wjpmr, 2021,7(6), 224-228

SJIF Impact Factor: 5.922

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

ANALYTICAL ESTIMATION AND VALIDATION OF GENOTOXIC IMPURITY IN PROMETHAZINE HYDROCHLORIDE OF INDIAN MARKETED FORMULATIONS USING GC-MS

Kandula Kiriti Kalyan Kumar[1*], Dr. NDVR Saradhi[2] and Dr. M. Venkata Reddy[3]

[1]Research Scholar, Shri JJT University, Rajasthan.
[2]Principal, Dept. of Pharmacy, Prabhath Institute of Pharmacy, Nandyal, AP.
[3]Director, Sree Dattha Institute of Pharmacy, Ibrahimpatnam, RR Dist, Telangana.

***Corresponding Author: Kandula Kiriti Kalyan Kumar**
Research Scholar, Shri JJT University, Rajasthan.

Article Received on 09/04/2021 **Article Revised on 30/04/2021** **Article Accepted on 20/05/2021**

ABSTRACT

The main aim of the work to develop a simple, sensitive and rapid method for the estimation of Dimethyl Amino Isopropyl Chloride in Promethazine API and formulations by GC MS method. The method is developed by using GC column of 30m*0.32 mm*1.80 um and Helium as carrier gas; The MS conditions are fixed with ESI at 220^{0}C with interference temperature of 250^{0}C and ions are trapped by ion collector and detected by photomultiplier tube detector using single ion monitoring mode (SIM) at m/e of 121 to determine Dimethyl amino isopropyl chloride in standard and samples. The peak obtained at 6.773 min for Dimethyl amino isopropyl chloride and 7.812 min for Promethazine. The analytical method is validated as per USFDA impurity guidelines for its System Suitability, Accuracy, Precision, Intermediate precision and Method precision whose %CV were found to be 9.21, 85 – 89%, 5.68, 5.68, 5.89 respectively for Dimethyl Amino Isopropyl chloride and Limit of Detection and Limit of Quantification were be at 0.075 and 0.025 ppm respectively and the range between 0 to 3.8 ppm. The method is applicable for the analytical estimation of Dimethyl amino isopropyl chloride in academic research, BA/BE studies in Promethazine API, marketed formulations and Biological samples.

KEYWORDS: Dimethyl Amino Isopropylene Chloride, Promethazine, GC-MS, Validation etc.

INTRODUCTION

The Promethazine is a pehnothiazines derivative, it works by changing the chemicals in the brain and also act as antihistamine, the IUPAC name is N, N-dimethyl-1-phenothiazin-10-ylpropan-2-amine.[16, 12] The Dimethyl amino Isopropylene chloride is a class 2 solvent impurity used in the manufacture of injection and tablet form of Promethazine, The Tablet form does contain this impurity.[18] The impurity in the Promethazine is Dimethyl Amino Isopropylene Chloride is added during the manufacturing process into tablets.[8] Promethazine structure and Dimethyl amino Isopropylene chloride are shown in fig 1 and 2 as.[1] The recent Literatures shows evidence of presence of genotoxic impurities in Promethazine marketed formulations this is due to the degradation of structure and/or obtained from intermediate products and raw materials used in manufacturing process of Promethazine tablets.[20,21,22]

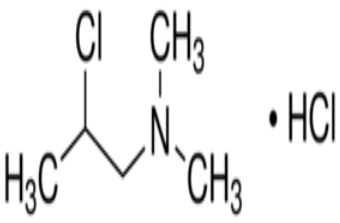

Fig 1: Dimethyl Amino Isopropyl chloride

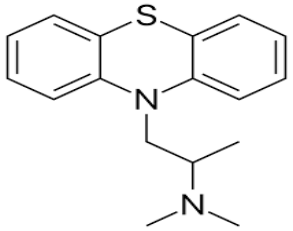

Fig 2: Promethazine

The gas Chromatography is a method used for the Qualitative and Quantitative analysis of Thermo labile and volatile natured organic compounds.[8] The Mobile Phase as Helium flowed through the column at 1ml/min, The Helium reduces the time of analysis for the most of the compounds and having high flow rate and

