Original Article

Development and Validation of Genotoxic Impurity in Esomeprazole Magnesium Trihydrate Active Pharmaceutical Ingredient by LC-MS/MS

Suresh Reddy Yelampalli[1]*, Venkata Shanmukha Kumar Jagarlapudi[1], Useni Reddy Mallu[2]

[1]Department of Chemistry, K.L. Educational foundation (Deemed to be K.L. University), Vijayawada, Andhra Pradesh, INDIA.
[2]Manasa Life Sciences, Hyderabad, Telangana, INDIA.

ABSTRACT

Objectives: A rapid, sensitive and selective analytical method has been developed and validated by liquid chromatography tandem Mass spectrometry (LC-MS/MS) for the quantification of traces of Cumene Hydroperoxide in Esomeprazole magnesium trihydrate active pharmaceutical ingredient. **Materials and Methods:** The chromatographic separation was carried out on a Develosil Phenyl phase-UG-5 150 x 4.6mm, 3µm column. The mobile phase consisting of 1% Ammonia solution buffer and acetonitrile, the flow rate was 0.9 mL/min with isocratic elution. **Results:** The retention time of Cumene Hydroperoxide was found 9.25 mins. The developed method was validated according to ICH guidelines. The system suitability was found 4.8% RSD and the linearity calibration curve was linear over the concentration range of 2.309 ppm to 12.747 ppm (r = 0.999). The intra-day and inter-day precision (RSD %) was 2.1% and the obtained recovery (%) was at LOQ to 150% is in between 92.4% to 102.8% respectively. **Conclusion:** The low RSD values and high recoveries of the method confirms the suitability of the method for quantification of Cumene Hydroperoxide in Esomeprazole magnesium trihydrate API.

Key words: LC-MS/MS, Esomeprazole magnesium trihydrate, Cumene Hydroperoxide, Method validation, ICH guidelines.

INTRODUCTION

Synthesis of active pharmaceutical ingredients often involves the use of reactive substances and hence, these reactive substances may be extant in the final drug substances as impurities. Such chemically reactive impurities may have unwanted toxicities, including carcinogenicity and genotoxicity and these impurities are to be controlled based on the maximum daily dose.[1] These limits normally fall at low µg/mL levels and hence conservative GC, HPLC methods (or final drug substance methods) are not suitable for their determination. Sophisticated techniques like LC–MS combine physical separation competences of chromatography (HPLC) with the mass analysis competences of mass spectrometry and have specificity and high sensitivity over conservative HPLC method. Their applications are oriented towards the potential identification and quantitation of trace level of impurities in drug substances.[2]

Cumene hydroperoxide[3] is genotoxic, including DNA damage and mutations in prokaryote systems. DNA single strand breaks were included in isolated rat hepatic nuclei, but single or double strand breaks were not induced in the DNA of lysed human adenocarcinoma cells. Cumene hydroperoxide enhanced asbestos-induced damage in calf thymus DNA.

Cumene hydroperoxide is relatively stable organic peroxide. It is typically used as an oxidizing agent. Its formula is $C_6H_5C(CH_3)_2OOH$ and chemical structure is depicted in Figure 1. Cumene hydroperox-

Submission Date: 05-05-2019;
Revision Date: 04-09-2019;
Accepted Date: 21-09-2019

DOI: 10.5530/ijper.53.4s.160

Correspondence:
Mr. Suresh Reddy Yelampalli ,
Department of Chemistry, K.L. Educational Foundation (Deemed to be K.L. University), Vijayawada, Andhra Pradesh, INDIA.
Phone: +91 9493453531
E-mail: sureshreddy.y777@gmail.com

www.ijper.org

ide available with a purity of ~80% in commercial market.

Esomeprazole is the enantiomer of omeprazole. Chemically it is 5-Methoxy-2- (S) [(4-methoxy-3, 5-dimethyl-2-pyridinyl) methyl] sulfinyl]-1H-benzimidazole magnesium salt trihydrate with molecular formula $C_{34}H_{36}MgN_6O_6S_2 \bullet 3H_2O$ $C_{17}H_{18}N_3O_3S \bullet Na$ and chemical structure depicted in Figure 2.

