Journal of Bioanalysis & Biomedicine - Open Access

Research Article
OPEN ACCESS Freely available online
doi:10.4172/1948-593X.1000029
JBABM/Vol.2 Issue 4

# Quantification of 4-Oxiranyl Methoxy-9h-Carbazole a Genotoxic Impurity in Carvedilol Drug Substances by Lc-Ms

**M. Srinivasa Rao[1]*, Sumathi V. Rao[1], U.K.Ray[1], G.Sri Siva Kumar[1], Hemant Kumar Sharma[1] and K.Mukkanti[2]**

*[1]Department of Analytical research, Aurobindo Pharma Research Centre, 313 Bachupally, Qutubullapur Mandal, Hyderabad - 500090, India*
*[2]Head of Centre for Pharmaceutical sciences, JNTU Institute of sciences & Technology, Kukatpally, Hyderabad - 500085, India*

## Abstract

LC-MS multiple reaction monitoring (MRM) method had been evaluated for the determination of the very low level of 4-Oxiranylmethoxy-9H-Carbazole in drug substances such as Carvedilol (U.S. Pharmacopoeia (USP), 32NF-27,British pharmacopoeia (BP), 2009, Martindale 35th edition) This 4-Oxiranylmethoxy-9H-Carbazole have been identified as potential genotoxic impurity. LC-MS was found to be more promising and the limit of quantification was 15 µg/gm.

**Keywords:** HPLC; LC-MS; Genotoxins; Carvedilol; 4-Oxiranylmethoxy-9H-Carbazole

## Introduction

4-Oxiranylmethoxy-9H-Carbazole is generally used as an important intermediate in the preparation of pharmaceutical compounds such as antihipertensives. However, this 4-Oxiranylmethoxy-9H-Carbazole is found to be a genotoxic product. This Genotoxic compounds affects the human genes, and the presence of these genotoxins in the pharmaceutical compounds can be controlled. In current regulatory practice (ICH Guidelines 2002), genotoxic compounds are usually considered to operate by a non-threshold mode of action and, thus any level of exposure carries at least theoretically a risk. This precautions view implies that pharmaceutical measurements should be guided by the so called 'ALARA' principle (As Low As Reasonably Achievable), i.e., where avoidance is not possible, genotoxic impurities must be kept to a low level. However, the draft guidelines from European agency (Guidelines on the limits of Genotoxic impurities CPMP/SWP/5199/02, EMEA/CHMP/QMP/251344/2006, EMEA/CHMP/SWP/431994/2007) and feedback from the US food and drug administration (USFDA) (USFDA, Guidance for Industry 2008) to pharmaceutical industries via drug application has enabled the pharmaceutical industries to establish interim strategies. Generally the daily intake of these genotoxins has been limited to a daily dose of 1.5 µg/day. Therefore it is preferable for the potential genotoxins to be controlled during the synthesis; where the levels cannot be controlled and no safety data yet exists it may be preferable for the pharmaceutical company to change the route of synthesis of the drug substances. As 4-Oxiranylmethoxy-9H-Carbazole is also a genotoxic compound, the regulatory team may be expected to estimate the levels of 4-Oxiranylmethoxy-9H-Carbazole to be controlled to 15 ppm in the drug substance. It was felt necessary to develop simple, sensitive validated method for 4-Oxiranylmethoxy-9H-Carbazole.

## Experimental

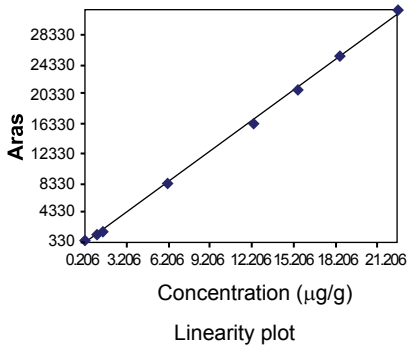
### Sample, chemicals and reagents

Carvedilol was synthesized from the CRD research department in APL Research centre, (A Division of Aurobindo Pharma Ltd.) (Bachupally, Quthubullapur, Hyderabad-90, INDIA). Acetonitrile (HPLC grade), Ammonium acetate (BDH grade), High pure water was prepared by using the Milli-Q plus purifier.