Viscosity[15] and a Non Polar silica column is build with 6% cyano propylene Phenyl and 94% Dimethyl Polysiloxane and an equivalent to USP G43 column[6] filled in glass SCOT column having 60m*0.32µ*0.18µ. The Mass Spectrometer is used to identify the positive ions formed during the ionization of the eluent from GC which is hyphenated with MS by Interference, the temperature is maintained at ionization and interference chamber at 220 and 250^{0}C respectively and the positively charged ions travel through Q3 Analyzer with Single ion monitoring (SIM)[17] The Q3 Mass Analyzer consists of two Quadruples in a series and a RF Quadruple analyzer is placed in between.[3] The ions collected at Ion traps and Detected at Photomultiplier tube. In GC MS the sample mixture is directly vaporized at 70-250^{0}C temperature slowly raising 20^{0}C for every 5 sec the components of the mixture are separated based on their affinity difference with bonded phase. The separated compounds exit the column and enter the vacuum system of the MS. The sample molecules are ionized and accelerated into a pre calibrated mass analyzer. Retention times, Molecular masses and fragmentation patterns are recorded.[9]

MATERIALS AND METHODS

The chemicals used are HPLC grade having purity not less than 99.8%. Dichloromethane is a solvent very volatile colorless liquid, soluble in water with characteristic odor have boiling point of 40^{0}C.[11] Dimethyl amino Isopropyl Chloride in is a class 2 solvent available in crystalline powder and Less Toxic procured from Sigma Aldrich, Promethazine Hydrochloride is white, faint yellow crystalline powder having 99.89% purity procured from Sigma Aldrich. The helium Gas is procured from local market Panchalingala Oxygen Gas Company and the water used is double distilled from milli Q and the sample tablets from Abott laboratories Phenergan 25 mg purchased from local market.

The Instruments used are Gas Chromatography make Shimadzu Model TQ 8040 NX equipped with triple Quad mass analyzer make of Agilent and GC MS empowered with smart Single reaction mode (SRM) software. Capillary column of make GS TEK model GsBP- 624 glass column having 30m*0.32mm*1.8um[19], The Semi microbalance of Make Satorius Secura 225D-10N is a advanced electronic weighing balance sensitive to 0.1mg[13], TQM mass Analyzer make of Analytica.

Gas Chromatography: Initially the GC is run with Helium as carrier gas at 2ml/min for 60 min to stabilize the system; Temperature is maintained at 200^{0}Cand split ratio of 1:5, and the split is in on Position. The oven is programmed initially 60^{0}C and slowly rise to 240^{0}C by 20^{0}Cper min hold for 2 min. The column is 30m*0.32mm*1.8um fixed in the temperature programmed oven and the sample volume 1µl is injected through programmed temperature vaporization (PTV), initial temperature set below the Boiling point of injected sample solvent.[14]

Mass Spectrometer: the effluent vapors are directly introduced into ion source of MS, using EI as ionizing source, TQM mass Analyzer (triple Quadruple Mass Analyzers) two are Quadruple are arranged in sequence and a radio frequency Quadruple analyzer in between them. Selected ion monitoring (SIM) fixed at m/z 121 with a dwell time of 100 ms, SIM is more advantageous in scanning analyte, the interference temperature is 250^{0}C and solvent cut off time is 4 min.

Preparation of Standard and Sample Solutions
Diluent: Methylene Chloride.

Standard and sample Stock Solution: 25.59%w/w of Dimethyl amino Isopropyl chloride in O- Xylene, take 1 ml of above solution make to 100 ml with Methylene chloride, pipette out 1 ml and make to 100ml with same diluent, Now the standard solution is approx 25 ppm.

