak shapes were found to be satisfactory in Column B. Therefore, ZB-624 with dimensions of 30 m × 0.53 mm, 3.0 µ proved to be the best column that could fulfill all the needs of the method, i.e., higher sensitivity, shorter runtime.

3.1.2 Thermal program and thermal gradient

A linear thermal gradient was chosen to provide elution of the Impurity' peak during the isothermal segment of the chromatographic run for better quantification. An initial hold of 5 min at 40ºC and a linear thermal gradient to 200ºC at 20ºC/min was found to give the best peak shape and retention.

3.1.3 Headspace method optimization

The Headspace method was optimized in such a way that maximum amount of the Impurity present in the sample get evaporated for the detection. For this the standard and sample vials

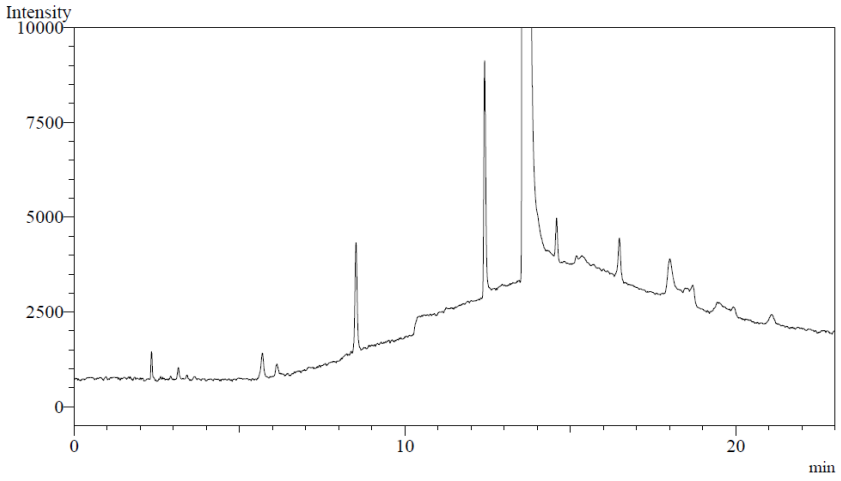
were heated at 70–110ºC for 20–30 min with constant Shaking. A combination of sample vial heating at 90ºC with 30 min shaking was found to be suitable for getting a good response.

3.2 METHOD VALIDATION

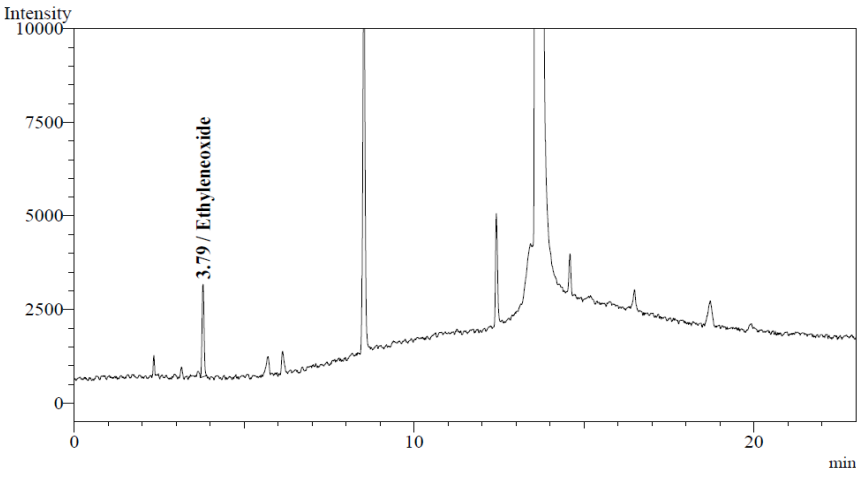
The method validation was done by evaluating Specificity, Repeatability, Linearity, Accuracy, Limit of Detection (LOD) and Limit of Quantification (LOQ), LOQ-Repeatability, LOQ-Accuracy, Ruggedness and Robustness.

3.2.1 Specificity

The Dipyridamole API sample was spiked with Ethylene oxide (1.25ppm) and sample was chromatographed to examine interference, if any, of the residual solvent peaks with each other. The retention time for standard Ethylene oxide 3.79 min, respectively. The Chromatograms as shown in Figure 1.