### Preparation of solution

**Preparation of standard stock solution:** Accurately weigh and transfer about 10 mg of 4-Oxiranylmethoxy-9H-Carbazole working standard into a 100 ml volumetric flask (0.10 µg/ml). Dilute 10 ml of this solution to 100 ml with diluents (0.01 µg/ml). Further dilute 10 ml of this solution to 100 ml with diluents (0.001 µg/ml).

| Concentration (µg/g) | Area | Statistical analysis | |
|---|---|---|---|
| 22.5 | 31715 | Slope | 1381 |
| 18 | 25327 | | |
| 15 | 20813 | Intercept | -191 |
| 12.5 | 16380 | | |
| 6 | 8407 | Residual Sum of Squares | 371 |
| 1.5 | 1884 | | |
| 1 | 1383 | Correlation Coefficient | 0.9995 |
| 0.2 | 330 | | |




Linearity plot

**Table 1:** Results of linearity study.

**Preparation of standard stock solution:** Transfer accurately 7.5 ml of 4-Oxiranylmethoxy-9H-Carbazole Stock solution in 100ml clean (15 µg/gm with respect to sample concentration 5 mg/ml). Spectrogram is shown in the Figure 1.

### Instrumentation

**Mass spectrometry:** The LC-MS/MS system was used for method

***Corresponding author:** M. Srinivasa Rao, APL Research Centre (A Division of Aurobindo Pharma Ltd.), 313, Bachupally, Quthubullapur, Hyderabad 500090, India, Tel: +91 40 23040261; Fax: +91 40 23042932; E-mail: metta_cnu@yahoo.co.in

**Received** July 02, 2010; **Accepted** August 23, 2010; **Published** August 23, 2010

**Citation:** Srinivasa Rao M, Rao SV, Ray UK, Siva Kumar GS, Sharma HK, et al. (2010) Quantification of 4-Oxiranyl Methoxy-9h-Carbazole a Genotoxic Impurity in Carvedilol Drug Substances by Lc-Ms. J Bioanal Biomed 2: 091-095. doi:10.4172/1948-593X.1000029