Weigh an Equivalent of 25 mg of Dimethyl amino Isopropyl chloride containing in a 1000 mg of Promethazine dissolved in 100 ml of Methylene chloride, pipette out 1 ml and make to 100ml with same diluent, now the sample stock solution is approx 25 ppm.

Both sample and standard solutions are filtered through 0.45u filter to remove undissolved solid particles and degas by applying vacuum.[4]

Preparation of working solutions: from the above stock solution precisely take 1.0 ml of standard and sample and dilute to 10 ml with same diluent. The standard and sample are approx 2.5 ppm.

Validation: The USFDA, CGMP, ISO/IEC, USEPA set certain rules for validation of a method.[10, 2, 5]

1. **System Suitability**: it is carried by injecting a blank followed by six replicate injection of standard followed by a blank at final, Rt, Peak Area are calculated mean, deviation and %CV are calculated, the %CV for impurity not more than 15.0.
2. **Selectivity**: the Selectivity of the method study by injecting a blank followed by six standard replicate injection, sample and spike sample finally standard as bracketing. The results of standard, sample and spike are compared to find the effect of solvent on sample and standard.
3. **Precision**: The system Precision is designed by injecting a blank initially and six replicate injections of standard solution of same concentration. The method precision is conducted by injecting initially a blank, six replicate standards followed by a six test samples finally concluded with a standard injection as bracketing. The Mean, SD and %CV calculated which should lay not more than 15.0%.

4. Linearity: a linearity/range is constructed by injecting various concentrations of Standard solution of impurity from LOQ to 150% of working standard solution. The graph is plotted against concentration and peak area, the correlation (r^2) determined between each concentration. R^2 value should lie in 0.99 – 1.00. A series of injections initially with Blank followed by six standards, Blank, LOQ level, 40%, 60%, 80%, 100%, 150%.

5. Accuracy: The accuracy of the method is designed by preparing a LOQ, 50%, 100%, 150% level with respect to the original standard solution and the each concentration were prepared in 3 sets and injected. Initially a blank followed by six working standard solutions, blank, 3 test samples, 3 sets of LOQ, 50%, 100%, 150% finally the working standard is injected. The recovery studies acceptable levels lies in 80-120%.

RESULTS AND DISCUSSION

Chromatograms

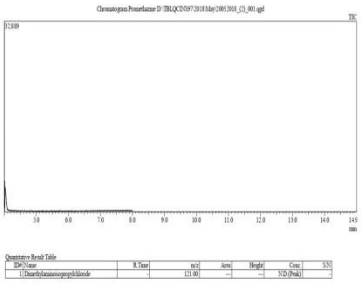

Fig 3: Blank/Placebo

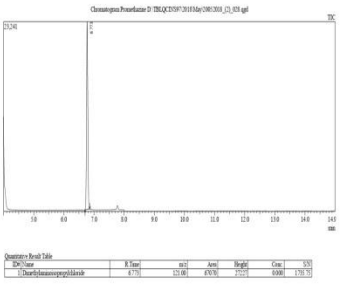

Fig 4: Std Dimethyl Isopropyl Chloride

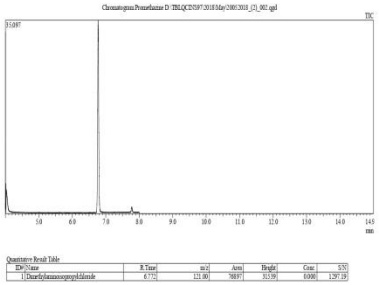

Fig 5: Promethazine Showing the peak of Dimethyl Isopropyl Chloride

System Suitability

$$\text{DimethylAmino Isopropyl content in ppm} = \frac{\text{AT x } 25.59 \text{ x1 x 1 x10}}{\text{AS x 100 x 100 x 100 x 10 x WT}} * 10^6$$

$$\text{DimethylAmino Isopropyl content in ppm} = \frac{60966 \text{ x } 25.59 \text{ x1 x 1 x10}}{75674 \text{ x 100 x 100 x 100 x 10 x 25}} * 10^6 = 2.06 \text{ PPM}$$

Table No 01: validation parameters.

