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# RESIDUAL OXYGEN IN SEALED GLASS INJECTION VIALS BY GAS-CHROMATOGRAPH

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**ABSTRACT**

A method for determination of residual oxygen in sealed pharmaceutical glass vials was developed and validated using gas chromatograph (GC) with thermal conductivity detector (TCD). The gas in the headspace of the vial is collected in a gas tight syringe and injected to GC maintained at 35°C using a molecular sieve packed column and helium as carrier gas. The GC conditions were maintained to separate oxygen from other interference thus to obtain a well resolved peaks. Known amount of sample and reference standard are injected through gas tight syringe and the concentration of residual oxygen present in the headspace of sample is quantitatively measured.

## INTRODUCTION

Evaluation of residual oxygen in pharmaceutical packaging containers arises to ensure the stability and potency of drug product. Besides a loss of efficacy and reduction in shelf life, exposure of such products to oxygen can results in product discoloration, changes in dissolution rate and profile, toxicity or other pharmacological properties associated with negative side effects.

Oxidation degradation of dry substances in pharmaceutical formulations is well documented (1). It imparts decrease in potency and reduces product shelf life (2). In such cases, extra care may be required to minimize or eliminate oxygen concentration. Inert gas purging over final products, to lower oxygen levels have long been used in manufacturing processes to protect formulations that are susceptible to oxidation. Due to technical limitations for nitrogen filling as well as the seal integrity of the

packages, the content of oxygen inside the package is necessary to monitor time to time. Monitoring headspace oxygen levels would greatly benefit the final quality assurance (3). Such analysis could also assess the container closure integrity i.e., no oxygen remaining into the vials. Head space oxygen levels are often monitored during the filling process as in-process control of the purging system used to bring headspace levels below the required specifications.

The rapid and accurate determination of headspace oxygen levels in various packaging forms used in pharmaceutical industry poses several unique analytical challenges. Below mentioned methods are suitable for samples sealed in vials/containers with rubber stoppers.

Method development and validation of residual oxygen in cefuroxime sodium injection vial was carried out are specificity, LOD, LOQ and precision.

## Experimental

### Instruments and Reagents

Gas Chromatograph: Shimadzu make, GC-2014 model, with thermal conductivity detector.

Gas tight syringe: 500 µl capacity, Hamilton make.

GC column: Packed, Molecular sieve 5A.

Headspace Vials: 20 ml capacity, with PTFE septa and Aluminum crimp cap.

Reference standard: Oxygen gas of known purity (ppm) in Nitrogen.

Gas: Oxygen, Nitrogen and Helium

Sample: Cefuroxime sodium injection, sealed in glass vials **with rubber s**epta and aluminum crimp cap.

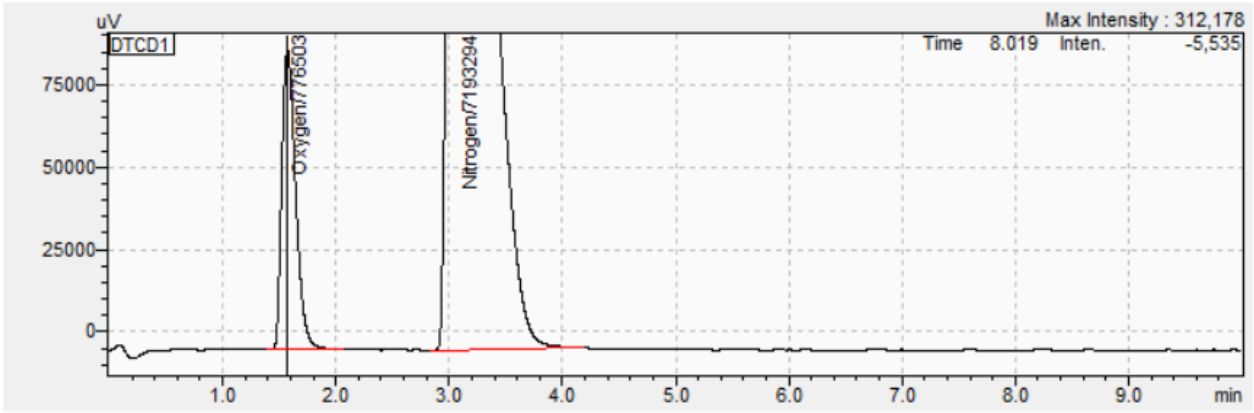
**Validation Parameters**:

The chromatographic conditions are developed by injecting oxygen and nitrogen gases separately for peak identification. Then injected atmospheric air to identify and to check the peak separation for oxygen and nitrogen. The conditions are optimized to get maximum resolution. After that injected known volume of the reference standard mixture and verified the resolution.

