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Method Development and Validation of 2-[(2-Methoxyphenoxy) Methyl] Oxirane Content in Ranolazine Drug Substance by LC-MS/MS

Rahul Dev* **and Rahul Kumar**

Department of Chemistry,
ShriVenkateshwara University, Gajraula, Amroha (Uttar Pradesh), INDIA.
Corresponding Author: r.kumar31284@gmail.com.

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ABSTRACT

A LC-MS/MS method was developed for determination of 2-[(2-methoxyphenoxy) - methyl] oxirane using Xselect CSH ® C18 (100 x 3.0) mm, 2.5µm and a mobile phase of buffer pH 5.50 : Methanol with gradient, at flow rate of 0.3 ml/min with MS detector. The mass of 2-[(2-methoxyphenoxy) - methyl] oxirane was found 181.17 in ESI positive mode and the retention time was found 4.9 minutes. The proposed method was validated for System suitability, Specificity, Linearity, LOD and LOQ, Recovery, Precision, and Range. All the parameters were found within the acceptable limits. Linearity of 2-[(2-methoxy phenoxy) - methyl] oxirane was in the range of LOQ to 150% of specification level. LC-MS/MS method was specific, accurate, precise and suitable for the analysis of 2-[(2-methoxy phenoxy) - methyl] oxirane in Ranolazine drug substance.

Keywords: Liquid chromatography with mass spectrometry (LC-MS/MS), Genotoxic impurity, ICH guideline and Method Validation.

INTRODUCTION

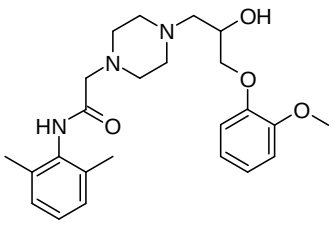
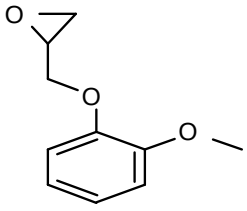
Genotoxic impurities are unwanted chemicals, have no therapeutic value and are potentially harmful. Therefore they need to be controlled in Active pharmaceutical ingredient and Drug products. Genotoxic substances are those which impact genetic material by means of mutations. Mutations can be chromosomal breaks, rearrangements, covalent binding or insertion into DNA during replication. Mutations may also occur indirectly by activating a cell to produce genotoxic substances. The focus of this study is on reactive substances they have a

potential to directly cause DNA damage when present low levels lading to mutations and there for potentially causing cancer. Because of this, it is important to identify genotoxic substances followed by monitoring and control at very low levels to ensure safety to the public.

The source of genotoxic impurities in pharmaceuticals (API & DP) can come from many places including starting materials, by products, reagents, intermediates, degradation products, ligands and catalysts, solvents or unwanted side reactions from the API synthetic process that get carried over into the final product. In addition, the Active pharmaceutical ingredient itself can decompose to form genotoxic impurities or they can form in the drug product by reaction between excipients or containers and the API. The employment of these compounds within the synthetic process is logical as these substances are reactive building blocks that come together to form complex drug substances.

Impurity guidelines have mainly been developed by international Conference on Harmonization (ICH). ICH Q3A regulates impurities in new drug substances with thresholds for reporting, identifying, and qualifying impurities. ICH Q3B is the equivalent guideline for impurities in new drugs. ICH Q3C controls residual solvent, and is the first time the ICH applied substance specific limits. Depending on their potential risk to human health. ICH Q3D is currently under development and will include elements and limits for heavy metal impurities. Currently released ICH guidelines for impurity limits are not suitable for most genotoxic impurities. The genotoxins material considered unsafe at any level. The limit for a genotoxin with an understood toxicity can be calculated based upon the konw PDE. The limit for the genotoxin without sufficient information must determine based upon TTC of 1.5µg/day.