Inj. No	System Suitability	System Precision	Method Precision	Intermediate Precision
Blank	0000	0000	0000	0000
01	87092	76897	76897	57862
02	76897	65734	65734	57968
03	65734	76598	76598	67070
04	76598	72238	72238	58606
05	72238	75489	75489	58854
06	75489	73045	73045	61245
Avg.	75674.67	73333.5	73333.5	60267.5
SD	6976.592	4172	4172	3551
%CV	9.21919	5.68	5.68	5.89
Acceptance*	LT 15.0	LT 15.0	LT 15.0	LT 15.0
Result	Passes	Passes	Passes	Passes

The table 1 represents the results obtained from the injections protruded into the GCMS of standard Dimethyl amino isopropyl chloride in dichloromethane and Promethazine in water with a concentration of 2.5 ppm. the results were excel calculated to its mean, standard deviation, % Coefficient variation and the results were compared with the USFDA impurity guidelines and all the results lies within the specified guidelines and passes the test.

Table No 2: Accuracy.

S.No	Injection ID	Standard	LOQ Level	Recovery 50% level	Recovery 100% level	Recovery 150% level
01	Blank	0.00	0.00	0.00	0.00	0.00
02	2	76897	689	35472	57862	107588
03	3	65734	554	29423	57968	92189
04	4	76598	553	29749	67070	92073
Mean		73333.5	598.7	31548.0	60966.7	97283.3
% Recovery		99.85	--	85.91	83.01	88.31
Acceptability		99–101%	--	80 –120%	80 –120%	80 –120%
Result		Pass	--	Pass	Pass	Pass

The table 2 represents recovery study of the standard and sample in the Promethazine tablets, the study made by spiking method and the % recovery lies in 85 - 89% for sample and 99.85% for the standard solution.

Table No 3: Linearity.

S.No	Description	Concentration	Peak Area	Peak Height
1	Blank	0	00000	0000
2	At LOQ level	0.025	599	222
3	At 40% level	1.2	28899	11451
4	At 60% level	1.8	44405	17828
5	At 80% level	2.4	58703	23704
6	At 100% level	2.5	62683	25557
7	At 150% level	3.8	94680	38653
r^2			0.999	0.999
Slope			24799	10071

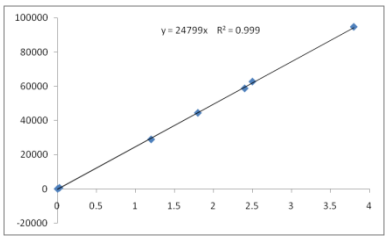

Figure 1: Concentration Vs Peak Area

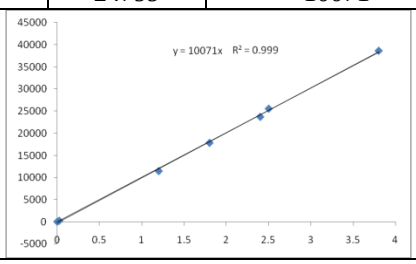

Figure 2: Concentration Vs Peak height

The table 3 shows the results for Dimethyl amino isopropyl chloride standard solution injected with various concentrations from blank to 150% level with respect to the original concentration of working standard solution. The results excel calculated to find correlation coefficient and slope it were found to be 0.999 and 24799 respectively for peak area of Dimethyl amino isopropyl chloride, 0.999 and 10071 for peak height.

