ABSTRACT OF THESIS

Chapter 1- In India, the BIS standards were developed and revised in the year 2005. Only pH, Ethanol %, Reducing Sugar, Total acidity, Volatile acidity and Total SO_2 have shown relevance in consequence with the standards of other wine producing and consuming countries. From last few years, the wine production as well as wine export graph is increasing at the rate of 20-30% every year. Other countries (EU, Australia, Nova-Scotia and International OIV) have made their own standards for wine quality and for exporting wine to these countries one needs to follow their standards. The standards developed by BIS are not holistic enough, keeping in view the standard of other. For example, the BIS standards do not include any specified limits for contaminants like pesticide residues, heavy metals, or spoilage compounds like 2,4,6-TCA, Biogenic Amine, 4 Ethyl phenol, etc. Therefore, there is a need to work extensively on wine quality. Till date, there has been no research published on the chemical quality of Indian wines and hence this study will help in establishing a baseline data for better understanding of the quality of Indian wine which will help in developing the quality standards that comply with the standards of other countries and also in harmony with the International standards.

Chapter 2- Analysis of physico-chemical parameters is of utmost importance for evaluation of the quality parameters of red, rose and white wines, since they contribute to wine organoleptic characteristics such as colour, aroma, astringency, and bitterness. Furthermore, several studies have indicated other biological properties of interest, related to their antioxidant capacity. The appearance and oxidation status of wine was determined by examining the colour intensity, hue by using UV-visible spectrophotometer oxidation by dissolve oxygen meter.

For evaluation of astringency and bitterness of the wine samples, pH, total acidity, volatile acidity and SO_2 contents were evaluated by standard methods. The sweetness of wine was evaluated by using Gold-cast method. All these parameters provide important information about acidic and sensorial characteristics of wine. The results of all parameters were found within the range of good quality of wine.

All the physico-chemical parameters were analysed for the evalution of the quality of wine. All the wines were found within the good wine quality range with respect to its colour, hue value varied upto 0.97 in red, 0.57 in rose and 0.23 in white

hence these wines did not exceed oxidation threshold, which indicate no browning of wine. pH varied from 3.3 to 3.7 in all wine, total acidity varied from 5.1 to 7.6 g L^{-1}, volatile acidity varied from 0.1 to 0.58 g L^{-1} ; on the basis of residual sugar concentration wines were from dry and sweet were present, Total SO_2 as well as free SO_2 content varied from 27 to 249 and 9 to 99.8 mg L^{-1} in all samples respectively, which comply BIS as well other country standards. Dry extract varied from 12 to 49 mg L^{-1}in all wine samples. In view of the above results, all wine samples comply BIS as well other international standards. Therefore Indian wines are of good quality in all respects.

A method is described for estimation of alcohols (ethanol, methanol and other alcohols) acetaldehyde, ethyl acetate in red and white wines. The sample preparation involved appropriate dilution of the sample with water for ethanol analysis and two times dilution with 12.5% ethanol for remaining compound. All selected analytes were estimated by gas chromatography flame ionization detector (GC-FID) within the chromatographic run time of 47 minutes with 2 µL splitless injection. Quantification was performed using solvent calibration curve in the range of 1-500 mg L^{-1} with r^2> 0.99 for all the test compounds. Limit of quantification for most analytes were 1-10 mg L^{-1}. Ethanol content of all tested wines was within 10 to 15% except for two wine samples in which the ethanol content was 16 and 17%. The concentration of methanol ranged from 0 to 195 ppm which is below maximum level specified by BIS. Acetaldehyde concentration ranged from 14 to 96 ppm while ethyl acetate concentration ranged from 0 to 44 ppm. Isoamyl alcohol was also quantified as higher alcohol, the concentration of which ranged from 21-183 ppm. The concentrations of all these four compounds in the analysed wines were below maximum level specified by BIS.