Following chromatographic conditions are used:

| | |
|---|---|
| Column | : Molecular sieve 5A, 2 m length, 2 mm dia. 80-100 mesh. |
| Inlet temperature | : 100°C |
| Carrier gas | : Helium |
| Flow | : 20ml/min |
| Oven temperature | : 35°C isothermal for 10min. |
| Detector used | : Thermal conductivity detector |
| Detector temperature | : 150°C |
| TCD current | : 160 mA |
| Injection volume | : 500 µl through gas tight syringe |
| Injection type | : Manual |

The Resolution obtained between oxygen and nitrogen is 3.5 and the retention time for oxygen is 1.5 min and of nitrogen is 3.1 min. shown in Figure: 1




Results View - Peak Table

Peak Table | Compound | Group | Calibration Curve

| Peak# | Ret. Tim | Area | Height | Name | Area% | S/N | Resolution(U |
|---|---|---|---|---|---|---|---|
| 1 | 1.572 | 776503 | 90908 | Oxygen | 9.743 | 209.68 | -- |
| 2 | 3.080 | 7193294 | 317686 | Nitrogen | 90.257 | 732.75 | 3.499 |
| Total | | 7969798 | 408593 | | 100.000 | | |

Figure: 1 Chromatogram showing Resolution and retention time

**Specificity:**

Column blank was performed to check any inbuilt contamination or interferences. No peak observed in the blank chromatogram at retention time 1.5 min (Figure: 2). 20 ml headspace vial was sealed and purged with nitrogen gas to remove atmospheric oxygen. This vial was then spiked with known amount of oxygen and it was observed that the area for oxygen increases in spiked vial (Figure: 3 & 4).