Chemical Structure of Ranolazine and 2-[(2-Methoxyphenoxy) methyl] oxirane :

Ranolazine: ***Chemical Name:*** *N-(2,6-dimethylphenyl)-2-(4-(2-hydroxy-3-(2-methoxyphenoxy)propyl)piperazin-1-yl) acetamide* ***Molecular weight:*** *427.54*	
2-[(2-Methoxyphenoxy) methyl] oxirane *Chemical Name: (2-[(2-Methoxyphenoxy) methyl] oxirane)* ***Molecular weight:*** *180.2*	

METHOD DEVELOPMENT

Instrument, Chemicals and Reagents

The following reagents and chemicals were used during the evaluation studies:

Sr. No.	Name of the materials	Grade	Make
1	Ammonia solution	LC-MS	Merck
2	Formic acid	LC-MS	Merck
3	Methanol	LC-MS	Merck
4	Acetonitrile	LC-MS	Merck
5	Water	LC-MS	Milli-Q

Instrumentation

A Waters Xevo-TQD Liquid chromatography system with mass spectrometer equipped with an auto sampler.Column was employedin the method was Water Xselect CSH ® C18 (100 x 3.0) mm, 2.5µm.The flow rate selected was 0.3ml/min. All the weighing in the experiments was done with Mattler toledo electronic balance capable of measuring with an accuracy of 0.01 mg.

Glassware

All the volumetric glassware used in the study was grade a quality Borosil.

Chromatographic Conditions for LC:

Parameters	Description
Mobile Phase A	Pipette 1 mL Ammonia solution (25%) in 1000 mL milli-Q-water (v/v), Adjust pH to 5.50 ± 0.05 by diluted (10%) formic acid.
Mobile Phase B	Methanol
Flow rate	0.3ml/min
Colunm Temp.	45°C
Injection voume	3µl
Run time	12 minutes

Gradient Program for LC:-

MS Conditions:

Time (min)	% MP-A	% MP-B
0.0	55	45
6.00	55	45
6.10	10	90
9.00	10	90
9.10	55	45
12.00	55	45

Source Parameters		
1.	Capillary voltage	3.50kV
2.	Cone voltage	28V
3.	Source Temperature	120°C
4.	Desolvation Temperature	500°C
5.	Cone Gas	50 L/hr
6.	Desolvation Gas	1000 L/hr
MRM Parameters		

1.	Collision Gas	ON
2.	Collision Energy	6.0 (for both Daughter Ions)
3.	Ionization Mode	ES Positive
4.	Parent Ion	181.17
5.	Daughters Ions-I	124.94
6.	Daughters Ions-II	151.02

Preparation of Diluent:

Prepare mixture of Acetonitrile and HPLC grade water in the ratio (70:30) v/v. Mix well and sonicate to degas.

Preparation of Standard Solution:

Weighed accurately about 5.0 mg of *2-[(2-Methoxyphenoxy) methyl] oxirane* standard into 100 mL volumetric flask, dissolve and make volume up to the mark with diluent. (Stock-I). (Approx.50 ppm)

Pipette out 1.0 mL of this solution into 100 mL volumetric flask and make volume up to the mark with diluent (Stock-II). (Approx.0.50 ppm)

Pipette out 0.6 mL of this solution into 10 mL volumetric flask and make volume up to the mark with diluent. (Approx. 0.030 ppm)

Preparation of Test Solution:

Weigh accurately about 400.0 mg of sample in to 10 mL volumetric flask add 6 mL diluent sonicate this solution till clear and make volume up to the mark with diluent. (Approx.40000 ppm)

VALIDATIONOF HPLC METHOD

Specificity:

The specificity is defined as the ability to assess and ensure that the impurities, degradation product and diluent do not affect the sample analyzed.