A reversed-phase high-pressure liquid chromatographic method is presented for the simultaneous separation and determination of malic, citric, lactic, formic and ascorbic acids in wine. Reversed-phase liquid chromatography, involving buffer in the mobile phase to suppress the ionization of the solutes was found suitable for chromatographic separation of carboxylic acids. The use of a linear photodiode array for detection was important in optimizing analyte responses and in avoiding spectral interferences. Thus, the proposed method is rapid and simple for quantification of organic acids in wine. After filtration and degasification, the organic acids in the

sample are separated on Purosphere RP 18 (C18) column and quantified by using a rapid diode array detector. The method is considered to be a suitable choice for accurate and precise determination of above acid compounds. Proposed method was applied for the determination of these compounds in collected wine samples. The major organic acids found in wine were 0.26-1.77 g L^{-1} tartaric acid, 0.098-3.7 g L^{-1} lactic acid, upto 0.417 g L^{-1} citric acid, upto 0.97 g L^{-1} malic acid, and upto 0.016 g L^{-1} fumaric acid and were within the range of good quality wine which posse quality for flavour and colour. These acids concentration indicates status of malolactic fermentation.

Chapter 3- Phenolic compounds are one of the most important quality parameters of wines, since they contribute to wine organoleptic characteristics such as colour, astringency and bitterness and are also responsible for neutraceutical properties. For the determination of individual phenolic compounds, direct injection to LC-MS/MS method was standardized. The antioxidant activity of Indian wines was measured by two different analytical methods: [1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, and ferric reducing/antioxidant power (FRAP). The reducing power (PR) was evaluated and correlated with the total phenolic contents determined by the Folin–Ciocalteu's reagent.

The total polyphenol concentration was found to vary from variety to variety and region to region. The content of phenolics decreased in the order: red > rose > white wine. The TPC contents of red, rose and white wines ranged from 1.07 to 2.62 g GAE/L (average 1.8), 0.24 to 0.49 (average 0.37) and 0.16 to 0.41 (average 0.27) g L^{-1} gallic acid equivalents (GAE). The antiradical activity/ free radical scavenging activity in red wines were higher than rose and white wines, which were ranging from 0.21 to 0.72 (average 0.51), 0.08 to 0.253 (average 0.17) and 0.017 to 0.09 (average 0.048) mM trolox equivalent, respectively. In terms of the reducing power, the red wines also had higher ferric reducing-antioxidant power (FRAP) than rose and white wines which ranged from 2.01 to 7.04 mM (average 3.79), 0.46 to 1.82 mM (average 1.06) and 0.07 to 0.71 (average 0.38) mM quercetin equivalent (QE), respectively.

It is verified that the red wines have higher phenolic content levels than white and rose wines and the same result is obtained for antiradical activity as well as ferric reducing antioxidant capacity. The amounts of phenolic content and antioxidant

activity vary considerably in different types of wines, depending on the grape variety, vintage year, and region. Total phenolics and antioxidant activity were highly correlated. There was a significant difference in total phenolic content and antioxidant capacity among the red, rose and white wine samples. Direct injection with LC- MS/MS has been used successfully to achieve good sensitivity and specificity without any loss in recovery during sample preparation or false identification. Individual phenolic composition of wine was possible within short chromatographic run time. It was observed that there was high correlation between the content of total phenolics as well as individual phenolic compounds, which further correlated with their reducing power and antiradical activity confirming that the individual phenolic compounds are likely to contribute to the antioxidant activity of these wines.