Esomeprazole is cost effective in treatment of gastric oesophageal reflux diseases.[4] Esomeprazole magnesium was developed as the S-isomer of omeprazole as an attempt to improve its pharmacokinetic properties.[5]

Esomeprazole is highly bound (97%) to plasma proteins and primarily metabolized by 2 cytochrome P450 (CYP) isozymes, CYP3A4 and CYP2C19, with CYP2C19 being the predominant metabolic pathway.[6] There are stereo selective differences in the metabolism of PPIs by the cytochrome P450 (CYP) isoenzymes 2C19 and 3A4 and this is the basis of the observed pharmacodynamic and clinical efficacy differences between Esomeprazole and omeprazole.

Literature survey reveals that very few analytical methods have been established for the determination of Esomeprazole[7-9] and Cumene hydroperoxide.[10] To the best of our knowledge, there is no reported LC-MS/MS method for determination of Cumene Hydroperoxide in Esomeprazole magnesium trihydrate active drug, previous to our work. Thus, efforts were made to develop fast, selective and sensitive analytical method for the determination of Cumene Hydroperoxide in Esomeprazole magnesium trihydrate raw material by using LC-MS/MS. In the current work, developed and validated[11] a simple, reliable and reproducible LC-MS/MS method, which was duly validated by statistical parameters like, precision, accuracy and recovery.

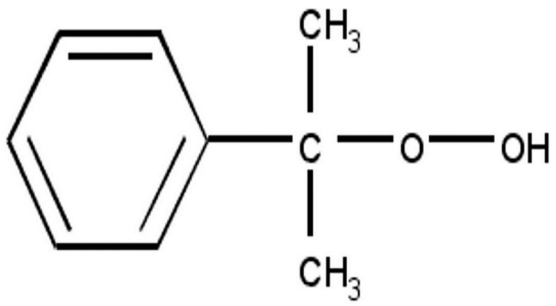


Figure 1: Chemical structure of Cumene Hydroperoxide.

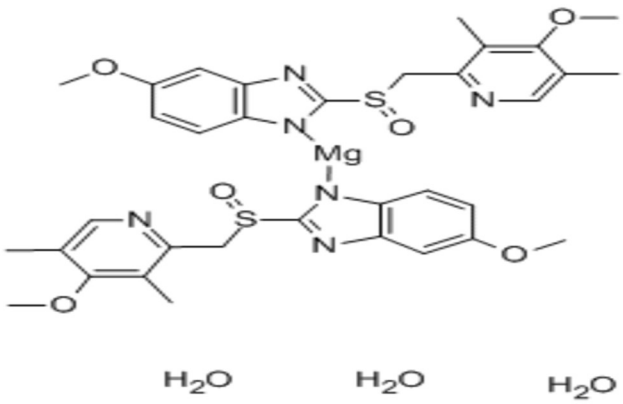


Figure 2: Chemical structure of Esomeprazole magnesium trihydrate.

MATERIALS AND METHODS

Esomeprazole magnesium trihydrate and Cumene Hydroperoxide was the generous gift from Everest organics limited, Hyderabad, India. HPLC grade acetonitrile and Methanol was procured from Qualigens, India. Ammonia solution and Formic acid were purchased from Loba Chemie, Mumbai, India. All other chemicals and solvents used analytical grade. Water used in the LC-MS/MS analysis was prepared by the water purifier (Arium®, 611UF, Sartorius, Germany). The mobile phase solution was filtered through a 0.45 µm Ultipor® N66® membrane filter (Pall Life Sciences, USA) prior to use.

Instrumentation and Chromatographic conditions

Instrument Name: LC-MS/MS coupled with ABSCIEX triple Quadruple Mass spectrometry.

Make and Model: ABSCIEX QTRAP 4500 or Equivalent

Column: Develosil PH-UG-5 150X 4.6mm

Flow rate (µL/min): 900

Injection volume: 20

Elution mode: Isocratic

Column temperature (°C): 30

Run time: 15 mins

Rinsing solvents: Methanol

MS-Parameter

Ionization mode: TIS

Scan type: MRM

Polarity mode: Positive

MS/MS-Parameters (For AB SCIEX)

CUR: 20.0

CAD: Medium

TEM: 500

IS(v): 5500

GS1: 60.0

GS2: 60.0

DP: 40.0

EP: 10.0

CE: 18.0
CXP: 10.0
Q1: 134.9(M+H)
Q3: 106.9
Dwell (msec): 200

Valco valve diverter program

Events	Time (min)	Position
1	0.0	A
2	0.5	A
3	0.6	B
4	5.9	B
5	6.0	A
6	15.0	A

MS/MS-Parameters (For Waters XEVO-TQS)

