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Identification & Quantification of Impurities in Red & White Wine By GC-MS

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Abstract- The work is based on to check the quality of wine. The method is designed as to find the impurity in wine. The extended of ethyl glycol and ethyl phenol in wine and its quantity was founded by this method using GC-MS. Solid phase extraction is used for finding nature of the impurity and was founded on GC-MS. the ethyl phenol and ethyl glycol is produced by the spoilage of yeast brettanomyces which may affect the human health.

Keyword-Agilent Single Quadrupole GC-MS, Food and Beverages, SPME, Red and White Wine, Impurities

I. INTRODUCTION

Gas chromatography and mass spectrometry (GC/MS) has historically been used by the wine maker to detect the quality and impurity of the wine. Now-a-days common techniques are used to check the quality of wine .Now they are depending on the Oenologist for evaluation and determination of quality of wine by identifying the defects responsible for the quality of the wine. But GC-MS has false the opinion of the expert of objective and quantitative information. While using SPME extraction method for GC-MS many advantages have been seen such as very small sample quantity, less preparation of the sample and analysis is rapid of targeted molecule. Several types of molecules, are hazardous to humans, affect health and quality, such as ethyl glycol, ethyl phenol, and volatile phenol compounds derived from ***Brettanomyces*** yeast metabolism. Repeated result to determine the compound is desirable as it is an automated technique and such method is provided by GC-MS. Sequential full scan of wine sample is carried out on a GC-MS system consisting of single-quadruple mass spectrometer. It was carried out at National Horticulture Research and Development Foundation Central Government Lab (NHRDF).In this report, we present the results of study, experimental method and concentration ranges.

II. LITERATURE REVIEW

1) Syed ZameerHussain and KhushnumaMaqbool-(2014)
GC/MS-a combination of two different analytical techniques, Gas Chromatography (GC) and Mass Spectrometry (MS), is used to analyze complex organic and biochemical mixtures. GC can separate volatile and semi-volatile compounds with great resolution, but it cannot identify them.

2) Simay Gunduz, HasibeYilmaz, Ahmet C.Gorenunder (2013)
Studied ethyl alcohol contents of different kinds of beverages, vinegars, vegetables and fruits collected from Turkish markets were investigated using HS-GC-FID method. The ethanol contents of fruits, vinegars and beverages were found to vary between 0.32x10-4-0.35% (w/w), apple vinegar and concentrated orange syrup were determined to contain as high as 0.44 and 0.68 % (w/w) ethanol, respectively.

3) R. Larcher , C. Puecher, S. Rohregger (2011)
The ability of cellulose acetate, cellulose acetate propionate (CAP), cellulose acetate butyrate and cellulose propionate (CP) fibres to reduce 4-ethylphenol and 4-ethylguaiacol, causing the off-flavour named ‘‘Brett character’’, was studied. CAP and CP performed best in a preliminary comparative test.

4) Sarah K. Brill & Mathew S. Wagner (2010)
Reported Alcohol determination in beverages using polar capillary gas chromatography mass spectroscopy and an acetonitrile internal standard. High concentration alcohol samples were diluted to ~5% ABV using ultrapure H2O.

5)R. Larcher,GNicolini,T. Roman villegas, D. Bertoldnand C. Puecher (2009)
The paper establishes a new HPLC method for measurement of the total content of gluconic acid (GA)in wine using a pulsed amperometric detector equipped with a gold electrode cell and an anion exchange column.

6) Carsten Fauhl and Reiner Wittkowski (2004)
The aim of the present study was to provide the official wine control authorities with an internationally validated method for the determination of 3-methoxy-1,2-propanediol(3-MPD) and

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cyclic diglycerols (CycDs)—both of which are recognized as impurities of technical glycerol—in different types of wine.

7) Mei-ling Wang, Youk-mengChoong, Nan-wei Su &Min-hsiung Lee(2003)

Develop a simple and rapid method to determine ethanol content in alcoholic beverages using megapore polar column with direct injection gas chromatography. Ethanol in sample was injected directly into GC for analysis, after adding suitable amount of internal standard, acetonitrile solution.

8) PilarVin˜as, Carmen Lo´pez-Erroz (2000)

A reversed-phase LC method, optimised for the separation of *trans*- and *cis*resveratrol, catechin, epicatechin, quercetinand rutin, is reported. Analyses were performed on a reversed-phase column by gradient elution. standards. The procedures were applied tothe determination of the phenolic compounds in different types of wines and musts.