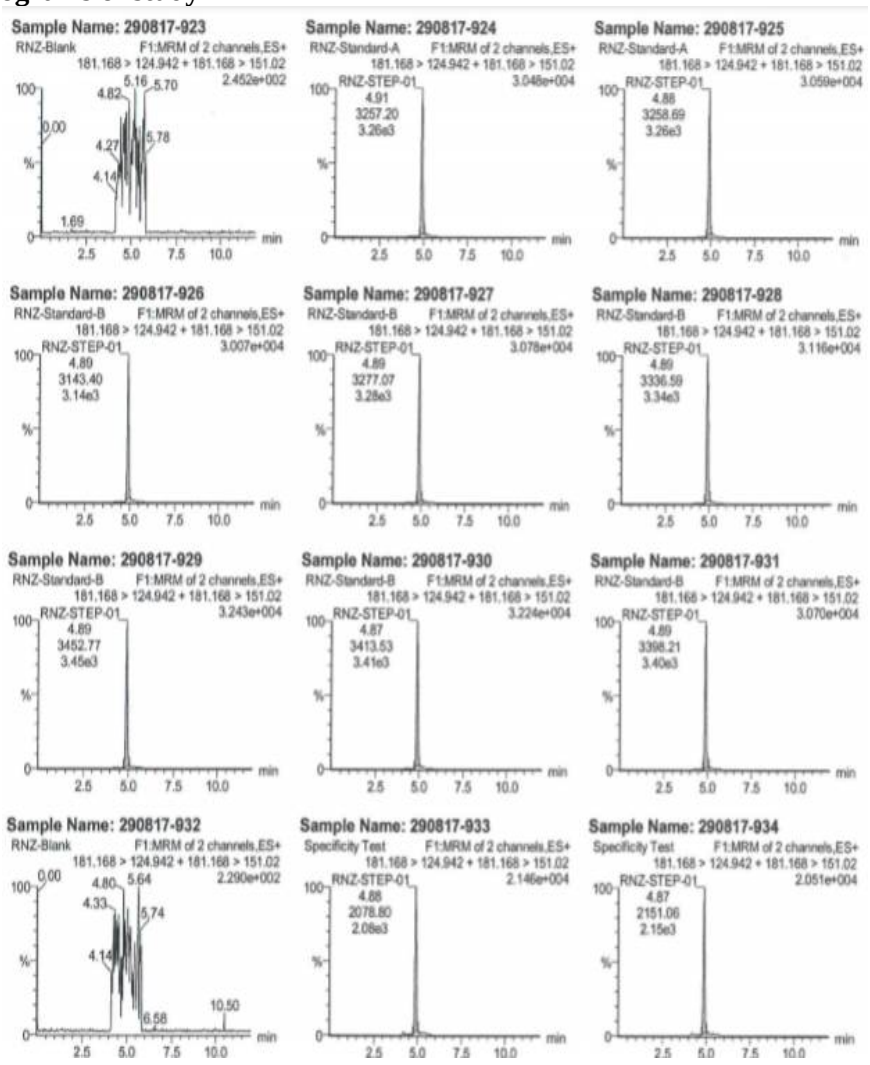
Inject the Blank (as Diluent), Standard solution, sample, individual impurities at specification level and spike sample of all impurities at specification level. Check the interference at the retention time and mass of analyte.

Table-I

Solution	***Peak Name***	***RT (min.)***	**Parent Ion**
Standard solution	*2-[(2-Methoxyphenoxy) methyl] oxirane*	4.91	181.168
Test solution	*2-[(2-Methoxyphenoxy) methyl] oxirane*	4.88	181.168
Spiked test solution	*2-[(2-Methoxyphenoxy) methyl] oxirane*	4.86	181.168
Related compound-A	--	4.69	--
Related compound-B	--	9.07	--
Related compound-C	--	2.19	--
Related compound-D	--	9.05	--
Related compound-E	--	8.56	--
Related compound-F	--	9.06	--
Related compound-G	--	5.16	--
Related compound-H	--	9.06	--
Related compound-I	--	3.01	--
Related compound-J	--	9.11	--
Related compound-M	--	6.03	--

No peak was observed in blank at the retention time of *2-[(2-Methoxyphenoxy) methyl] oxirane* peak and all peaks are well separated from *2-[(2-Methoxyphenoxy) methyl] oxirane*. There is no interference of blank and other components presents in sample matrix.