(a)



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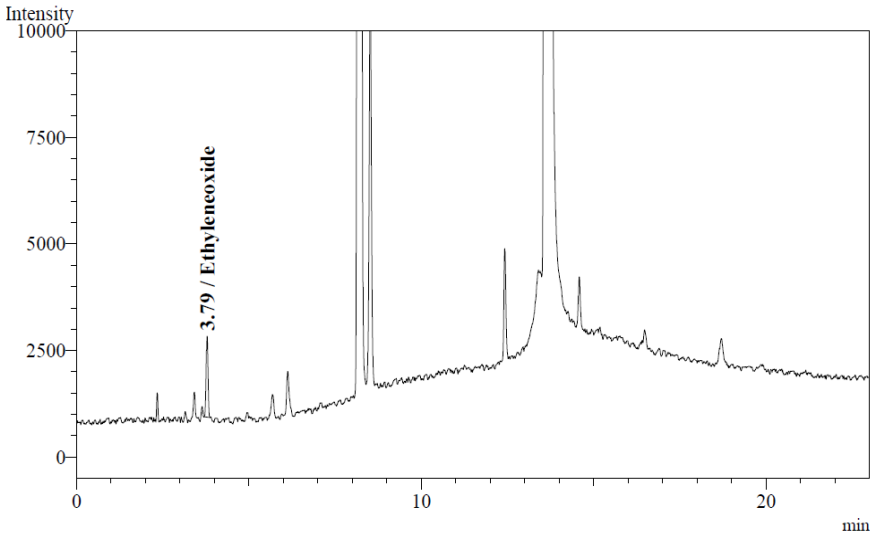


(c)

Fig. 1 Typical chromatograms of (a) Blank (DMA) (b) Ethylene oxide (c) Ethylene **oxide and Dipyridamole Spiked.**

3.2.2 Repeatability

The Ethylene Oxide was prepared at 1.25 ppm absolute with respect to Sample concentration and injected in six replicates. The RSD(n=6) values obtained for the area of Ethylene oxide is 8482.The %RSD for Ethylene oxide peak area response of Standard six injections should not more than 15%.The Repeatability data and Chromatogram are shown in Figure 2 and Table 1.

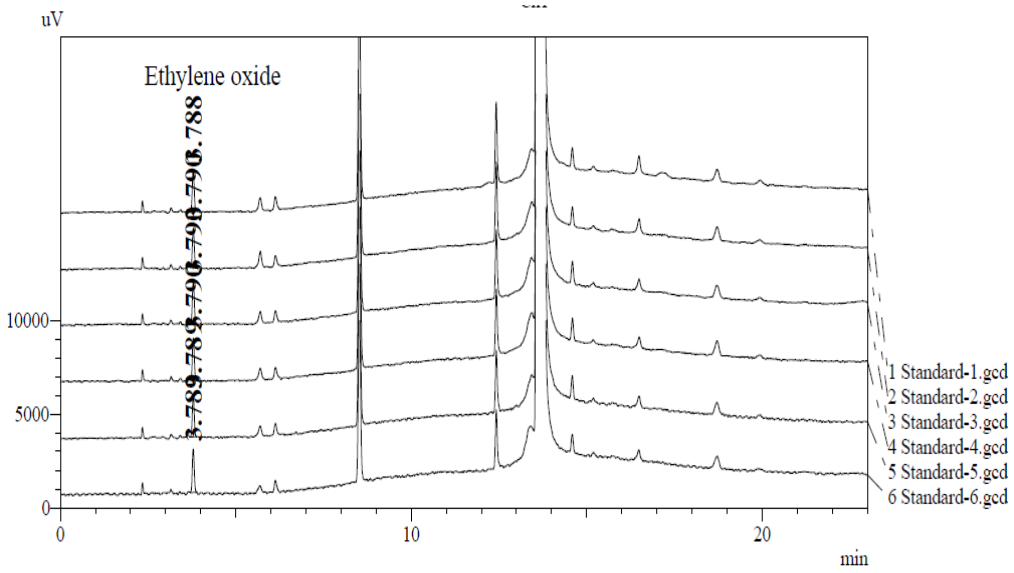


Fig. 2 Typical chromatogram showing %RSD for Ethylene oxide.

Table 1: Repeatability data for Ethylene oxide.