9) E. Falque Lopez and E. Fernandez Gamez(1996)

A method to simultaneously identify and quantitate the major carboxylic acids, sugars, glycerol, and ethanol in wines and grape musts is proposed. The technique involves isocratic separation, an ion-exchange column, and refractive index and U V detection (at214 nm) without sample preparation.

[10] Goodman Donald E.-Patent(US3896659A)

The volumetric ethanol content of a liquid mixture containing water and ethanol (e.g., an alcoholic beverage) is determined by gas-solid chromatography using aqueous ethanol of known composition as an external calibration standard. A pre-determined volume of sample mixture to be analyzed is injected into a gas chromatographic column containing a solid hydrophobic microporous adsorbent and chromatographed.

Figure-1 GC-MS.

III. SYSTEM ARCHITECTURE

a) Method

For this experiment, two targeted molecule types that affect wine quality were analyzed using an Agilent Single Quadruple GC-MS system (Figure 1). Table 1 and 2 contains brief description of the two target molecule

EHYL GLYCOL		
1	Chemical formula	$C_2H_6O_2$
2	Molar mass	62.07 g·mol^{-1}
3	Appearance	clear, colorless liquid
4	Odor	Odorless
5	Density	1.1132 g/cm^3
6	Melting point	−12.9 °C (8.8 °F; 260.2 K)
7	Boiling point	197.3 °C (387.1 °F; 470.4 K)
8	Solubility in water	Miscible
9	Solubility	soluble in most organic solvents
10	Vapour pressure	0.06 mmHg (20 °C)
11	Viscosity	1.61 × 10^{-2} N*s / m^2
12	EU classification	 Xi

Table-1 Properties of Ethyl Glycol.

EHYLPHENOL		
1	Chemical formula	$C_8H_{10}O$
2	Molar mass	122.16 g/mol
3	Appearance	White solid
4	Odor	Powerful woody-phenolic
5	Density	1.1132 g/cm^3
6	Melting point	42 to 45 °C (108 to 113 °F; 315 to 318 K)
7	Boiling point	218 °C (424 °F; 491 K)
8	Solubility in water	Slightly soluble
9	Solubility	soluble in most organic solvents
10	Vapour pressure	0.0372mmHg (25 °C)
11	EU classification	 Xi

Table-2 Properties of Ethyl Phenol.

Preparation of sample of wine for GC-MS

1) Wine sample (1L) were filtered through whatmann filter paper no. 1 under ambient conditions

2) 10ml of sample was drawn in 15ml centrifuge tube.

3) Add 1ml Methyl Tertiary Butyl Ether (MTBE), 4 anhydrous $MgSO_4$ and 1g NACL.

4) Vortex for 2 min and centrifuged for 5 min (600RPM,-10^{0}C)

5) Supernatant was collected in 2ml Eppendorf tube and the tube was placed for 15 min at 20^{0}C

6) Immediately cleaned by dispersive solid phase extraction (DSPE) with $CaCL_2$ (100mg), MgSO4 (50mg) and PSA (25mg) and further vortex for 1min

7) Centrifugation at 10,000 rpm for 3min.

8) Filter through 0.2µm poly tetra fluoro ethylene membrane filter.

9) 2µL of the extract was injected in the SIR mode into the GC-MS

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SR. NO	PARAMETER	TEST VALUE					STD
		Coldconda Premium	Rio	Nine Hill	Fort Wine	Madira	
1	Ph	2.8	2.92	3.40	3.30	3.48	2.9-3.9
2	Briks	12.0	14.0	9.0	15.0	8.0	1.3455
3	Alcohol%	13.97/ (14%)	15.41/ (16%)	13.8/ (14%)	13.63/ (14%)	13.8/ (12%)	Figure mentioned in bracket
4	Volatile acidity	0.36	0.96	0.54	0.66	0.84	0.08-0.12
5	Titrable acidity	6.07	4.50	4.70	4.32	8.52	0.4-1.5
6	Reducing sugar	21.35	22.30	7.2	16.70	13	0.1-9.6
7	Total sulphur dioxide	56.0	64.0	96.0	88.0	122.0	20

Result Of Wine Sample For Other Physical Parameter

The above are the physical parameter of different wines. As we can see all the parameter tested are not as per their standards. Which may be hazardous to the individual consuming the wine. Whatever tested values of the of seven physical parameter are compare to the government norms.