Chapter 4- A fast, sensitive, cost-effective and accurate method is presented for the determination of 23 target (Esters, Aldehyde, Terpene alcohol, varietal aromatics e.g. methoxy pyrazine and contaminant TCA and 4-Ethylphenol) and 22 non-target volatiles in wine by GC-TOFMS. Several factors including solvent selection and sample to solvent ratio for extraction, cleanup and GC-TOFMS parameters were optimized. The sample preparation involved extraction of volatile compounds in wine samples (10 mL, red and white wine) with 1 mL methyl tertiary butyl ether (MTBE) in presence of 4 g anhydrous $MgSO_4$ and 1 g NaCl. The upper organic phase was separated by centrifugation, cleaned by dispersive solid phase extraction with 100 mg $CaCl_2$, 50 mg $MgSO_4$, and 25 mg PSA and analyzed by GC-TOFMS in full scan mode with electron impact ionization. The limit of quantification for most of the compounds was <25 ng mL^{-1}. While the method detection limit was 1.3 ng mL^{-1}. The linearity was established in the calibration range of 10-1000 ng mL^{-1} (r^2>0.999). The method was validated at 10, 25 and 50 ng mL^{-1} and the recoveries (n=6) were within 90-110% with associated relative standard deviations ranging between 1-7% indicating satisfactory intra-laboratory precision. MTBE was found as a promising solvent for the extraction of volatile compounds in wine as well as for injection into the GC-MS system as far as analysis of volatile compounds is concerned. The method was applied for the analysis volatile compounds from 25 red and 25 white wine samples obtained from wineries in India.

Chapter 5- A multiresidue method has been established and validated for simultaneous estimation of 83 pesticides and 12 dioxin-like polychlorinated

biphenyls (PCB) in red and white wines. The sample preparation involved extraction of 20 mL wine (acidified with 20 mL of 1% HCl) with 10 mL ethyl acetate (+ 20 g sodium sulphate). The co-extracted fatty acids were removed as insoluble salts on reaction with anhydrous calcium chloride. The extract was further cleaned by dispersive solid phase extraction (DSPE) with 200 mg florisil. The final extract (5 mL portion) was solvent exchanged to 1 mL of cyclohexane:ethyl acetate (9:1) and again cleaned by DSPE with 25 mg primary secondary amine sorbent. The residues of all the 95 analytes were estimated by gas chromatography time of flight mass spectrometry (GC-TOFMS) within the chromatographic run time of 31 minutes with 2 µL splitless injection. Quantification was performed using matrix-matched calibration curve in the range of 10-500 µg L^{-1} with r^2> 0.99 for all the test compounds. Limit of quantification for most analytes were ≤10-20 µg L^{-1}. Acidification of wine prior to extraction prevented hydrolysis of organophosphorous pesticides as well as dicofol. Solvent exchange to cyclohexane:ethyl acetate (9:1) further minimized the traces of co-extractives. For selected synthetic pyrethroids, e.g. cyfluthrin and cypermethrin, higher LOQ (> 20 µg L^{-1}) was observed. For organochlorines and dioxin-like PCB, the LOQ was in the range of 1-5 µg L^{-1}. Recoveries at 5, 10 and 20 µg L^{-1} were >80% for most analytes. For certain compounds like cyprodinil, buprofezin and iprodione, lower recoveries (<70%) could be due to instability, matrix interference, or inefficient desorption from the DSPE sorbents. The expanded uncertainties at 10 µg L^{-1} were <20% for most analytes. Intra-laboratory precision in terms of Horwitz ratio of all the analytes was below 0.5, suggesting ruggedness of the method. Effectively, the method detection limit for most analytes was as low as up to 1 µg L^{-1} in both red and white wine, except for cyfluthrin and cypermethrin. Out of the 50 commercial wine samples, pesticides could be detected in only four samples at less than 10 ng mL^{-1} (precisely 2-6 ng mL-1), which is less than the specified maximum residue limits of the European Union. The compounds detected included lambda-cyhalothrin in Cabernet Sauvignon, triadimefon in Ugni Blanc and chlorpyriphos and myclobutanil separately in two different samples of Chenin Blanc. PCB was not detected in any of the samples.

A fast, sensitive, cost-effective and accurate method is presented for the determination of 2,4,6-trichloroanisole (2,4,6-TCA) in wine by GC-MS. 2,4,6-TCA is a spoilage compound and as a contaminant adds mouldy-musty odour to wine.