S.No.	Ethylene oxide Area
1	8403
2	8127
3	8849
4	8554
5	8243
6	8714
Average area	**8482**
Standard Deviation	**277**
% of RSD	**3.26**

3.2.3 Linearity

The linearity of the method was determined by making injections of Standard Ethylene oxide solvent over the range 50-150% LOQ. Three replicates were performed at each level. The calibration curves were obtained with the average of peak area ratios of three replicates. The correlation coefficient (R^2) value for Ethylene oxide was found to be higher than 0.998 and the calibration curves were linear within the range. These results revealed an excellent linearity. The linearity values and Graph for the Ethylene oxide as shown in Table 2 and Figure 3.

Table 2: Linearity data for Ethylene oxide.

S.No.	Concentration level	Run-I Area	Run-II Area	Run-III Area	Average Area
1	50% (1.25ppm)	4494	4671	4634	4600
2	80% (2.0ppm)	6882	6306	6279	6489
3	100% (2.5ppm)	8494	8488	7979	8320
4	120% (3.0ppm)	9903	9495	9746	9715
5	150% (3.75ppm)	12305	12204	12531	12347

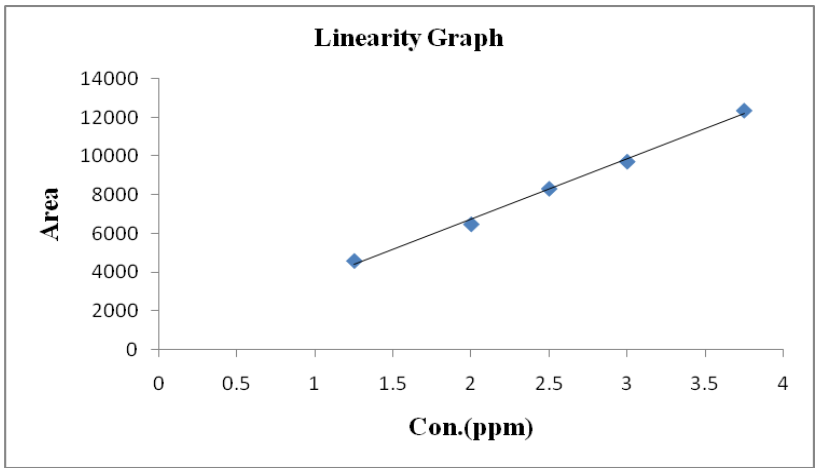


Figure 3:Linearity Graph of Ethylene oxide.

Conc.(ppm)	Area
1.25	4600
2.0	6489
2.5	8320
3.0	9715
3.75	12347

3.2.4 Accuracy (%recovery)

A known amount of Dipyridamole sample (about 1.0gr) was taken separately in three different vials and spiked with 2.0 ml of Ethylene oxide at three different levels (50, 100 & 150 % of Quantization Limit) in triplicate. From accuracy data, the % recovery of Ethylene oxide was found within the limits (100 ±15%).Results indicates that the method has an acceptable level of accuracy. The results are present ed in below Table 3.

Table 3: Accuracy data for Ethylene Oxide.

S.No	50% Area	100% Area	150% Area
1	4540	8260	11406
2	4560	8413	11754
3	4620	8249	11830
Average area	**4573**	**8307**	**11663**
%Recovery	**107.82**	**97.93**	**91.67**
Standard Ethylene oxide Avg. Area : 8482			

3.2.5 Limit of Detection (LOD) and Quantitation (LOQ)

The LOD and LOQ were calculated by instrumental and statistical methods. For the instrumental method, LOD is determined as the lowest amount to detect, and LOQ is the lowest amount to quantify, by the detector. The LOD and LOQ of Ethylene oxide in Dipyridamole API was determined based on Linearity. The area of Ethylene oxide at LOD Concentration (0.21 ppm) is 689 and Standard average area of Ethylene oxide at LOQ Concentration (0.71 ppm) is 1597.The linearity also passed at LOQ Concentration(R^2=0.995). The Values, linearity graph and Chromatograms of LOD and LOQ as shown in Table 4, Figure 4 & 5.

Table 4: Linearity Graph data for Ethylene oxide at LOQ Concentration.