Capillary (Kv): 3.50
Cone (V): 30.0
Source offset (V): 50.0
Source Temperature (°C): 150
Desolvation Temperature (°C): 400
Cone Gas Flow (L/Hr): 150
Desolvation Gas Flow (L/Hr): 850
Collision gas flow: (mL/Min): 0.2
Nebuliser Gas Flow (Bar): 7.0

Analyser

LM-1 Resolution: 2.8
HM-1 Resolution: 15.0
MS Mode Collision Energy: 2.0
MSMS Mode Collision Energy: 20.0
LM-2 Resolution: 2.7
HM-2 Resolution: 15.0
Ion Energy 2: 0.6
Gain: 1.0
Collision Energy: 12
Ch1:135.16(M+H)
Daughter mass: 107.00

Preparation of Ammonia buffer

Take 1.0 mL of ammonia solution in 1000mL of Milli Q water. Filter and degas through 0.22µm membrane filter paper.

Preparation of solution A

Use buffer

Preparation of solution B

Prepare a mixture of buffer and acetonitrile in the ratio of 10:90 (%v/v)

Preparation of mobile phase

Prepare a mixture of solution A and solution B in the ratio of 25:75 (%v/v)

Preparation of diluent

0.25% formic acid in methanol.

Preparation of Cumene Hydroperoxide standard stock solution

Weigh accurately and transfer about 10.0 mg of Cumene Hydroperoxide standard into 100 mL volumetric flask, dissolve and dilute the volume with diluent and mix well. Transfer 2.55 mL of above solution into a 100 mL volumetric flask and dilute the volume with diluent and mix well.

Preparation of Cumene Hydroperoxide standard solution

Transfer 5.0 mL of above Cumene Hydroperoxide standard stock solution into a 50 mL volumetric flask and dilute the volume with diluent and mix well.

Preparation of test solution

Weigh accurately and transfer about 150 mg of test sample into 5 mL volumetric flask, dissolve and dilute the volume with diluent then sonicate and mix well.

Specification

Not more than 8.5 ppm

Method validation

The developed method was validated as per United States Pharmacopoeia general chapter <1225>[12] and ICH Q2 (R1) guidelines

System suitability

The system suitability test was performed using six replicate injections of standard and evaluated the system suitability.

Specificity

The method was validated for specificity by injecting diluent as a blank solution in triplicate and evaluated for blank peaks interference at the retention time of Cumene Hydroperoxide (Mass:135.16).

Precision

Precision of the method was evaluated by injecting the six impurity spiked test samples preparations from a homogeneous Esomeprazole magnesium trihydrate API. The % relative standard deviation of six spiked test samples was calculated for Cumene Hydroperoxide. Intermediate precision of the method was also evaluated as same like precision by using different instru-

ment and different column by injecting the six impurity spiked test samples preparations.

Limit of detection and limit of quantification

The LOD and LOQ are expressed as a known concentration of Cumene Hydroperoxide at a specified signal to noise ratio, usually for LOQ 10:1, for LOD 3:1 can be quantitated and detected under the stated LC/MS method.

Linearity

Linearity was conducted by preparing the six levels of linearity solutions for Cumene hydroperoxide from the range of LOQ (2.309 ppm) level to 150% (12.747 ppm) level. The pure form of Cumene Hydroperoxide was used to prepare the linearity solutions. Draw a linearity graphs for the peak area against concentration.

Accuracy

The recovery of the method was evaluated by spiking the Cumene Hydroperoxide in test sample at the concentration of LOQ, 50%, 100% and 150% of the specification level. The recovery samples were prepared in triplicate at each level and injected in the proposed LC-MS/MS method. The % recovery of Cumene Hydroperoxide was calculated at each level. The acceptance limit for recovery of Cumene Hydroperoxide was 90.0 to 110.0%.

Solution stability

The bench top stability of Cumene hydroperoxide standard and spiked sample preparation established up to 12 hrs, after preparation ($n = 1$) at bench top condition. Solution stability was evaluated by similarity of standard and % absolute difference of test sample.

Robustness

The robustness of the method was evaluated by deliberately altering the method conditions from the original method parameters on same concentration of the Cumene hydroperoxide. Test sample was analyzed with three different preparations. Robustness of the method was assessed by varying the instrumental conditions such as flow rate (± 10%) and column temperature (± 5°C). The robustness study was evaluated by the calculation of the % content of Cumene hydroperoxide in spiked sample.