b) Instrumental Analysis

The GC Agilent and MS Waters used for this analysis was set to perform sequential full scan. It was equipped with a standard split/split less injector. The split/split less injector temperature was set to 220 °C, and a split less injection was used. The GC-MS parameters are summarized in Table 3. The analytical column used was 15 m 0.25 mm × 0.25 µm film .TCA d5 was used as an internal standard; its ions are 215 and 217. The results were analyzed using Chem Station software. The expected retention time (RT) and areas is automatically tested by Chem Station software

MS CONDITIONS(WATERS)		
1	Injector Temperature	220^0C
2	Split flow	80ml/min
3	Source Temperature	300^0C
4	Function type	Full scan
5	Solvent Delay	0-0.50 min
6	Scan Range	35-270
7	Scan Time	0.10sec
GC CONDITIONS (AGILENT)		
1	Initial Temperature	40^{0C}
2	Initial Time	2 min
3	Rate	25^0C/min -250^0C/min
4	Hold time	4 min

Table-3 GC-MS Conditions.

IV. RESULT AND ANALYSIS

For the detection of impurity GC-MS had been used and Red and White wine used as a sample which was been purchased form the local market. The spectra of the impurity EG 1 can be seen in Figure 2 for White wine sample but EP 1 has not found in White wine sample can be seen in Figure 3.The spectra of the impurity EG 1 can be seen in Figure 4 for sample of Red wine and EP 1 for sample of red wine can be seen in Figure 5. The calibration curves of the target molecules can be seen in Figure 6 and 7. The calibration curve of the EP 1 and EG 1 at various ppb solution can be seen in Figure 8 and 9 .Name of impurity, retention time, area, and quantity of impurity can be seen in Table 4.

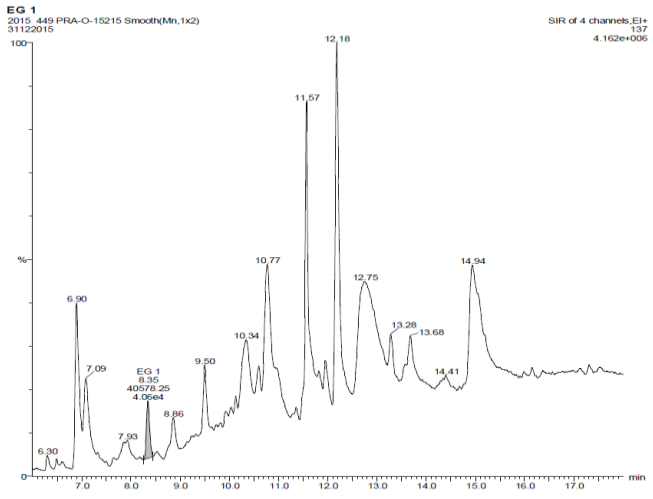

Figure-2 Spectra for EG 1for White wine

For the detection of impurity GC-MS had been used and White wine used as a sample which was been purchased form the local market. The spectra of the impurity EG 1 can be seen in Figure 2

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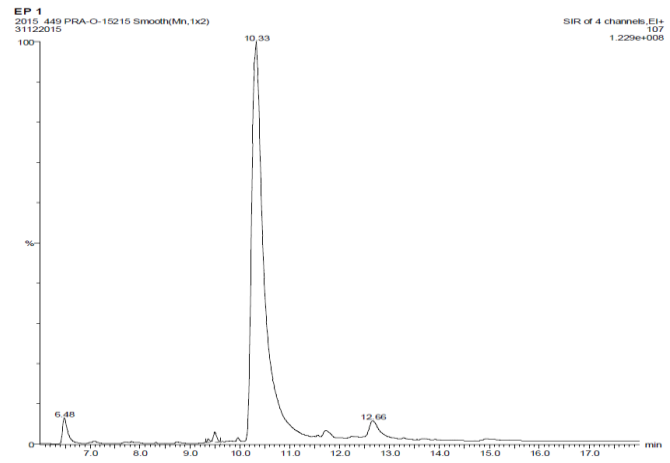

Figure-3 Spectra for EP 1 for White wine

White wine sample but EP 1 has not found in White wine sample can be seen in Figure 3.