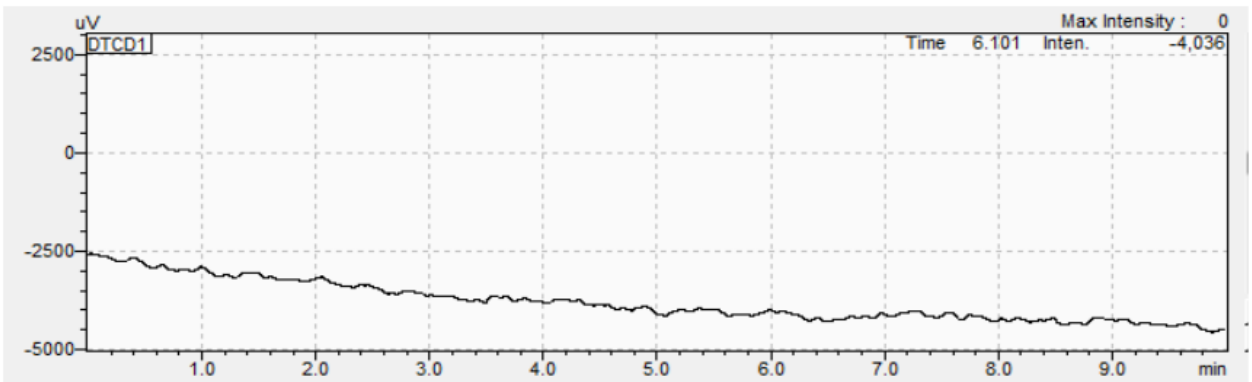



Figure: 2 Chromatogram showing column blank

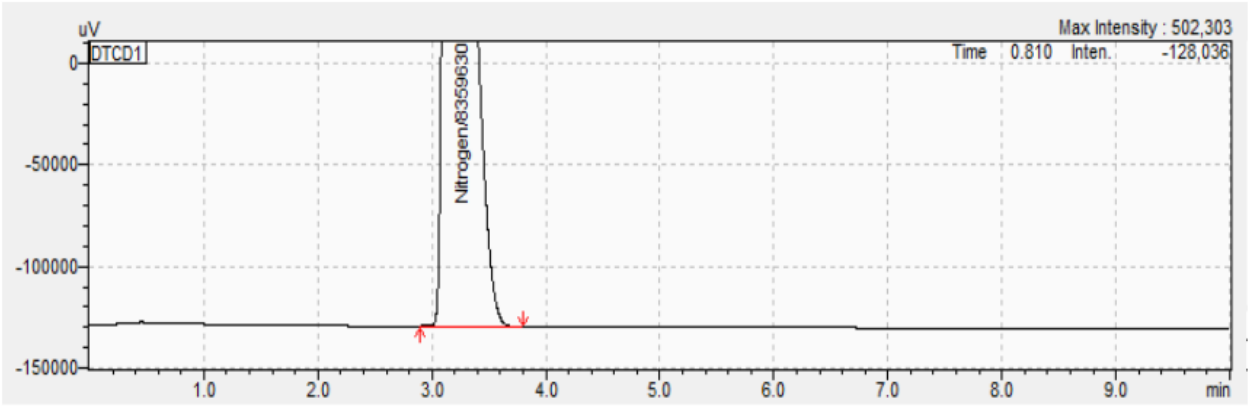



Figure: 3 Chromatogram showing vial purged with nitrogen gas

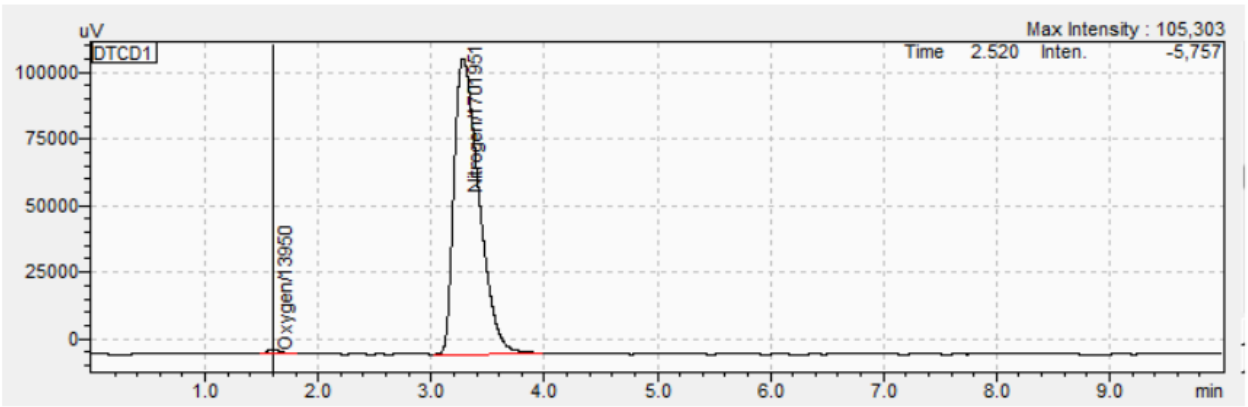



Figure: 4 Chromatogram showing vial spiked with oxygen gas

**Precision:**

System precision was done using standard reference gas (10.03 %) and method precision was done using six different sample vials (Figure: 5 & 6). Higher concentration standard was used since the sample contains higher level of residual oxygen. The results are shown in table: 1.