RESULTS AND DISCUSSION

Method development

Initially the method was developed on HPLC by using the mixture of ammonia buffer and acetonitrile in the ratio of 70:30 as a mobile phase with 1.2mL flow rate by using 257nm with UV Detector. In the HPLC method the response of Cumene hydroperoxide at specification level was very low and the obtained area response was not sufficient and not reproducible for the quantifying the Cumene hydroperoxide and alternatively developed and optimized a liquid chromatography tandem Mass spectrometry (LC-MS/MS) with using of 1% Ammonia solution buffer and acetonitrile as mobile phase and phenyl phase column with Mass detector and the obtained area response was reproducible and the retention time of the Cumene Hydroperoxide was about 9.25 min.

System suitability

The system suitability of the method was demonstrated by means of % RSD of area of six replicate standards. The obtained results of % RSD for six replicates of Cumene hydroperoxide peak area from standard was less than 5.0%. System suitability parameter results are presented in Table 1.

Specificity

Specificity study was performed to check the no interference of blank peaks at the retention time of Cumene Hydroperoxide. The chromatograms of blank, standard, test sample and spiked test sample were shown in Figure 3-6 respectively, no blank peaks are present at the retention time of Cumene hydroperoxide.

Precision

Precision of the method was evaluated by injecting the six impurity spiked test samples preparations from a homogeneous Esomeprazole magnesium trihydrate API. The % relative standard deviation of six spiked test samples from precision and intermediate precision study was 1.3 and 1.1 respectively. The cumulative % RSD of Method precision and intermediate precision

Table 1: System suitability results.

Standard Replicate injections	Cumene hydroperoxide Peak Area
1	66738
2	65765
3	71597
4	68001
5	71902
6	63742
Mean	67957.5
%RSD	4.8

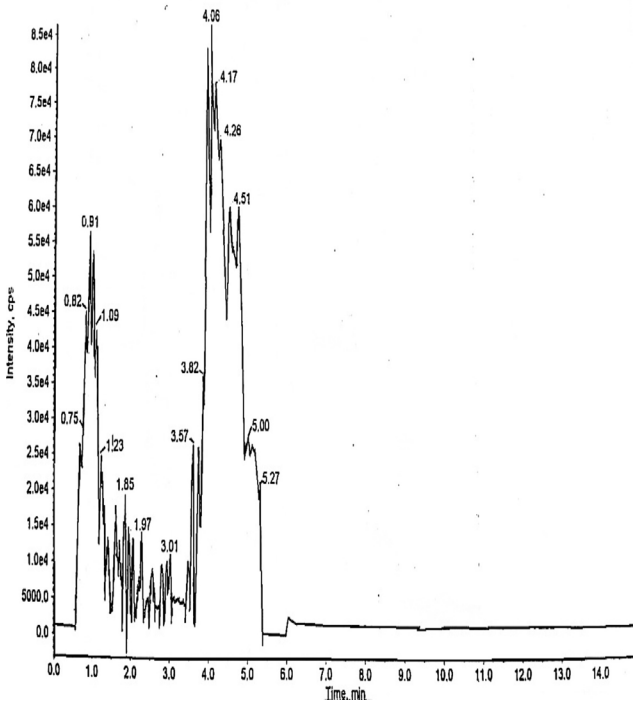


Figure 3: Typical Chromatogram of Blank.

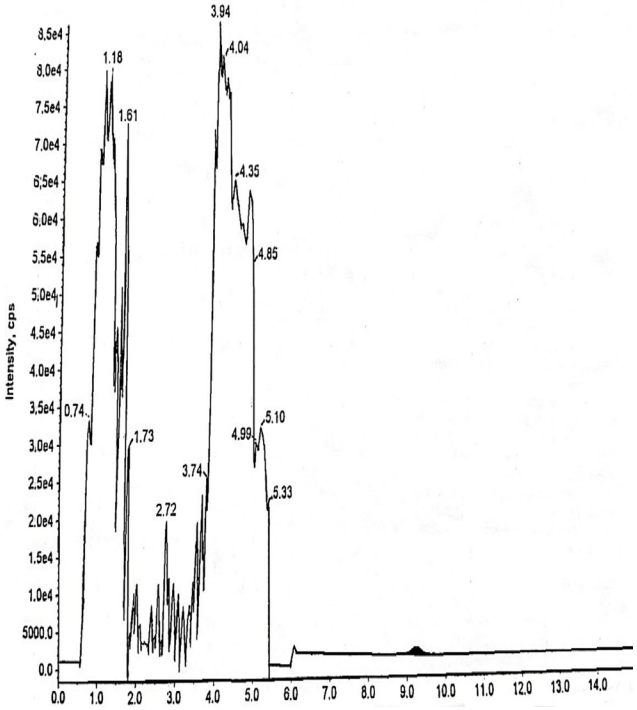


Figure 5: Typical Chromatogram of Test sample.