Chromatograms of study



Note: *2-[(2-Methoxyphenoxy) methyl] oxirane* = (RNZ)

Linearity:

A linearity study verifies that the sample solutions are in a concentration range where analyte response is linearly proportional to concentration of analyte. The linearity of method

was determined using different concentration of *2-[(2-Methoxyphenoxy) methyl] oxirane*. Calibration curve found to be linear from LOQ to 150% of specification level.

Preparation of 2-[(2-Methoxyphenoxy) methyl] oxirane standard stock solution for linearity:

Accurately weighed and transferred 5.382 mg of *2-[(2-Methoxyphenoxy) methyl] oxirane* working/reference standard into 100.0 mL volumetric flask. Added 20 mL of diluent to dissolve the content and diluted to the volume with diluent.

Pipette out 1.0 mL of this solution into 100 mL volumetric flask and make volume up to the mark with diluent.

Linearity solutions have been prepared as follows:

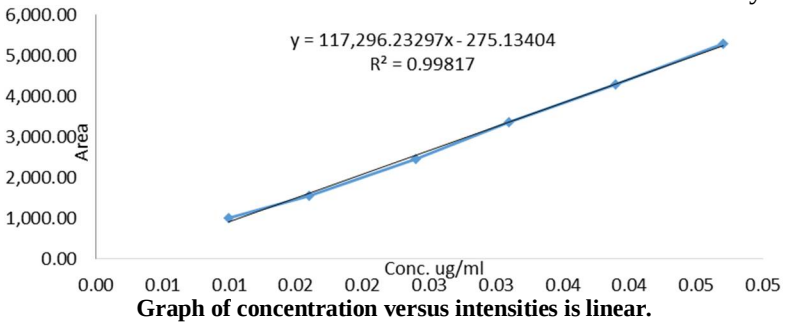
Linearity solution	Standard stock (mL)solution for Linearity	Volume (mL) make up to
QL	0.20	10
50%	0.30	10
75%	0.45	10
100%	0.60	10
125%	0.75	10
150%	0.90	10

Correlation coefficient for the linearity curve of *2-[(2-Methoxyphenoxy) methyl] oxirane* in Ranolazine drug substance found >0.99. For details, refer Table-II.

Table-II

Conc. level	Conc. (%w/w)	Mean area
QL	0.010	997
50%	0.016	1559
75%	0.024	2444
100%	0.031	3361
125%	0.039	4281
150%	0.047	5296
Square correlation		0.99817
Slope		117296.2329743
Y-intercept		-275.13404
Y-intercept at 100%		-8.19
Residue sum of square		24790.00000

The area response increases with the increase in the concentration of the analyte.



Graph of concentration versus intensities is linear.

Limit of detection (LOD):

Prepared standard solution as per methodology. Injected blank and standard solution and checked the acceptance criteria for system suitability. The lowest concentration of *2-[(2-Methoxyphenoxy) methyl] oxirane* shall be prepared and injected to obtain the detection limit.

Preparation of standard Solution:

Weighed accurately about 5.260 mg of *2-[(2-Methoxyphenoxy) methyl] oxirane* standard into 100 mL volumetric flask, dissolve and make volume up to the mark with diluent. (Stock-I).

Pipette out 1.0 mL of this solution into 100 mL volumetric flask and make volume up to the mark with diluent. (Stock-II).

Pipette out 0.6 mL of this solution into 10 mL volumetric flask and make volume up to the mark with diluent.

Preparation of DL solution

Pipette out 0.2 mL of stock-II solution into 20 mL volumetric flask and make volume up to the mark with diluent.

