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A VALIDATED STABILITY METHOD FOR DIMETHYL AMINO ISOPROPYL IN PROMETHAZINE HCL BY GC-ESI –MS

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ABSTRACT

The aim of present work is to develop a simple and sensitive method for a genotoxic impurity like Dimethyl Amino Isopropyl Chloride (DAIC) in the Promethazine (PRMT) bulk and tablets by hyphenated GC MS method. A Novel Method is developed by online coupling of GC and MS. Column of 30m*0.32 mm*1.80 um, Helium as carrier gas, automatic column oven temperature from 40°C slowly rose to 220^{0}C. The MS conditions are fixed with ESI at 220^{0}C and interference temperature of 250^{0}C and the positive ions are separated as per m/z ratio by triple Quad mass analyzer and the ions trapped by ion collector and detected by photomultiplier tube detector using single ion monitoring mode (SIM) and lab solutions insight software. The impurity peak obtained at 6.773 min for DAIC and 7.812 min for PRMT both in standard and sample formulations. The analytical method is validated for System Suitability, Accuracy, Precision where %CV were found to be 9.21, 85 – 89%, 5.68, 5.68, 5.89 respectively for DAIC and LOD and LOQ 0.075 and 0.025 ppm correspondingly and the range between 0 to 3.8 ppm. The validated 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.

INTRODUCTION

The Promethazine (PRMT) is a pehnothiazines derivative, it works by changing the chemicals in the brain, also act as antihistamine, chemically it is N, N-dimethyl-1-phenothiazin-10-ylpropan-2-amine [(1, 2)]. The DAIC is a class 2 solvent impurity used in the manufacture of injection and tablet form of Promethazine [(3, 4)]. The Genotoxic impurities (GTIs) in Pharmaceuticals could impact negatively on human health [(5)]. Promethazine structure and DAIC are shown in **fig 1 and 2** [(6)].

The GTIs in Promethazine due to the degradation of structure and/or obtained from intermediate products and raw materials used in manufacturing process of Promethazine tablets [(7-9)]

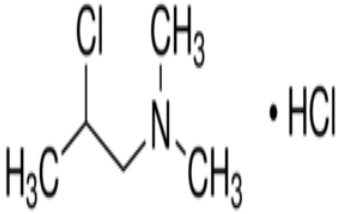

Fig 1: Dimethyl Amino Isopropyl chloride

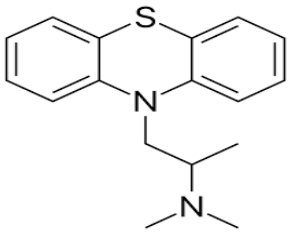

Fig 2: Promethazine

The gas Chromatography is a method used for the Qualitative and Quantitative analysis of Thermo labile and volatile natured organic compounds [(10)]. The Helium as carrier gas at 1ml/min, reduces the time of analysis for the most of the compounds and having high flow rate and Viscosity [(11)] and a Non Polar silica with 6% cyano propylene Phenyl and 94% Dimethyl Polysiloxane equivalent to USP G43 column[(12)] filled in glass SCOT column having 30m*0.32mm*0.18µ. The Mass Spectrometer is used to identify the positive ions formed during the ionization of the eluent from GC. The temperature is sustained at ionization and interference chamber at 220°C and 250°C. The ions travel through Q3 Analyzer with Single ion monitoring (SIM) [(13)] The Q3 Mass Analyzer consists of two Quadruples in a series and a RF Quadruple analyzer is placed in between [(14)]. The ions collected at Ion traps and Detected by Photomultiplier tube. In GC MS the sample mixture is directly vaporized at 70-250°C temperature slowly raising 20°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 [(15)].

Screening of Literatures reveals there are no validated methods has identified in assay of Dimethyl amino isopropyl chloride GTIs in Promethazine marketed formulations. The novel method is developed and validated according to USFDA, ICH guidelines.

Materials and Methods: The chemicals used are HPLC grade 99.8% purity, Dichloromethane [(16)]. Dimethyl amino Isopropyl Chloride (DAIC) is a class 2 solvent available in crystalline powder and Less Toxic procured from Sigma Aldrich, Promethazine Hydrochloride 99.89% purity gifted from Pharma Train laboratory, Hyd. The helium Gas procured from local market Panchalingala Oxygen Gas Company and the water used is double distilled from milli Q and the sample tablets Phenergan 25 mg from Abbott laboratories purchased from local market.

The GC-MS make Shimadzu Model TQ 8040 NX equipped with triple Quad mass (TQM) analyzer make of Agilent and GC MS empowered with lab solutions insight software. Capillary column GS TEK model GsBP- 624 glass column having 30m*0.32mm*1.8um[(17)], The Semi microbalance Sartorius Secura 225D-10N is an advanced electronic weighing balance sensitive to 0.1mg [(18)], Agilent GC Autosampler Syringe (8010-0371) 10µl[(19)].

Gas Chromatography Conditions: 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°C and split ratio of 1:5, and the split is in on Position. The automatic oven from 60°C and slowly rise to 240°C by 20°C per 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 [(20)].