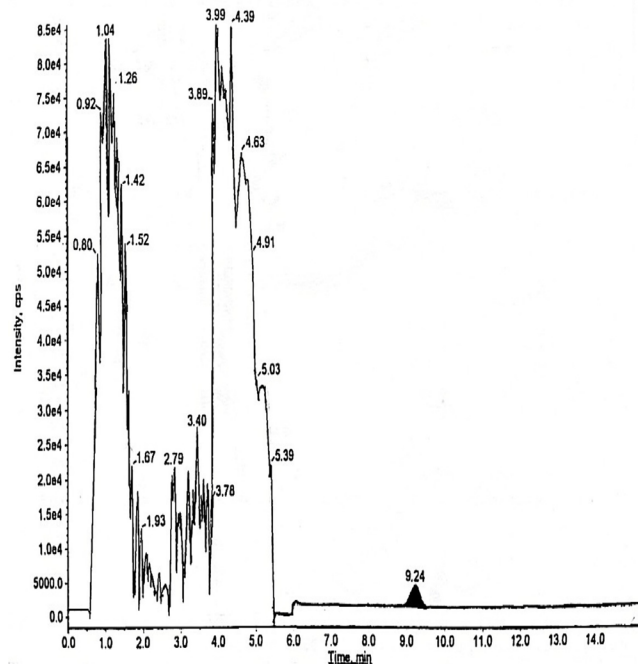


Figure 4: Typical Chromatogram of Standard.

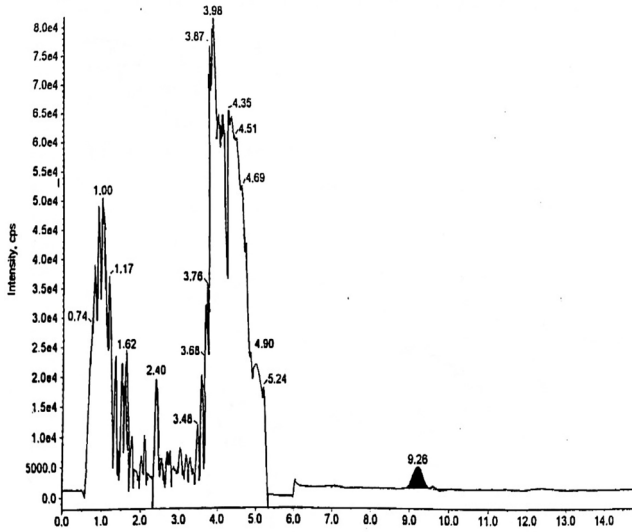


Figure 6: Typical Chromatogram of Spiked test sample.

study was 1.2. The graphical presentation of Precision and Intermediate precision study shown in Figure 7.

Limit of detection and limit of quantification

The LOD and LOQ values are established by signal to noise ratio method, the obtained S/N value of Cumene hydroperoxide were 10.9 at LOQ concentration 2.309 ppm, 3.6 at LOD concentration 0.0775 ppm with respect to test concentration. The Chromatograms for Detection limit and Quantification limit level were shown in Figure 8 and Figure 9 respectively.

Linearity

Linearity was conducted by preparing the six levels of linearity solutions and found linear from LOQ (2.309 ppm) level to 150% (12.747 ppm) level for Cumene hydroperoxide. Drawn a linearity graph for Cumene Hydroperoxide peak area against its concentration and linearity graphs shown in Figure 10 and linearity data is presented in Table 2.