Precision at detection limit:

Table-III

Injection	Area of 2-[(2-Methoxyphenoxy) methyl] oxirane	S/N Ratio
1	408	137
2	401	154
3	422	103
4	419	112
5	409	176
6	446	198
Mean	418	137
STDEV	15.93424	147
%RSD	3.8	

As per obtained signal / noise and visual observation DL within the limit and the *2-[(2-Methoxyphenoxy) methyl] oxirane* peak can be detected reliably in six replicate injections at DL level for *2-[(2-Methoxyphenoxy) methyl] oxirane* peaks within the limit. Hence obtained concentration can be considered DL level for *2-[(2-Methoxyphenoxy) methyl] oxirane*.

Table-IV

Name	(ppm (w/w) with respect to test concentration)	(ppm)
2-[(2-Methoxyphenoxy) methyl] oxirane	0.130	0.0052

Limit of quantitation (LOQ):

The Quantitation limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

The limit of quantification is determined by establishing the signal to noise ratio. Inject the blank sample and the spiked sample at LOQ level in six replicates and calculate signal to noise ratio and the % RSD at LOQ level.

Preparation of QL solution

Pipette out 0.2 mL of stock-II solution into 10 mL volumetric flask and make volume up to the mark with diluent.

Precision at quantitation limit:

Table-V

Solution	Area of 2-[(2-Methoxyphenoxy) methyl] oxirane	S/N Ratio
1	935	351
2	921	347
3	903	275
4	993	269
5	994	128
6	1005	325
Mean	959	282
STDEV	43.93518	
%RSD	4.6	

As per obtained signal / noise and visual observation QL within the limit and the % RSD of six replicate injections at QL level for *2-[(2-Methoxyphenoxy) methyl] oxirane* peaks within the limit. Hence obtained concentration can be considered QL level for *2-[(2-Methoxyphenoxy) methyl] oxirane*.

Table-VI

Name	(ppm (w/w) with respect to test concentration)	(ppm)
2-[(2-Methoxyphenoxy) methyl] oxirane	0.260	0.0104

Recovery:

Recovery means the percentage of the true concentration of a substance recovered during the analytical procedure.

Prepared blank and standards solution as per methodology. Injected blank and standards solution and checked the acceptance criteria for system suitability. Accuracy was carried out accuracy (recovery) for *2-[(2-Methoxyphenoxy) methyl] oxirane* at QL level, 100% and 150% of specification level.

Solutions were prepared to determine the accuracy of the method in below mentioned manner:

S. No	Solution Name	Wt. of Sample (mg)	Stock-II Added	Volume make up to
1	Test Solution-1	404.61	-	10
2	Test Solution-2	402.72	-	10
3	Test Solution-2	403.61	-	10
4	Recovery at QL level Set-1	400.15	0.2	10
5	Recovery at QL level Set-2	404.64	0.2	10
6	Recovery at QL level Set-3	403.68	0.2	10
7	Recovery at 100% level Set-1	402.14	0.6	10
8	Recovery at 100% level Set-2	402.97	0.6	10
9	Recovery at 100% level Set-3	402.71	0.6	10
10	Recovery at 150% level Set-1	401.08	0.9	10
11	Recovery at 150% level Set-2	404.99	0.9	10
12	Recovery at 150% level Set-3	403.07	0.9	10

Injected all preparations of control sample, accuracy at QL level, accuracy at 100% target limit and accuracy at 150% target limit.
Average and individual % Recovery should not be less than 80 and not more than 120

Table –VII

Average test area in recovery				2224		
Recovery levels	**Average Area**	**Corrected Area**	**Amount added (ppm)**	**Amount recovered (ppm)**	**% Recovery**	**Average Recovery**
QL	3178	954	0.0104	0.0088	84.62	98.1
	3310	1086	0.0104	0.0099	95.19	
	3520	1296	0.0104	0.0119	114.42	
100%	5820	3596	0.0313	0.0331	105.75	107.4
	6044	3820	0.0313	0.0351	112.14	
	5779	3555	0.0313	0.0326	104.15	
150%	7479	5255	0.0470	0.0484	102.98	101.8
	7494	5270	0.0470	0.0481	102.34	
	7345	5121	0.0470	0.0470	100.00	

Obtained % individual and average recovery results found within the acceptance criteria, hence method is accurate.