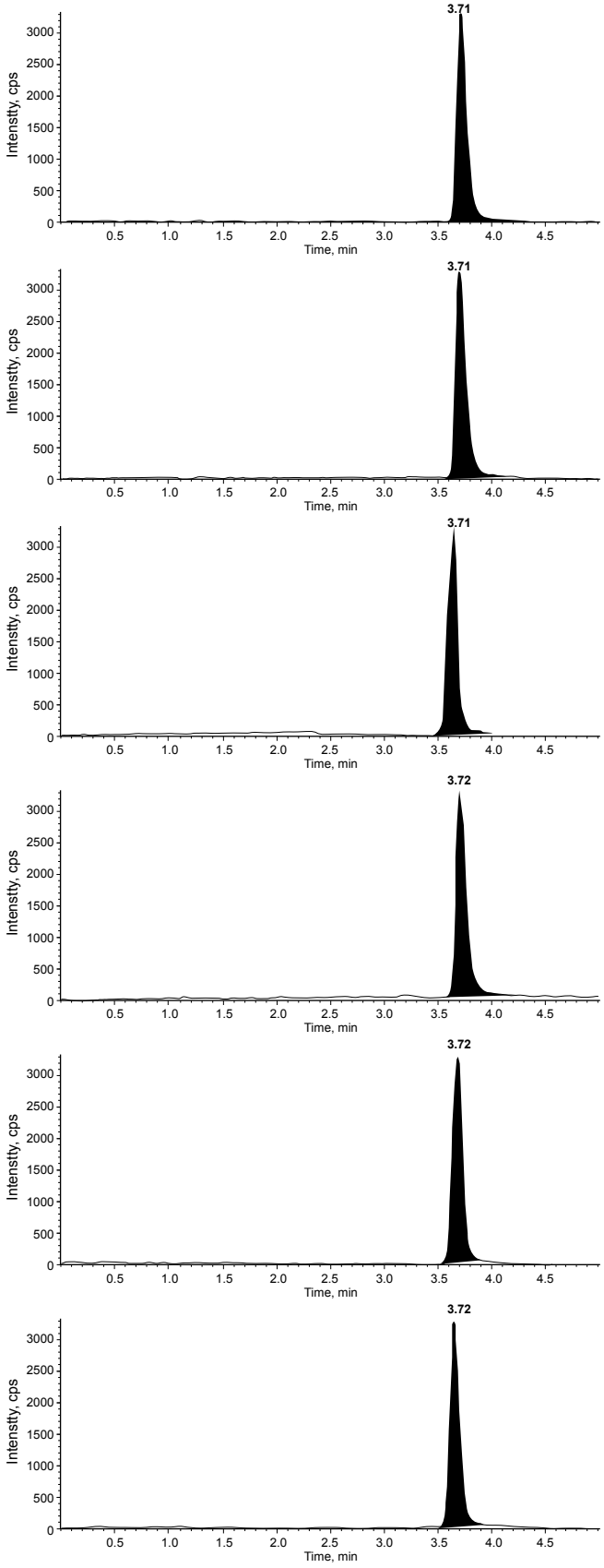



**Figure 1:** System Precision.

development and validation was done in Applied Biosystems Sciex API 2000 model coupled with Shimadzu HPLC system with mass detector and auto sampler was used in the experiment. Data acquisition and processing were conducted by using the Analyst software.

## LC-MS/MS analysis

LC-MS/MS analysis was carried out using Perkin Elmer triple

| Injection ID | Area | |
|---|---|---|
| | 4-Oxiranylmethoxy-9H-Carbazole | |
| | LOD | LOQ |
| 1 | 1148 | 3426 |
| 2 | 988 | 3693 |
| 3 | 1140 | 3655 |
| 4 | 1044 | 3922 |
| 5 | 1115 | 3833 |
| 6 | 1273 | 4153 |
| Mean | 1118 | 3780 |
| SD | 98 | 249 |
| % RSD | 8.8 | 6.6 |
| Conc. (µg/g) | 0.825 | 2.68 |