Limit of Quantification (LOQ)

$$LOQ = \frac{Concentration\ of\ Standard\ Injected}{\frac{S}{N} ratio} = \frac{2.5}{99.78} = 0.02505\ PPM$$

S/N ratio for 2.5 PPM is 997.8 and LOQ is 10:1 Hence S/N is ($\frac{997.8}{10}$ = 99.78)

Limit of Detection (LOD)

$$LOD = \frac{Concentration\ of\ Standard\ Injected}{\frac{S}{N} ratio} = \frac{2.5}{33.26} = 0.075\ PPM$$

S/N ratio for 2.5 PPM is 99.8 and LOD is 3:1 Hence S/N is ($\frac{99.8}{3}$ =33.26)

CONCLUSION

The aim was achieved by using helium as carrier gas, Rt at 6.773 min Dimethyl Amino isopropyl Chloride obtained and Promethazine at 7.812 min. The Analytical method is validated for System Suitability, System Precision, Intermediate Precision, Method precision, accuracy, Linearity, LOQ and LOD for standard and

sample preparations of Dimethyl Amino isopropyl chloride in standard and Sample Promethazine marketed formulations. The study was concluded that the Indian marketed formulations may contain the genotoxic impurity value lies within the specified guidelines of USFDA and FSSAI.

ACKNOWLEDGEMENTS

I Especially thanks to Dr M Santhiramudu Garu Chairman RGM group of institutions, Andhra Pradesh and Dr C Madhusudhan Chetty Garu principal Santhiram College of Pharmacy, Nandyal, AP for supporting in completing this research work.

Conflict of Interest: Nil.

REFERENCES

1. aldrich, s. (2019). *2-chloro-n, n-dimethylpropylamine hydrochloride.* merck.
2. cder. (2015). *analytical procedures and method validation for drugs and biologicals.* usa: u.s. department of health and human services.
3. finnigan, t. (2018). *mass spectrometer scan types.* thermo scientifics.
4. fssai. (2015). *manual of methods of analysis of foods.* new delhi: food safety and standards authority of india.
5. ich. (2016). *ich q2 analytical method validation.* switzerland: international conference for hormonization of human health.
6. ji, z. et al (2017). *general separation technologies.* gs tek column broucher.
7. li, d. et al (2018). review on the progress in the synthesis of antihistamines and promethazine. *international conference on new materials and intelligent manufacturing*, 187-190.
8. liu, c. t. et al (2016). qualitative and quantitative analysis of volatile components of zhengtian pills using gas chromatography mass spectrometry and ultra-high performance liquid chromatography. *journal of analytical methods in chemistry*.
9. lowa state university, d. o. (2015). *mass spectrometry tutorial.* lowa: department of chemistry.
10. nk, s. et al (2014). usfda guidelines on process validation. *international journal of pharmtech research*, 920-923.
11. pubchem. (2016). *dichloromethane.* national library of medicine.
12. pubchem. (2018). national library of medicine national centre for biotechnology information. *pub chem*.
13. scientific, f. (2016). *sartorius secura semi micro balance.* usa: thermo fisher scientifics.
14. shimadzu. (2016). *fundamanetal guide to gc.* shimadzu - global analytical and measuring instruments.
15. sidisky, l. et al (2011). *carrier gas selction for capillary gas chromatography.* usa: sigma aldrich.
16. sinha, s. et al (2018). promethazine drug information. *drug bank*.
17. wikipedia. (2018). *gas chromatography - mass spectrometry.* google.
18. young, r. e. et al (2011). *trace analysis of volatile and semi volatile genotoxic impurities in drug products.* health and medicine.
19. zhenghua.et al (2016). *gsbp gc columns.* usa: gs tek.
20. B, D. et al (2003). Spectrophotometric method of the determination of gold (III) by using imipramine hydrochloride and promethazine hydrochloride. *world wide science.org*, 76-82.
21. FDA. (2016). *Phnergan.* USA: Wythe Pharma.
22. Salins, C. D. et al (2018). *Annual report 2018.* French : french national agency for medical and health products saferty.