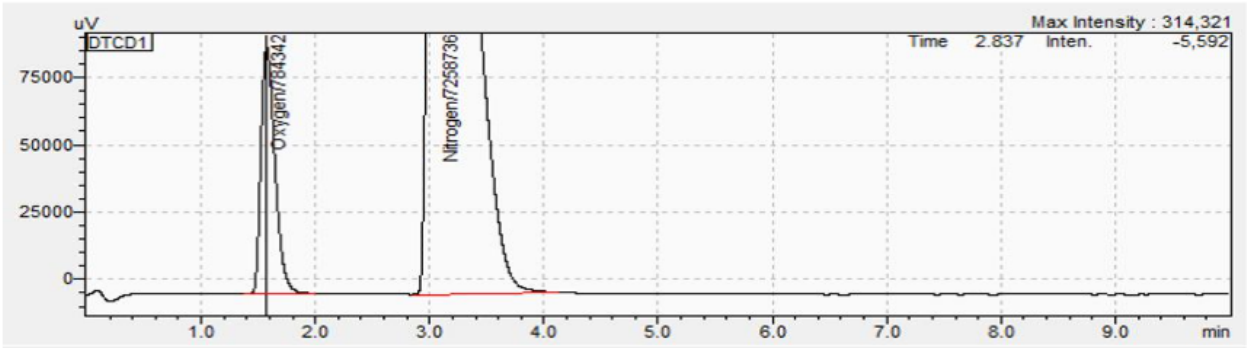



Figure 5.Chromatogram for 10.03% Standard Oxygen

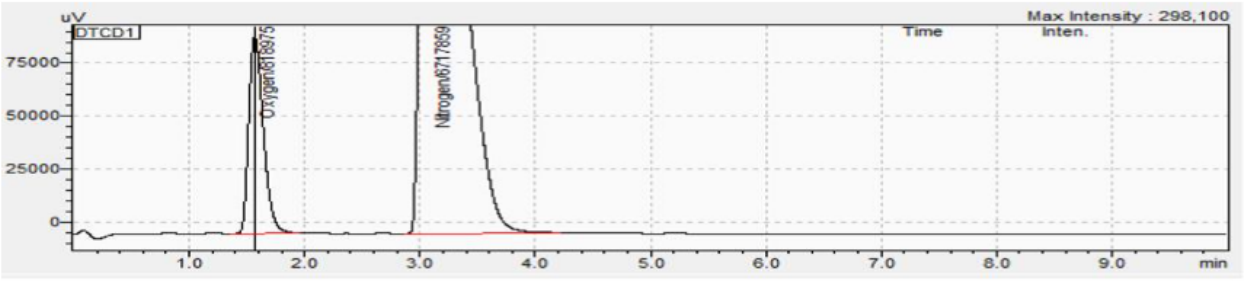



Figure 6. Chromatogram for Sample vial

Table: 1 System precision and method precision

| No. of runs | System precision | Method precision |
|---|---|---|
| 1 | 777370 | 803496 |
| 2 | 778387 | 818975 |
| 3 | 784342 | 698936 |
| 4 | 775019 | 692069 |
| 5 | 776503 | 815710 |
| 6 | 784076 | 823369 |
| Average | 779282.83 | 775425.83 |
| Standard Deviation | 3973.38 | 62297.48 |
| % RSD | 0.51 | 8.03 |

Calculate the concentration of oxygen present in the headspace as:

$$\frac{\textbf{Average area for sample x Concentration of gas (ppm or \%) x Volume of sample injected (ml)}}{\textbf{Average area for standard x Volume of standard injected (ml)}}$$

Reference standard gas used must be of same concentration as of sample. If the concentration of sample is lower than the available reference standard, inject lower volume of reference standard.

Precision was carried out at limit of detection (LOD) 50 ppm (Figure: 7) and limit of Quantification (LOQ) 100 ppm (Figure: 8), the results obtained are shown in table: 2.