Several factors including solvent selection for it's extraction, sample to solvent ratio, cleanup and GC-MS parameters were optimized. Wine samples (60 mL, red and white wine) were extracted with 2 mL toluene in presence of 24 g anhydrous $MgSO_4$ and 2 g NaCl. The upper organic phase was separated by centrifugation, cleaned by dispersive solid phase extraction with 100 mg $CaCl_2$ + 50 mg $MgSO_4$, followed by 25 mg PSA and analysed by GC-TOFMS and GC-MS/MS with electron impact ionization. The linearity was established in the calibration range of 0.5-500 ng mL^{-1} (r^2>0.999). The method detection limit (MDL) was 0.0083 ng mL^{-1}. The method was validated at 0.04, 0.2 and 0.8 ng mL^{-1} and the recoveries (n=6) were 90±5, 93±4 and 97±3%, respectively. The Horwitz ratios at 0.04, 0.2 and 0.8 ng mL^{-1} were 0.07, 0.05, and 0.04 indicating satisfactory intra-laboratory precision. Strength of high selectivity and sensitivity of tandem mass spectrometry and high sensitivity of time-of-flight mass spectrometry in association with the peak-find and deconvolution tools of the chromatography software were useful in screening wines for 2,4,6-TCA residues even at parts per trillion levels. Under optimized conditions, the method was sensitive enough to comply with the requirement of lowest odour threshold of 0.01-0.04 ng mL^{-1}. 2,4,6-TCA was found in all the 5 tested incurred samples in the range of 5-20 ng L^{-1} while the 2,4,6-TCA concentration in 4 Argentinean wines ranged within 180-280 ng L^{-1}. Out of the 50 commercial samples analysed, 2,4,6-TCA was detected only in four samples (3 red and 1 white) at less than 10 ng L^{-1} (precisely 8-10 ng mL^{-1}), which is less than the specified odour threshold.

Free amino acid and biogenic amine content of the Indian wine was studied. Simultaneous determination of 20 underivatized amino acids and 5 biogenic amines were carried out by ESI-MS/MS with PDFOA ion pairing reagent and acidified mobile phase. The total amount of free amino acids and biogenic amines was higher in red wines than in white and rose wines. The main amino acids were proline and arginine, while the major biogenic amines were tyramine, histamine and spermidine. In all these 50 wine samples tyramine, histamine, cadaverine, spermidine were found in low concentration. Tyramine, histamine, cadaverine, and spermidine were higher in the red wines than white and rose wines because of malolactic fermentation and this was due to growth of lactic bacteria that induces an increase of amines content in wines. In red wine tyramine ranged between 0-2.1 mg L^{-1}, spermidine were 0-8.5 mg L^{-1}, histamine were ranged 0.42-1.99 mg L^{-1}, cadverine content were 0.4-1.86 mg L^{-1} while in white wines and rose wines tyramine concentration were very low (0-0.729

mg L-1), histamine content were 0.38-1.98 mg L^{-1}, spermidine were 0-1.98 mg L^{-1}, cadverine content were 0-0.77 mg L^{-1}. However, in these wines, the content in total biogenic amines was low and did not represent a toxicological hazard for human health.

In this study, by using standardized method we quantified Na, Mg, Al, Si, K, Ca, Ba, Be, V, Cr, Fe, Mn, Ni, Co, Cu Zn, As, Ag, Ba, Pb and Cd by using ICPMS. All samples comply the BIS as well as international standard specified limit. Heavy metal content in Indian wines does not represent a possible toxicological problem for human health.

This endeavor will evolve into a comprehensive quality standard for Indian wine in harmony with different international standards. Along with the routine parameters it also recommends monitoring of the compounds characteristic to specific grape varieties; safety assessment by screening for the residues of spoilage compounds and variety of contaminants that might find their place in wine from direct and indirect sources. Thus a holistic quality assessment will be possible, which will improve the image of Indian wine in the international arena and establish its own footprint in quality.

The thesis ends up with the list of publications and conferences/seminar attended.