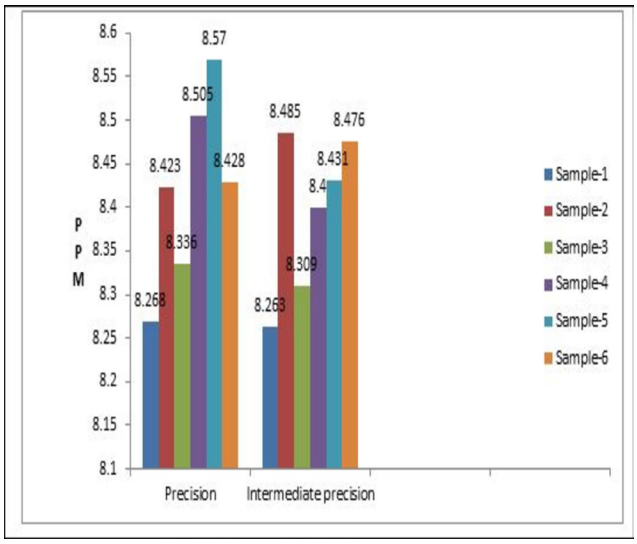


Figure 7: Graphical presentation of Precision and Intermediate precision study.

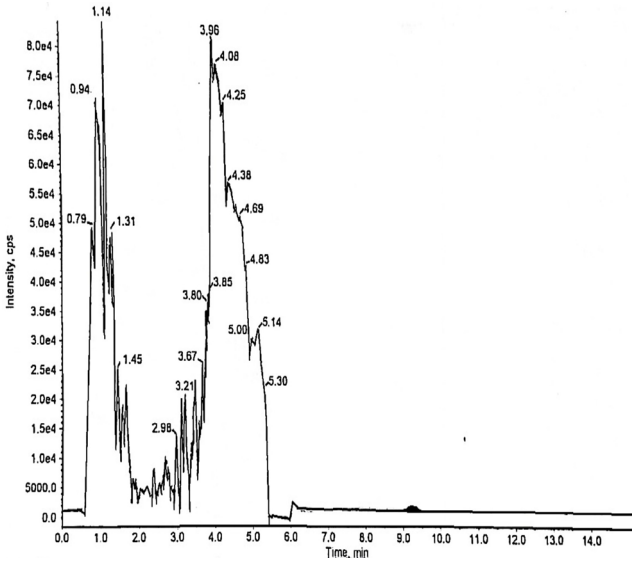


Figure 9: Typical Chromatogram of Quantification limit.

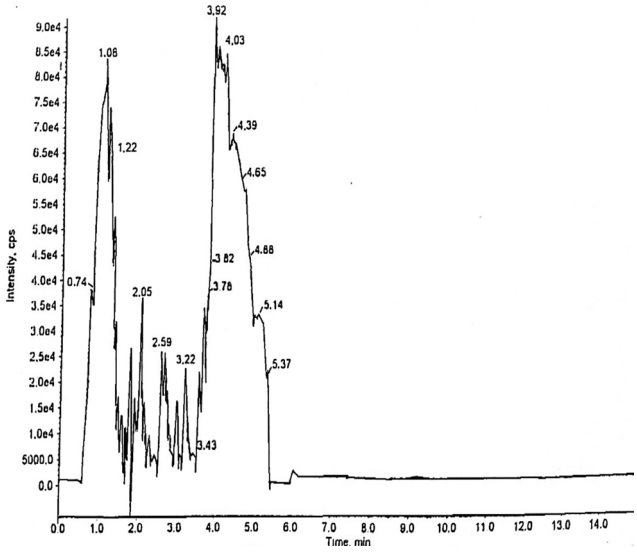


Figure 8: Typical Chromatogram of Detection limit.

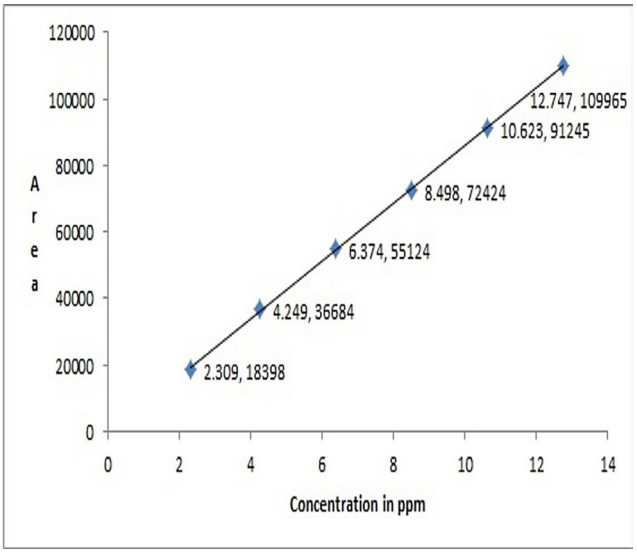


Figure 10: Linearity graph for Cumene Hydroperoxide.