Note: SD: Standard deviation, RSD: Relative Standard deviation

**Table 2:** Results of LOD and LOQ study.

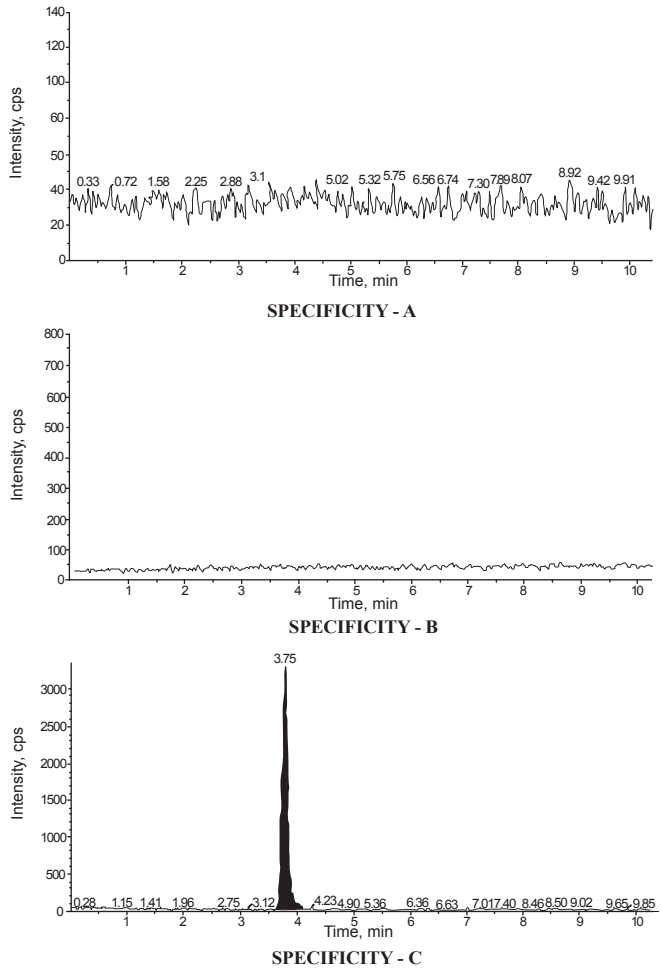



**Figure 2:** Specificity-**A**: Mass Spectrogram of Carvedilol Drug Substance (Control sample). Specificity-**B**: Mass Spectrogram of Carvedilol Drug Substance spiked with all related Compounds without 4 -Oxiranylmethoxy-9H-Carbazole at specification level. Specificity-**C**: Mass Spectrogram of Carvedilol Drug Substance spiked with all related. Compounds with 4 -Oxiranylmethoxy-9H-Carbazole at specification level.

Research Article
OPEN ACCESS Freely available online
doi:10.4172/1948-593X.1000029
JBABM/Vol.2 Issue 4

quadrupole mass spectrometer (API 2000, PE SCIEX) coupled with Shimadzu HPLC equipped with SPD 10 A VP UV-VIS detector and LC 10 AT VP pumps. Analyst software was used for data acquisition and data processing. The turbo ion spray voltage was maintained at - 4.5 Kv and temperature was set at 375°C. The auxiliary gas and sheath gas used was high pure Nitrogen. Zero air was used as Nebulizer gas. The analysis was carried out using YMC PACK C8 250 X 4.6 mm column with 5 µm particle diameter with mobile phase consisting of a mixture of 0.01M Ammonium acetate pH-5 in water and acetonitrile in the ratio of 15:85 v/v. The flow rate was 1ml/min with flow rate split down to 0.2 ml/min in to the LC-MS system. The column was monitored at 55°C.The injection volume was 20 µl. Electrospray ionization in negative mode was used with a multiple reaction monitoring (MRM) mode was used as MS method for quantification of 4-Oxiranylmethoxy-9H-Carbazole in drug substances. In this method 4-Oxiranylmethoxy-9H-Carbazole is monitored by its molecular ion value of 238.10 (M-H) and its daughter ion 181.0 with focusing potential-270, declustering potential -25, entrance potential -10 , and the curtain gas flow 25 (psi) respectively.

## Results and Discussion

The main target of LC-MS/MS method was to quantification of 4-Oxiranylmethoxy-9H-Carbazole impurity in the Carvedilol active ingredient. During the method development we used different reversed phase stationary and mobile phase was used and finally chromatographic separation was achieved on a YMC PACK C8 250*4.6,5mic column (YMC) in isocratic mode using with 0.01M Ammonium acetate pH-5 in water and Acetonitrile in the ratio of 15:85 v/v. The flow rate was 1.0 ml/min split down to 0.2 ml/min in to LC-MS system.