S.No.	Conc, ppm	Area
1	1.25	4600
2	1	6489
3	2.5	8320
4	3	9715
5	3.75	12347
Slope		**3116**
STEYX		**220**
LOD		**0.21 ppm**
LOQ		**0.71 ppm**

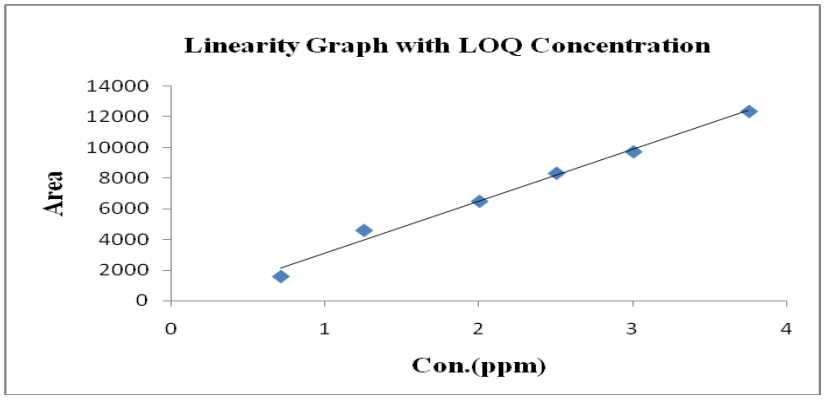
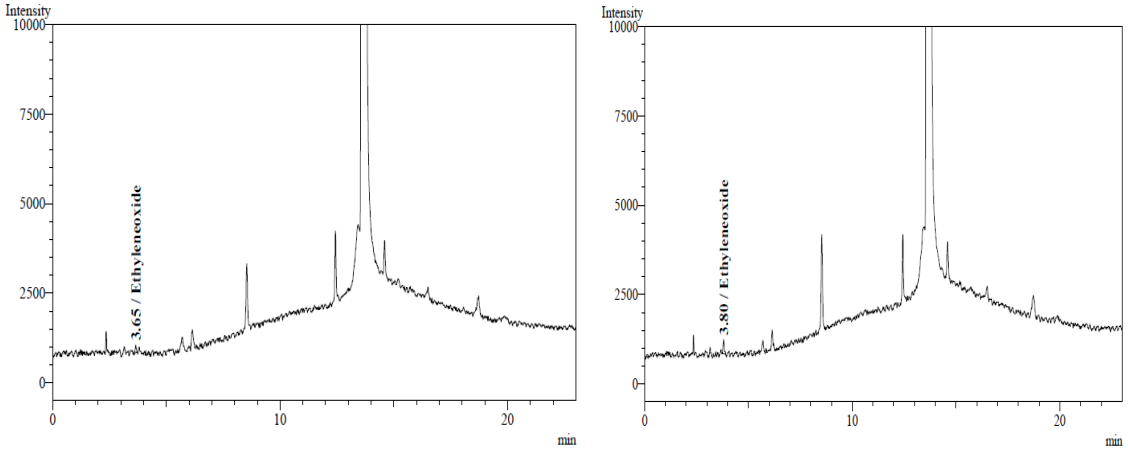


Fig.4 Linearity Graph with LOQ Concentration.



LOD **LOQ**

Fig.5 LOD and LOQ Chromatograms of Ethylene oxide.

3.2.6 LOQ Precision

The Ethylene oxide was prepared at LOQ level (0.71 ppm) absolute and injected in six replicates. The RSD(n=6) values obtained for the area of Ethylene oxide is 1597.The %RSD for Ethylene oxide peak area response of Standard six injections should not more than 15%. The results are presented in below Table 5.

Table 5: LOQ Precision data of Ethylene oxide.

S.No.	Ethylene oxide Area
1	1526
2	1631
3	1555
4	1575
5	1638
6	1655
Average Area	**1597**
Standard Deviation	**52**
% of RSD	**3.25**