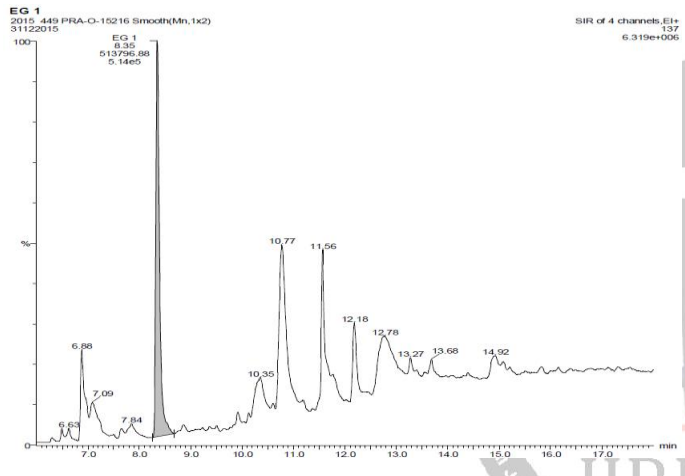

Figure-4 Spectra for EG 1for Red wine

The spectra of the impurity EG 1 the pick is high early time can be seen in Figure 4

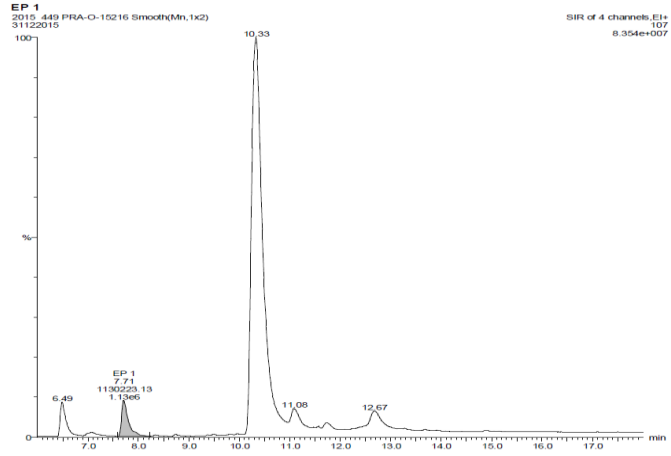

Figure-5 Spectra for EP 1for Red wine

Red wine and EP 1 for sample were found some amount in red wine can be seen in Figure 5.

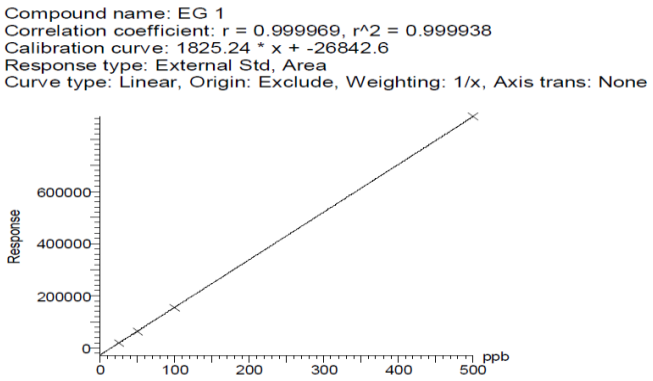

Figure-6 Calibration curve for EG 1

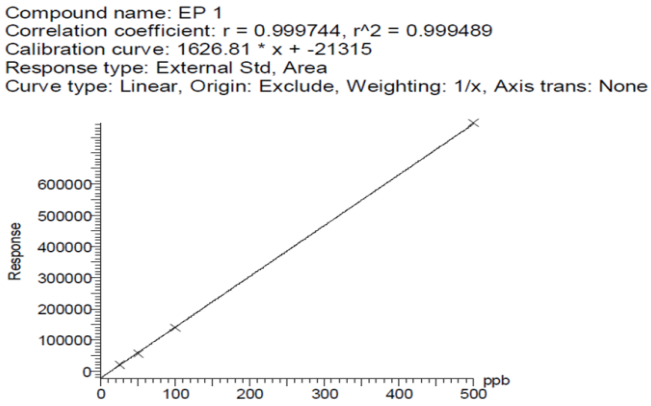

Figure-7 Calibration curve for EP 1

The calibration curves of the target molecules EP1 can be seen in Figure7. As point is gradually increases and constantly.

The calibration curves of the target molecules EG1 can be seen in Figure 6. As point is gradually increases and constantly.

The calibration curve of the EG 1 at various ppb solution can be seen in Figure 9. In different concentration we get different spectra. Whenever ppb get increases then spectra get down.

Table-4 Name, retention time, area and concentration of impurity in White and Red wine

		White wine		
Serial No	Name	Retention time	Area	Concentration (PPB)
1	Ethyl glycol	8.35	137	36.9
Red wine				
1	Ethyl glycol	8.35	137	296.2
2	Ethyl phenol	7.71	107	707.9

The ethyl glycol concentration in white wine is less than the Red wine. If we see in the table-4 the retention time & Area are taken content in both wine and just concentrated on the concentration (PPB). If more concentration than more harmful for the health that ways the concentration is detected at content retention time & Area.