Precision:

System precision:

Prepared and injected blank, standard solution-1, standard solution-2 and test solution as per methodology. Prepared following solution for repeatability study:

Preparation of standard Solution:

Weighed accurately about 5.124 mg of 2-[(2-Methoxyphenoxy) methyl] oxirane standard into 100 mL volumetric flask, dissolve and make volume up to the mark with diluent. (Stock-I).

Pipette out 1.0 mL of this solution into 100 mL volumetric flask and make volume up to the mark with diluent. (Stock-II).

Pipette out 0.6 mL of this solution into 10 mL volumetric flask and make volume up to the mark with diluent.

% RSD should not be more than 5.0 of six replicate of standard solution.

Table-VIII

S. No	Area of standard
1	2181
2	2116
3	2138
4	2206
5	2164
6	2264
Average value	2178
Std. Dev.	52.590557
%RSD	2.4

System precision found within the acceptance criteria for 2-[(2-Methoxyphenoxy) methyl] oxirane.

Method precision (Repeatability)

Prepared and injected blank, standard solution-1, standard solution-2 and test solution as per methodology. Prepared following solution for repeatability study:

Standard solutions preparation can be referred from System precision.

Preparation of repeatability solutions

S. No	Solution Name	Wt. of Sample (mg)	Stock-II Added (mL)	Volume made up(mL)
1	Test solution-1	405.24	--	10
2	Spiked test solution-1	403.69	0.60	10
3	Spiked test solution-2	404.35	0.60	10
4	Spiked test solution-3	402.21	0.60	10
5	Spiked test solution-4	402.89	0.60	10
6	Spiked test solution-5	402.43	0.60	10
7	Spiked test solution-6	403.82	0.60	10

% RSD should not be more than 10.0 of obtained 2-[(2-Methoxyphenoxy) methyl] oxirane content values in six preparations.

Table-IX

S. No	Solution Name	Average area	(ppm(w/w)
1	Test solution	1434	0.484
2	Spiked test solution-1	3809	1.290
3	Spiked test solution-2	3744	1.266
4	Spiked test solution-3	3560	1.210
5	Spiked test solution-4	3532	1.198
6	Spiked test solution-5	3473	1.179
7	Spiked test solution-6	3425	1.163
Average value			1.212
Std. Dev.			0.04999
%RSD			4.1

%RSD of obtained six assay results found within acceptance criteria, hence method is repeatable.

Range

Range of 2-[(2-Methoxyphenoxy) methyl] oxirane content in Ranolazine drug substance method was found between QL to 150% of specification level.

DISCUSSION

A chromatographic method involves demonstrating specificity, which is the ability of the method to accurately measure the 2-[(2-Methoxyphenoxy) methyl] oxirane response in the presence of all potential sample components. The chromatographic and mass spectroscopy parameters were fixed and LC-MS/MS system was studied for suitability of residual analysis. The developed method was performed for linearity, precision, Accuracy, specificity, LOD and LOQ.

CONCLUSION

A simple and sensitive method for the determination 2-[(2-Methoxyphenoxy) methyl] oxirane in Ranolazine drug substance by using LC-MS/MS was developed, validated and applied for the analysis of Ranolazine drug substance samples. The sample of Ranolazine drug substance was prepared with diulent. The method was validated to ensure the feasibility of the method for its application in routine analysis. The LOQs achieved through this method were lower than the genotoxic impurities limit.

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