Mass Spectrometer: the effluent vapors are directly inlet to ion source of MS, the Interference temp 250°C and using ESI ionization, TQM mass Analyzer set to selected ion monitoring (SIM) fixed at m/z 121 with a dwell time of 100 ms, SIM is more advantageous in scanning selected analyte 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/v of DAIC in O- Xylene, take 1 ml of above solution make to 10 ml with Methylene chloride, now the standard solution is approx 25 ppm. Sample solution is prepared by weighing counterpart of 1000 mg of PRMT dissolved in 100 ml of Methylene chloride, pipette out 1 ml and make to 100ml with same diluent. Both sample and standard solutions are sonicated, filtered through 0.45µ filter to remove undissolved solid particles and degas by applying vacuum [(21)].

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 Concentration is approx 2.5 µg/ml.

Validation: The USFDA, CGMP, ISO/IEC, USEPA set certain rules for validation of a method; [22-31].

1. **System Suitability**: it is carried by injecting a blank Methylene Chloride followed by six replicate injection of standard DAIC 2.5 µg/ml and concluded with a blank at final. The Peak Area calculated for mean, ±SD and %CV, the accepting limit of %CV not more than (NMT) 15.0.

2. **Selectivity**: the Selectivity of the technique, study by injecting a blank followed by six standard replicate injection, sample and spike sample, finally standard as bracketing. The selectivity of the method used to investigate any interferences of blank with standard and sample at peak region. The results of standard, sample and spike are compared to find the effect of solvent on sample and standard. The Rt of DAIC is 6.776 and for the PRMT is 7.812 min correspondingly. The mean Rt, ±SD and %CV were found to be NMT 15.0

3. **Precision**: The system Precision is designed by injecting a blank initially and six replicate injections of standard solution of 2.5µg/ml concentration. The method precision is conducted by injecting initially a blank, six replicate standards (2.5µg/ml) followed by a six test samples finally concluded with a standard injection as bracketing. The Mean, ±SD and %CV calculated the accepting limit should NMT 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:

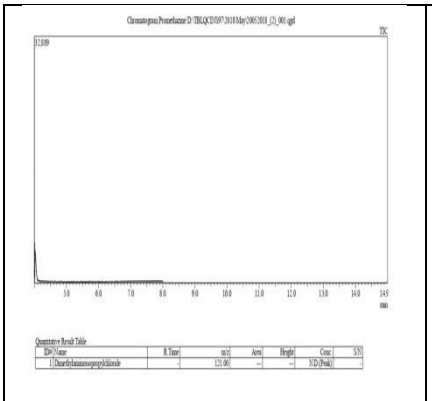

Fig 3: Blank/Placebo

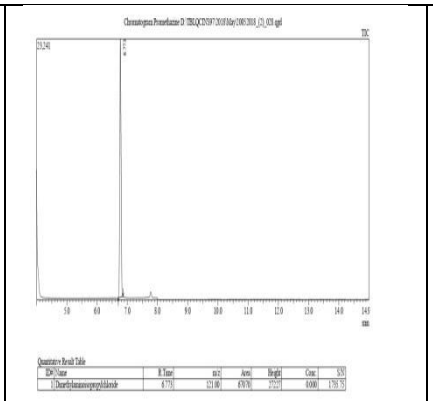

Fig 4: Std Dimethyl Isopropyl Chloride

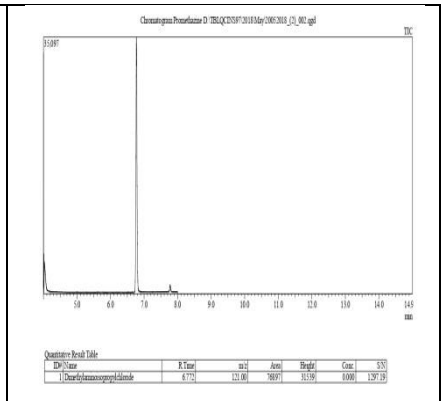

Fig 5: Promethazine Showing the peak of Dimethyl Isopropyl Chloride

System Suitability:

$$\text{DAIC content in } \mu g/ml = \frac{AT \times 25.59 \times 1 \times 1 \times 10}{AS \times 100 \times 100 \times 100 \times 10 \times WT} * 10^6$$

$$\text{DAIC in } \mu g/ml = \frac{60966 \text{ x } 25.59 \text{ x1 x } 1 \text{ x10}}{75674 \text{ x } 100 \text{ x } 100 \text{ x } 100 \text{ x } 10 \text{ x } 25} * 10^6 = 2.06 \text{ PPM}$$

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	NMT 15.0	NMT 15.0	NMT 15.0
Result	Passes	Passes	Passes	Passes

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

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

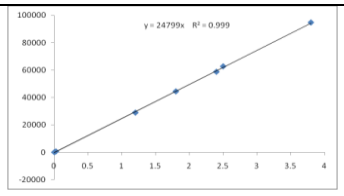

Figure 1: Concentration Vs Peak Area

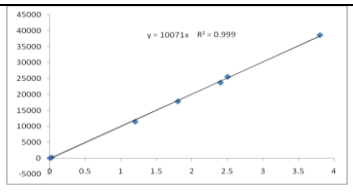

Figure 2: Concentration Vs Peak height

The table 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.

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.

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):

$\frac{2.5}{99.78} = 0.02505\ PPM$

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): $\frac{2.5}{33.26} = 0.075\ PPM$

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.

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Authors Contributions:

Dr NDVR Saradhi concepted the research work, collected materials and experimented and authored the manuscript, Mr KK Kalyan kumar collected the data and analyzed. Dr M Venkata Reddy provided the research support and analyzed the data.

Conflict of Interest:

All Authors declare that they have no conflicts of interest in publishing this research article.

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