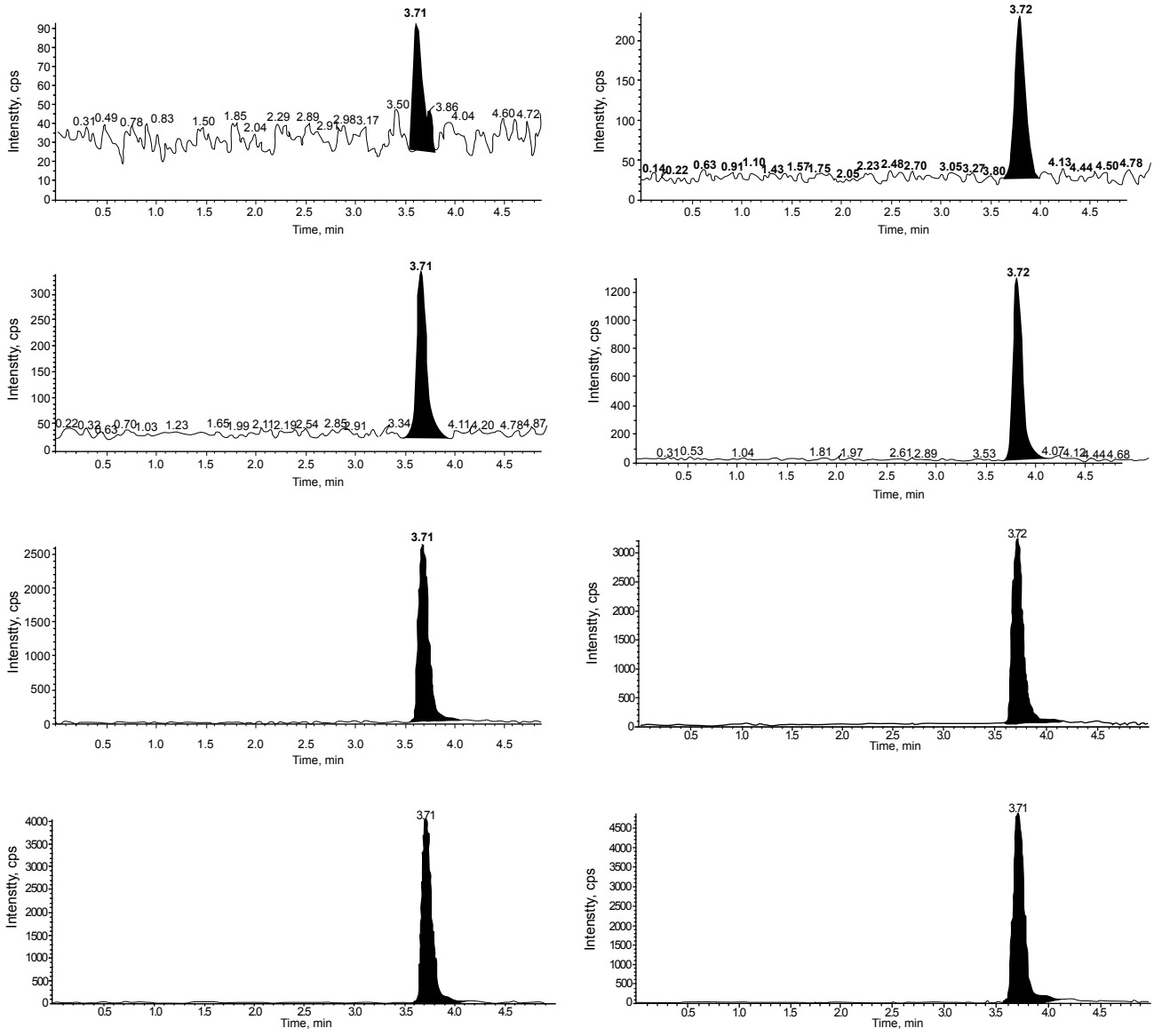



**Figure 3:** Linearity mass spectrogram of 4 -Oxiranylmethoxy-9H-Carbazole.

| Sample ID | Amount added (µg/g) | Amount found (µg/g) | % Recovery | Statistical Analysis | |
|---|---|---|---|---|---|
| LOQ Level Sample 1 | 2.679 | 2.7600 | 103.0 | Mean | 96.1 |
| LOQ Level Sample 2 | 2.681 | 2.4240 | 90.4 | SD | 6.39 |
| LOQ Level Sample 3 | 2.680 | 2.5410 | 94.8 | % RSD | 6.6 |
| 100% Level Sample 1 | 15.445 | 15.5480 | 100.7 | Mean | 97.7 |
| 100% Level Sample 2 | 15.491 | 14.2500 | 92.0 | SD | 4.91 |
| 100% Level Sample 3 | 15.454 | 15.5000 | 100.3 | % RSD | 5.0 |
| 150% Level Sample 1 | 23.181 | 24.4420 | 105.4 | Mean | 101.5 |
| 150% Level Sample 2 | 23.204 | 22.5190 | 97.0 | SD | 4.24 |
| 150% Level Sample 3 | 23.195 | 23.6960 | 102.2 | % RSD | 4.2 |
| Overall Statistical Analysis | | | | | |
| Mean 98.4 | SD 5.16 | % RSD 5.2 | 95% Confidence Interval | ± 12.8 | |