Accuracy

The accuracy was evaluated by spiking the Cumene hydroperoxide in sample solution corresponding at LOQ, 50%, 100% and 150% of specification level. Three spiked samples were prepared at 50% and 100% levels and six spiked samples were prepared at LOQ and 150% level of specification. Each spiked sample solution was injected and all the found results were satisfactory and the results are presented in Table 3.

Solution stability

The bench top stability of Cumene hydroperoxide standard and spiked sample established up to 12 hr and both standard and test sample stable up to 12 hr. Similarity factor of standard at 12 hr was found 0.99 and the absolute difference of Cumene Hydroperoxide content in ppm was with in the 0.20%
Absolute difference.

Robustness

Robustness of the method was assessed by varying the instrumental conditions such as flow rate (± 10%) and column temperature (± 5°C). The deliberate changes in the method have no significant changes in the % content of Cumene hydroperoxide in spiked sample. The robustness results were presented in Table 4.

CONCLUSION

The developed and validated method is simple, sensitive, innovative and economical method for determine

Table 3: Accuracy results for Cumene Hydroperoxide.

Level	% Recovery	Mean %Recovery	%RSD
LOQ	94.8	94.5	2.4
	92.6		
	92.4		
	98.5		
	95.2		
	93.4		
50 %	100.2	100.4	0.7
	101.2		
	99.8		
100 %	100.5	100.7	0.7
	101.5		
	100.2		
150 %	101.6	101.0	1.2
	102.8		
	101.6		
	99.9		
	100.0		
	100.1		

Table 4: Robustness study results.

Parameter	Variation	Cumene hydroperoxide (ppm)		
		Test sample-1	Test sample-2	Test sample-3
Flow Rate mL/min	Low Flow	8.347	8.247	8.228
	High Flow	8.467	8.201	8.397
Column temperature	25°C	8.310	8.131	8.437
	35°C	8.362	8.155	8.237
As such condition		8.476	8.331	8.400

Table 2: Linearity results for Cumene Hydroperoxide.

Linearity levels	Cumene hydroperoxide	
	Concentration (%)	Area response
1	2.309	18398
2	4.249	36684
3	6.374	55124
4	8.498	72424
5	10.623	91245
6	12.747	109965
Correlation Coefficient (r)	0.9999	
Intercept	- 966.37	
Slope	8697.28	

the low level Cumene Hydroperoxide content by liquid chromatography tandem Mass spectrometry (LC-MS/MS). The validation study performed as per USP and ICH guidelines and it revealed that the method is specific, linear, precise, accurate and robust over the range of 2.309 ppm to 12.747ppm. Hence it is concluded that the proposed method can be used for routine and stability analysis in quality control laboratories in pharmaceutical industries.

ACKNOWLEDGEMENT

The authors express their thanks to K.L. University for support and providing the research facility to carrying out the work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

RP-HPLC: Reverse Phase High Performance Liquid Chromatography, **UV:** Ultraviolet, **ICH:** International Conference on Harmonization, **USP:** United States Pharmacopoeia, **LOQ:** Limit of Quantitation, **LOD:** Limit of Detection, **RSD:** Relative Standard Deviation.