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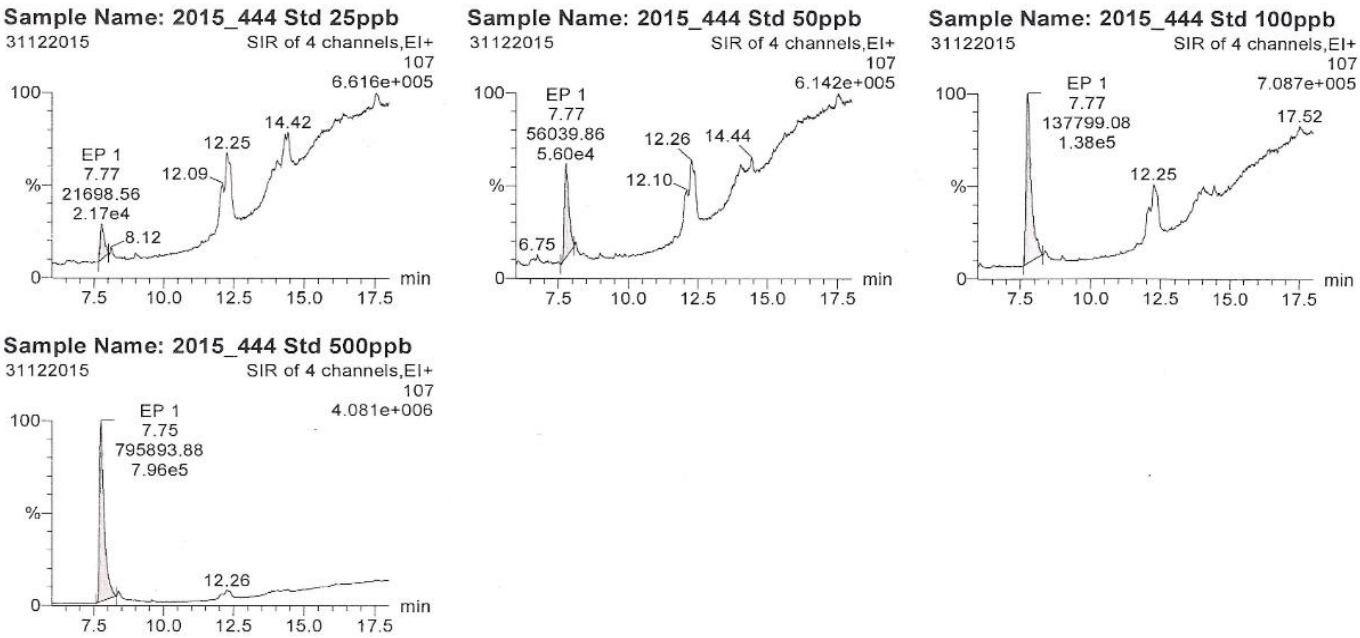

Figure-8 Calibration curve for EP 1 in various ppb solution

The calibration curve of the EP 1 at various ppb solution can be seen in Figure 8. In different concentration we get different spectra. Whenever ppb get increases then spectra get down.