Note: SD: Standard deviation, RSD: Relative Standard deviation

**Table 3:** Results of recovery study.

## Validation of the method for 4-oxiranylmethoxy-9H-carbazole in carvedilol

**Specificity of 4-Oxiranylmethoxy-9H-Carbazole:** Solutions are prepared using Carvedilol drug substance (Control Sample), Spiked with all the related substances in Carvedilol drug substance except 4-Oxiranylmethoxy-9H-Carbazole (Specificity-A) and Spiked with all the related substances including 4-Oxiranylmethoxy-9H-Carbazole in Carvedilol at specification level and subjected for LC-MS/MS study for the evaluation of specificity. The sample spiked with all other impurities without 4-Oxiranyl methoxy-9H-Carbazole at specification level, do not show any relevant response in spectrogram. The sample spiked with all other impurities along with 4-Oxiranylmethoxy-9H-Carbazole (Specificity-B) at specification level shows response equivalent to standard in spectrogram at retention time of 4-Oxiranylmethoxy-9H-Carbazole (m/z-238.10 M-H and 181.0) hence the method is specific and selective. Spectrogram is shown in the Figure 2.

**Linearity of 4-Oxiranylmethoxy-9H-Carbazole:** By selecting ion monitoring, the linearity of 4-Oxiranylmethoxy-9H-Carbazole was satisfactorily done a series of solutions were prepared using 4-Oxiranylmethoxy-9H-Carbazole at concentration levels from around detection level to 150% and the concentration levels are 22.5 (µg/g), 18 (µg/g), 15 (µg/g), 12.5 (µg/g), 6 (µg/g), 1.5 (µg/g), 1 (µg/g) and 0.2 (µg/g) respectively. The peak area versus concentration data was done by linearity plot slop, intercept, and residual sum of squares analysis. The calibration curve was given based on response over the concentration range for 4-Oxiranylmethoxy-9H-Carbazole. The correlation coefficients for 4-Oxiranylmethoxy-9H-Carbazole were 0.999. Linearity of the 4-Oxiranylmethoxy-9H-Carbazole spectrogram was shown in the Figure 3 and the results are tabulated in Table 1.

**Limit of detection (LOD) and Limit of Quantification (LOQ) for 4-Oxiranylmethoxy-9H-Carbazole:** The LOD and LOQ values of 4-Oxiranylmethoxy-9H-Carbazole were predicted from the linearity data. Each predicted concentration was verified for precision by preparing the solutions at about predicted concentration and injecting each solution six times for LC-MS/MS study and the predicted concentration for LOQ was 2.68 (µg/g) and LOD was 0.825 (µg/g). The