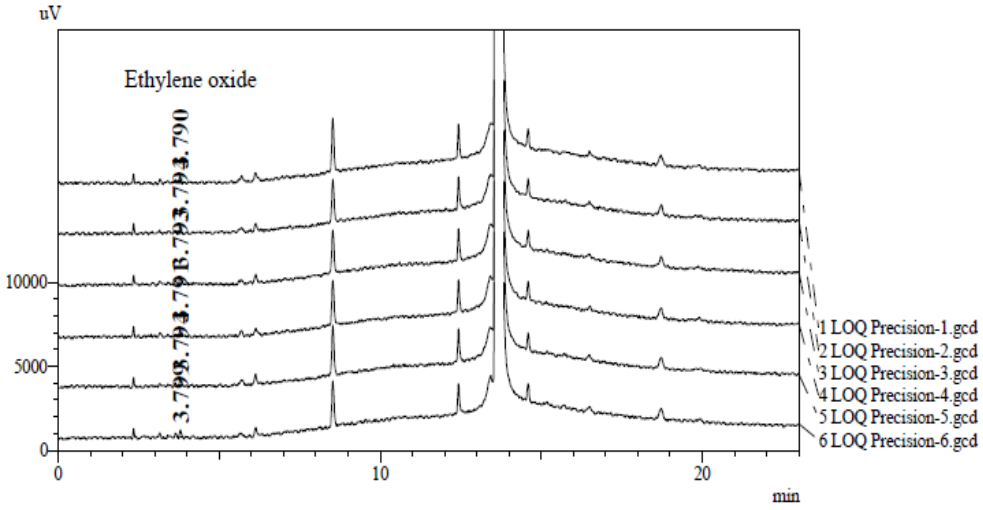


Fig. 6 Typical %RSD chromatograms at LOQ.

3.2.7 LOQ Accuracy

A known amount Dipyridamole sample (about 1.0gr) was taken separately in three different vials and spiked with 2.0 ml of Ethylene oxide at LOQ Level (0.71 ppm) in triplicate. From accuracy data, the % recovery of Ethylene oxide was found within the limits (100 ±20%).

Results indicate that the method has an acceptable LOQ level of accuracy. The results are presented in below Table 6.

Table 6: Accuracy data for Ethylene Oxide at LOQ Concentration.

S.No	Sample+LOQ Level Area
1	1483
2	1210
3	1562
Average area	**1418**
Standard Average Area	**1597**
%Recovery	**88.79%**

3.2.8 Ruggedness

Ruggedness of the method was evaluated by performing the sample analysis in six replicates using different analyst on different days and the results are summarized as shown in bellow Table 7. The %RSD values of less than 15.0% for Ethylene oxide content indicate that the method adopted is rugged.

Table 7: Ruggedness data for Ethylene oxide.

SST Parameter	Day-1			Day-2			Analyst-1	Analyst-2
	Analyst 1	Analyst 2	Analyst 1&2	Analyst 1	Analyst 2	Analyst 1&2	Day-1&2	Day-1&2
%RSD	3.17	3.09	3.02	3.08	3.04	2.98	3.09	3.22

3.2.9 Robustness

This study was performed by making small but deliberate variations in the method parameters. The effect of variations in flow rate of carrier gas and Vail temperature was studied. Under all the variations, system suitability requirement is found to be within the acceptance criteria and hence the proposed method is robust.

The relative standard deviation of area counts for Ethylene oxide peak obtained from six replicate injections of standard solution should be not more than 15.0%.The data of Robustness is following Table 9&10.

Table 9: Ethylene oxide Robustness (Flow variation).

System Suitability Parameter	2.59mL/min (Flow Minus)	3mL/min (Control)	3.5mL/min (Flow Plus)
% RSD	2.88	3.02	2.77

Table 10: Ethylene oxide Robustness (Vail Temperature variation).

System Suitability Parameter	85°C (Temperature Minus)		90°C (Control)	95°C (Temperature Plus)
% RSD	3.08	2.89	2.93	

4. CONCLUSION

A single, rapid and highly selective HSGC method was developed and validated for the quantification of Ethylene oxide present in Dipyridamole API through an understanding of LOD, LOQ, and nature of stationary phases of columns. In linearity study the correlation coefficient values observed were more than 0.99.The residue Ethylene oxide was determined in ppm levels also. The method was shown to be specific for Dipyridamole API and was

applied successfully to monitor and control impurity level. Further to the validation study, three batches of Dipyridamole were analyzed for the content of Ethylene oxide and the results showed that the Ethylene oxide was absent in all the three batches. Hence, after studying the data obtained from the validation activity and analysis of three batches, this Method was applicable for the routine analysis of the Dipyridamole API in pharmaceutical industry.

ACKNOWLEDGEMENT

We would like thank to for financial support and providing facilities my experiment to the Head of the Department K.Umamaheswara rao, and K.V.R, K.V.R & M.K.R College, Khajipalem.

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