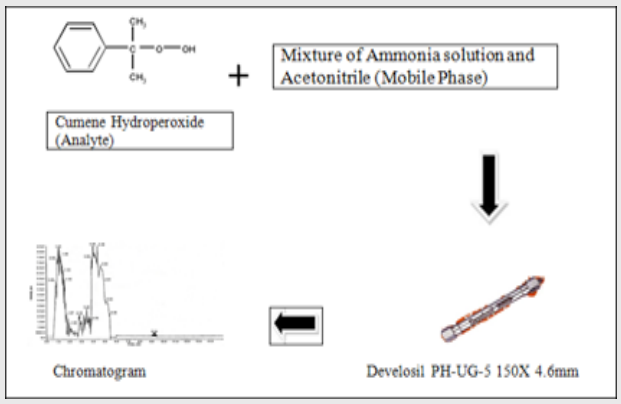
REFERENCES

1. Guideline EM. Guideline on the limits of genotoxic impurities. https://www.ema.europa.eu.genotoxic impurity
2. Raman NV, Prasad AV, Reddy KR. Strategies for the identification, control and determination of genotoxic impurities in drug substances: A pharmaceutical industry perspective. Journal of Pharmaceutical and Biomedical Analysis. 2011;55(4):662-7. https://www.ncbi.nlm.nih.gov/pubmed/21193280
3. Zhang M, Wang L, Ji H, Wu B, Zeng X. Cumene liquid oxidation to cumene hydroperoxide over CuO nanoparticle with molecular oxygen under mild condition. Journal of Natural Gas Chemistry. 2007;16(4):393-8. https://doi.org/10.1016/S1003-9953(08)60010-9.
4. Sharma S, Sharma MC. Densitometric method for the Quantitative determination of Esomeprazole and Domperidone. American-Eurasian Journal of Toxicological Science. 2011;3(3):143-8. http://www.i-scholar.in/index.php/AEJTSIDOSI/article/view/59257.
5. Kulkarni S, Tripathi S, Mehta PD, Lodhi NS, Sengar NP. Esomeprazole in the treatment of acidic disorder: An overview. Asian Journal of Biochemical and Pharmaceutical Research. 2011;1(2):562. https://issuu.com/ijcp/docs/ijcp_nov_2012/20.
6. Isaza C, Henao J, Martínez JH, Arias JC, Beltrán L. Phenotype-genotype analysis of CYP2C19 in Colombian mestizo individuals. BMC Clinical Pharmacology. 2007;7(1):6. https://www.ncbi.nlm.nih.gov/pubmed/17623107.
7. Andersson T, Hassan-Alin M, Hasselgren G, Röhss K, Weidolf L. Pharmacokinetic studies with esomeprazole, the (S)-isomer of omeprazole. Clinical Pharmacokinetics. 2001;40(6):411-26. http://europepmc.org/abstract/med/11475467.
8. Gopinath S, Kumar RS, Shankar MB, Danabal P. Development and validation of a sensitive and high-throughput LC-MS/MS method for the simultaneous determination of esomeprazole and naproxen in human plasma. Biomedical Chromatography. 2013;27(7):894-9. https://doi.org/10.1002/bmc.2878.
9. Onal A, Oztunc A. Development and validation of high performance liquid chromatographic method for the determination of esomeprazole in tablets. Journal of Food and Drug Analysis. 2006;14(1):12.You ML. Thermal hazard evaluation

of cumene hydroperoxide-metal ion mixture using DSC, TAM III and GC/MS. Molecules. 2016;21(5):562.Guideline IH. Validation of analytical procedures: Text and methodology Q2 (R1). International conference on harmonization, Geneva, Switzerland 2005:11-2. https://www.ich.org › Quality › Q2_R1 › Step4 › Q2_R1_ Guideline.

10. United States Pharmacopeial Convention. General chapter <1225>: Validation of compendial procedures. Rockville, MD: United States Pharmacopeial Convention. https://hmc.usp.org › documents › HMC › GCs-Pdfs › c1225_1SUSP40

PICTORIAL ABSTRACT



About Authors

Suresh Reddy Yelampalli, Department of Chemistry, K L Education Foundation (Deemed to be K.L. University), Vijayawada, Andhra Pradesh, India.

Venkata Shanmukha Kumar Jagarlapudi, Head of the Department, Chemistry, K L Education Foundation (Deemed to be K.L. University), Vijayawada, Andhra Pradesh, India.

Useni Reddy Mallu, Managing Director, Mansa Life Sciences, Hyderabad, Telangana, India.

SUMMARY

- The present study describes the use of LC-MS/MS method for those molecules which are not having high response in HPLC with UV detection.
- The Principle behind the LC-MS/MS method is identifying the components based on their mass in LC-MS/MS chromatogram.
- Method was developed on Develosil Phenyl phase-UG-5 150 x 4.6 mm, 3 µm column. The mobile phase consists of 1% Ammonia solution buffer and acetonitrile, the flow rate was 0.9 mL/min with isocratic elution. The retention time for Cumene hydroperoxide was found about 9.25 mins (Mass: 135.16).
- The developed method was validated as per ICH Q2 (R1) guidelines and USP general chapter <1225>.
- The developed method is simple, innovative, specific, robust, accurate and economical. This method can be used for regular analysis in quality control and research laboratory.

Cite this article: Suresh Reddy Yelampalli SR, Kumar VJS, Mallu UR. Development and Validation of Genotoxic Impurity in Esomeprazole Magnesium Trihydrate Active Pharmaceutical Ingredient by LC-MS/MS. Indian J of Pharmaceutical Education and Research. 2019;53(4s):s642-s649.