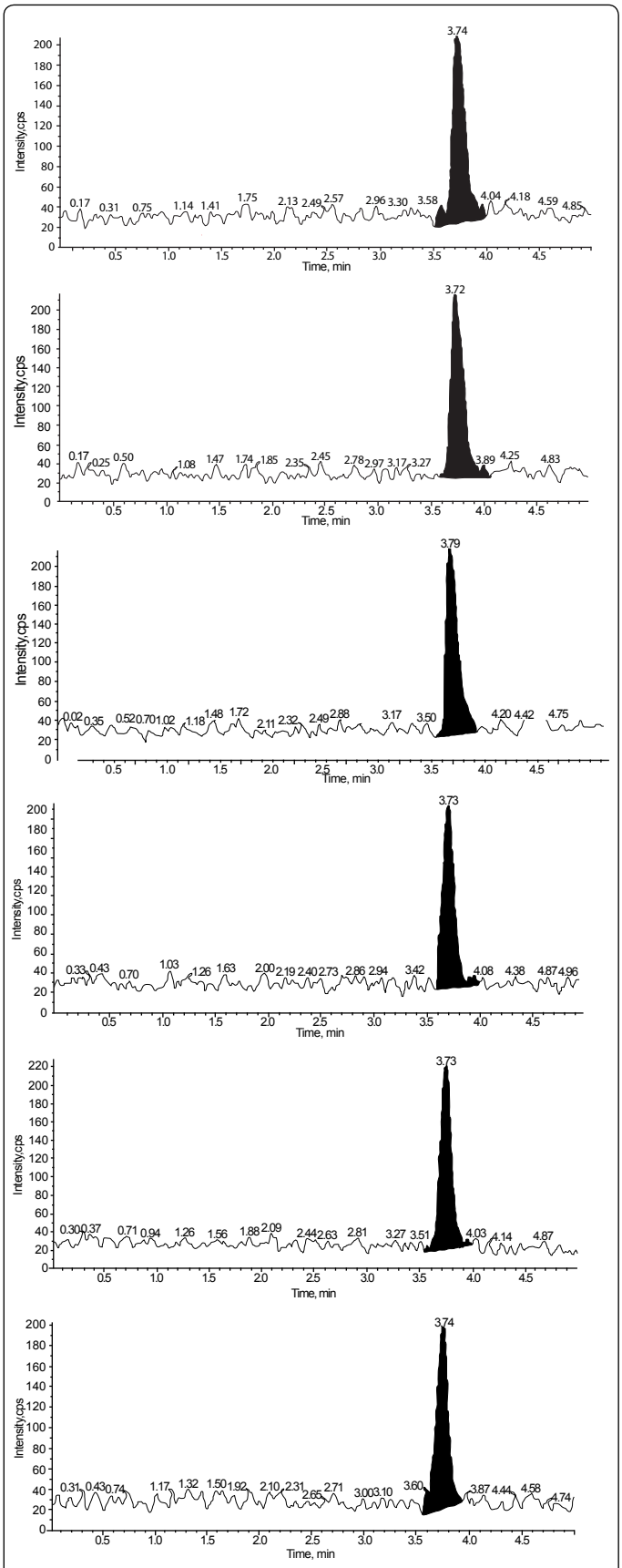



**Figure 4:** LOD precision mass spectrogram of 4 -Oxiranylmethoxy-9H-Carbazole.

Research Article
OPEN ACCESS Freely available online
doi:10.4172/1948-593X.1000029
JBABM/Vol.2 Issue 4

spectrograms are shown in Figure 4 and Figure 5 (Figure 5 is included in supplementry data). Further the results are tabulated in Table 2.

**Recovery of 4-oxiranylmethoxy-9H-carbazole from the API:** The accuracy of the method was evaluated in sample solutions were prepared in triplicate by spiking 4-Oxiranylmethoxy-9H-Carbazole at LOQ level to 150% with Carvedilol drug substance and injected each solution in to LCMS as per methodology. The percentage of recovery was calculated. A satisfactory value of 4-Oxiranylmethoxy-9H-Carbazole (96.10%, 97.70% and 101.5%) was found. At such low levels these recoveries and % RSD were satisfactory. Accuracy at LOQ to 150% level spectrogram is shown in Figure 6 and Figure 7 (Figure 6 and Figure 7 is included in supplementry data), further the results are tabulated in Table 3.

## Conclusion

LC-MS/MS method has been suitable for quantification of 4-Oxiranylmethoxy-9H-Carbazole for highly sensitive method of analysis with limit of detection 0.825 ppm. The methodology has one of the restrictions of 4-Oxiranylmethoxy-9H-Carbazole in Carvedilol sample.

### Acknowledgement

The authors gratefully acknowledge the management of Aurobindo Pharma Limited, in general and APL Research Centre in particular for allowing us to carry out the present work. The authors are also thankful to the colleagues of Analytical research department and Chemical research department.

### References

1. British pharmacopoeia (BP), pg374 2009.
2. EMEA Q&A EMEA/CHMP/SWP/43994/2007.
3. Guidelines on the limits of Genotoxic impurities EMEA-CIIMP/QMP/251344/2006, 28 June, 2006.
4. International conference on Harmonization (ICH) Guidelines, Q3A(R): Impurities in new drug substance February 2003.
5. Martindale 35th edition pg1114.
6. The European medicines agency (EMEA), committee for medical products for Human use (CHMP), Guidelines on the limits of Genotoxic impurities CPMP/SWP/5199/02.
7. U.S. Pharmacopoeia. (USP32 NF-27) (2007) 4036.
8. US FDA, Guidence for industry: genotoxic and carcinogenic impurities in drug substances and products: recommended approaches (draft), December 2008.