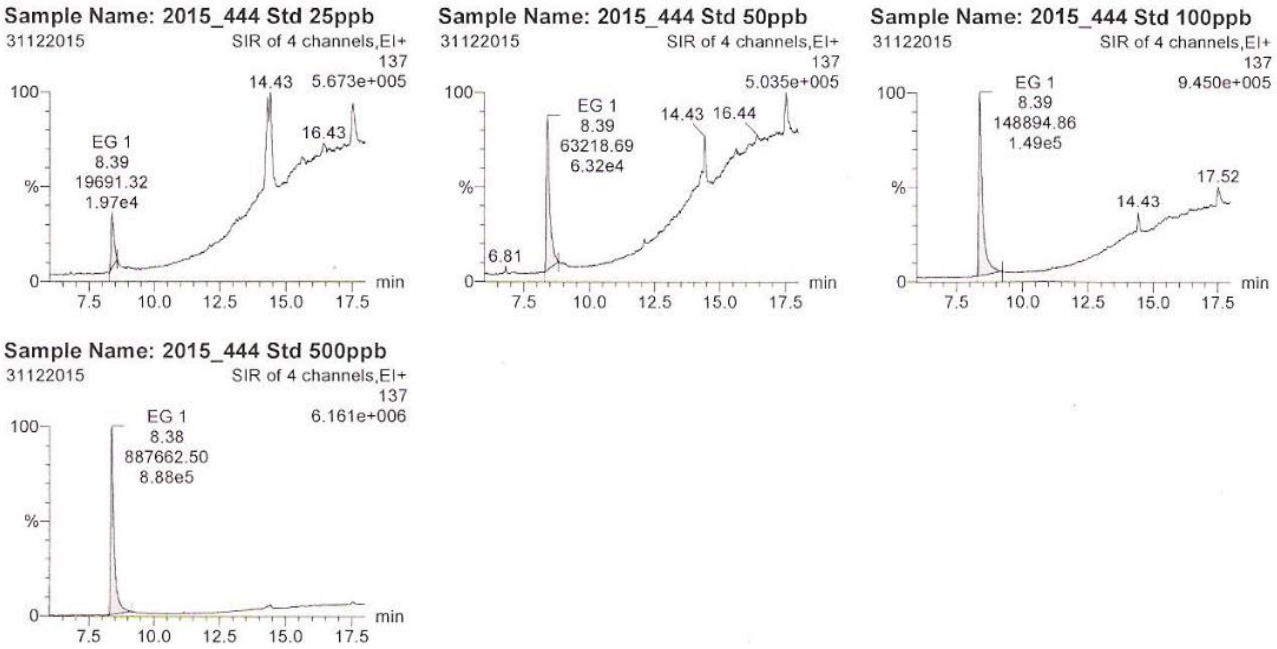

Figure-9 Calibration curve for EG 1 in various ppb solution

V. CONCLUSION

The actual foregoing objective of this report is to determine the impurities, efficient content level of sugar and alcohol in various wine by using an advanced analytical technique Gas chromatography–mass spectroscopy & to discover the effectiveness of technique as in description stated in report. As the standards value are not given for impurities such as Ethyl Glycol and Ethyl Phenol in any official documents or books but the class of these impurities are given Hazards to human health. PPB levels found in the sample shows impurities are marginally higher. To know the rapidity & accuracy of the technique utilized for analysis. The impurities found are highly injurious, and may cause hazard to the health of the individual consuming the Wine.

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REFERENCES

[1]Mei-ling Wang, Youk-meng Choong, Nan-wei Suand Min-hsiung Lee (2003). A Rapid Method for Determination of Ethanol in Alcoholic Beverages Using Capillary Gas Chromatography, Journal of Food and Drug Analysis, 11.

[2]Sarah K. Brill and Matthew S. Wagner (2012). Alcohol determination in beverages using polar capillary gas chromatography and an internal standard, Concordia College Journal of Analytical Chemistry, 3.

[3]British Pharmacopoeia Commission office, London, U.K (2009), I, 1024.

[4]Indian Pharmacopoeia, Government of India, Ministry of Health and Family Welfare, Delhi (2007) 95-96.

[5]Carsten Fauhl ;Reiner Wittkowski (2004).Gas Chromatographic/Mass Spectrometric Determination of 3-Methoxy-1,2-Propanediol and Cyclic Diglycerols, By-Products of Technical Glycerol, in Wine, Journal of Aoac International , 87.

[6]E. Falque Lopez and E. Fernandez Gomez (1996). Simultaneous Determination of the Major Organic Acids, Sugars, Glycerol, and Ethanol by HPLC in Grape Musts and White Wine Journal of Chromatographic Science, 34.

[7]NIOSH Pocket Guide to Chemical Hazards #0272". National Institute for Occupational Safety and Health (NIOSH).

[8] Ethyleneglycol (2008). National Institute for Occupational Safety and Health. Emergency Response Database. August 22, 2008. Retrieved December 31.

[9] Eiceman, G.A. (2000). Gas Chromatography. In R.A. Meyers (Ed.), Encyclopedia of Analytical Chemistry: Applications, Theory, and Instrumentation Chichester: Wiley. ISBN 0-471-97670-9.

[10]Jeff Cox "From Vines to Wines (1999)the Complete Guide to Growing Grapes and Making Your Own Wine" Storey Publishing ISBN 1-58017-105-2.

[11]Noble, A.C., & Bursick, G.F. Am. J. Enol. Vitic (1984) 35.

[12]Annex J summary of current food standards as of 04 April 2013

 DOI : 10.18231/2454-9150.2017.0064