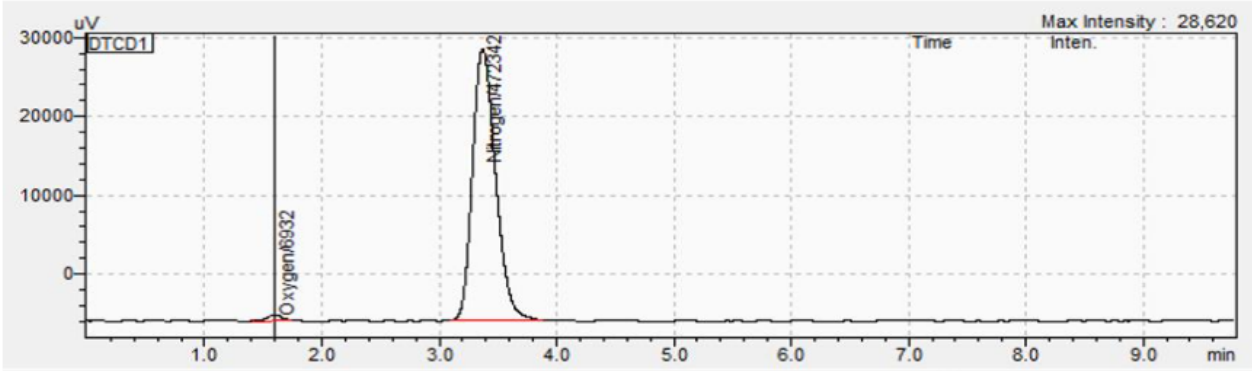



Figure: 7. Chromatogram for 50 ppm oxygen (LOD)

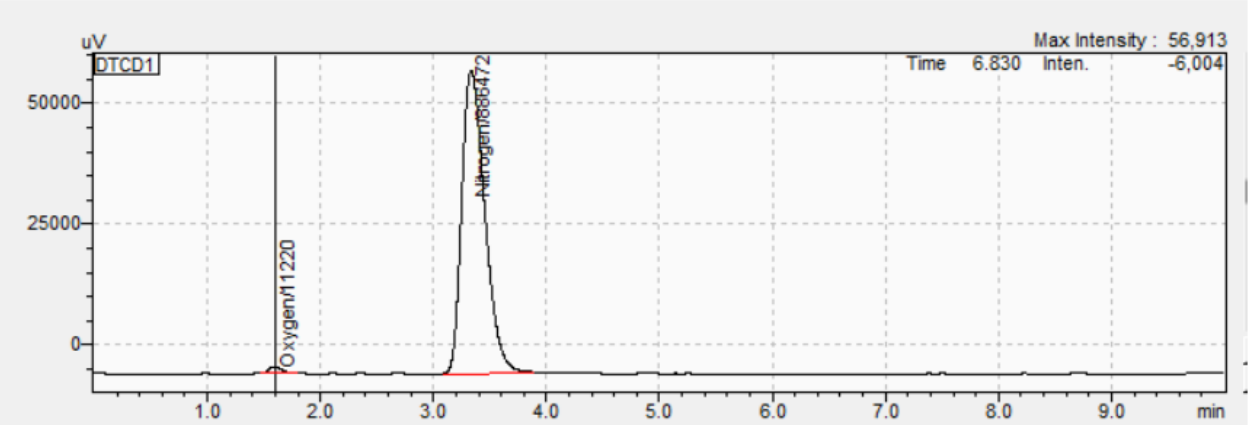



Figure: 8. Chromatogram for 100 ppm oxygen (LOQ)

Table: 2 Precision for LOD and LOQ

| No. of runs | Area at LOD | Area at LOQ |
|---|---|---|
| 1 | 6517 | 12593 |
| 2 | 10065 | 11351 |
| 3 | 7871 | 11220 |
| 4 | 6608 | 12715 |
| 5 | 6932 | 10742 |
| 6 | | 11151 |
| Average | 7598.60 | 11628.67 |
| Std dev | 1479.26 | 820.79 |
| % RSD | 19.47 | 7.06 |

It was observed that the precision at LOQ level is less than 10 %

## Conclusion:

Analysis of residual oxygen in injection vials are of high importance because it is used IM/IV to patients so to maintain the potency of drug is important with appropriate shelf life. There are different types of techniques and instruments available to measure residual headspace oxygen in sealed containers. Headspace gas analyzers are available in the market, but in case the test gas obtained by the detector probe is inadequate, the test would prove ineffective. All headspace gas analyzers normally collect gas by penetrating the seal are with the help of a probe, hence the test results will be affected to some extent by system respond time.

Above described method used for determination of residual headspace oxygen is valid and simple. Can detect and quantitate residual oxygen from parts per million (ppm) to percentage (%) level. Which gives an opportunity for quantitative determination of residual oxygen in headspace of sealed bottles precisely with data support.

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