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Analytical Methods

Development and validation of a simple analytical method for the determination of 2,4,6-trichloroanisole in wine by GC–MS

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ABSTRACT

A novel method for the residue analysis of wine spoilage compound 2,4,6-trichloroanisole is reported. Wine (60 ml) was extracted with 2 ml toluene in presence of 24 g $MgSO_4$ and 6 g NaCl. Cleanup of the toluene phase by dispersive solid phase extraction with mixture of 100 mg $CaCl_2$, 25 mg primary secondary amine and 50 mg $MgSO_4$ was effective in minimising co-extractives and matrix effects. Time-of-flight and tandem mass spectrometric parameters were optimised to achieve linearity over 0.25–500 ng ml^{-1} and method detection limit 0.0083 ng ml^{-1} which is well below the odour threshold of 0.04 ng ml^{-1}. Recoveries at 0.04, 0.2 and 0.8 ng ml^{-1} were within 80–110% (±8%). The method was reproducible when tested for Argentinean wines with intra-laboratory Horwitz ratios being <0.20 in white and red wines at both the laboratories of India and Argentina. The method could be successfully applied for incurred wine samples.

1. Introduction

Cork taint is considered as a major organoleptic defect in wine, which produces mouldy, musty aroma, and could result in significant financial loss to the wine industry. 2,4,6-Trichloroanisole (2,4,6-TCA) is the main compound responsible for this defective aroma (Buser, Zanier, & Tanner, 1982). Other factors, like storage conditions, transportation, and handling also could be responsible for appearance or increase in the 2,4,6-TCA concentration in the final product (Silva, Figueiredo, & San Romão, 2000). Although the perception threshold for 2,4,6-TCA lies within 10.0 ng l^{-1} (Evans, Butzke, & Ebeler, 1997; Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 1998), the concentration considered as a defect in wine ranges from 10 to 40 ng l^{-1} (Silva et al., 2000).

Considering the physicochemical properties of this semi-volatile compound, gas chromatography based techniques has been the choice of its analysis. One of the main problems for 2,4,6-TCA estimation is the low concentrations at which this analyte could be present in any sample and hence it is essential to count on efficient pre-concentration techniques. As per literature, the sample preparation techniques for estimating 2,4,6-TCA involves liquid–liquid extraction (LLE) (Bayonove & Leroy, 1994), stir bar sorptive extraction (SBSE) (Maggi, Zalacain, Mazzoleni, Alonso, & Salinas, 2008), solid phase extraction (SPE) (Soleas, Yan, Seaver, & Goldberg, 2002), and head-space solid-phase microextraction (HS–SPME) analysis (Riu, Mestres, Busto, & Guasch, 2002). All these methods suffer various limitations including lengthy and multi-step procedure, high analysis cost, poor selectivity and sensitivity, and high degree of matrix effect. Although HS-SPME has been successfully applied in water and wines (Evans et al., 1997; Fischer & Fischer, 1997), it also suffers from inadequate selectivity and sensitivity.

The aim of the present work was to develop a simple, fast, efficient, precise and cheap sample preparation method for the determination of the 2,4,6-TCA residues in white and red wine by gas chromatography-mass spectrometry (GC–MS) with method detection limit less than 10 ng l^{-1}.

2. Materials and methods

2.1. Reagents and materials

Certified reference standard of 2,4,6-Trichloroanisole (99% purity) was purchased from Sigma Aldrich (Steinheim, Germany).

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Sodium chloride was purchased from Sisco Research Laboratory (Mumbai, India). Anhydrous magnesium sulphate (analytical reagent grade) was purchased from Merck India Ltd. (Mumbai), and further purified by heating at 650 °C for 4 h before use and stored in desiccators. Anhydrous calcium chloride was purchased from Himedia (Mumbai, India). Primary secondary amine (PSA, 40 μm, Bondesil) sorbent was purchased from Varian Inc. (Palo Alto, USA). The organic solvents viz. toluene, ethyl acetate, acetonitrile, dichloromethane and hexane were of dried residue analysis grade from Thomas Baker (Mumbai, India).

Standard stock solution (1000 μg ml^{-1}) was prepared by dissolving 25 mg of 2,4,6-TCA in 25 ml toluene in volumetric flask (certified "A" class). An intermediate standard solution was prepared by diluting it with toluene to achieve a working standard concentration of 10 μg ml^{-1}. The calibration standards of strength 0.25, 0.5, 1, 10, 100, 250 and 500 μg l^{-1} were prepared by successive dilutions. Matrix-matched standards at the same concentrations were simultaneously prepared in both white and red wine.

2.2. Wine samples

Red wine (variety: Cabernet Sauvignon) and white wine (variety: Sauvignon Blanc) free from any traces of 2,4,6-TCA were obtained from the experimental winery of the National Research Centre for Grapes (NRCG), India and used for the method development and validation studies.

2.3. Instrumental conditions

2.3.1. GC–TOFMS analysis

An Agilent 6890 N GC system (Agilent Technologies, USA) hyphenated to Pegasus IV TOF-MS (Leco, St. Joseph, MI, USA) was used for analysis. The separation was performed by injecting (splitless, injector port set at 250 °C) 2 μl of the sample extract on a VF-5MS capillary column (5% phenyl polysilphenylene-siloxane; 30 m × 0.25 mm, 0.25 μm). A gooseneck liner (78.5 × 6.5 mm, 4 mm) from Restek Corporation (PA, USA) was used and the helium gas flow rate was set at constant 1.0 ml min^{-1}. The transfer line temperature was maintained at 290 °C. Electron impact ionisation was achieved at 70 eV and the ion source temperature was set at 240 °C. The mass spectrum of perfluorotributylamine was used to tune the mass spectrometer. The detector voltage was set at −1750 V and the data acquisition was carried out within the mass range of 45–400 m/z at acquisition rate of five spectra per second at one-dimension (1-D) mode with solvent delay of 500 s. The temperature program was 100 °C (1 min hold); ramped at 10 °C min^{-1} to 110 °C (7 min hold), then at 15 °C min^{-1} up to 150 °C and finally at 20 °C min^{-1} to 270 °C with a hold for 4 min. Identification was done by matching the full scan spectra with the corresponding NIST library spectrum with the match threshold of >80%.

2.3.2. GC–MS/MS analysis

A GC 7890A (Agilent Technologies, USA) coupled with triple quadrupole mass spectrometer (Micromass Quattro micro, Waters corporation, Milford, USA) was employed for the analysis of 2,4,6-TCA by tandem mass spectrometry (MS/MS). The system included 7683B series injector, 7683 series auto sampler and 4 mm i.d. hollow glass liner (Agilent Technologies). The ion source temperature was set at 200 °C with electron multiplier set at 650 V. Filament emission current was 1300 μA at 70 eV and the filament multiplier delay was set at 10 min. A DB-5MS (5% phenyl polysilphenylene-siloxane; J&W scientific, Folsom, CA, USA) capillary column (30 m × 0.25 mm, 0.25 μm) was used for separation. The sample extract (2 μl) was injected in splitless injection mode into split/splitless injector port maintained at 250 °C. Ultra-pure grade helium (BOC India Ltd., Kolkata) was used as the carrier gas at constant 1 ml min^{-1} flow. The system was controlled using MassLynx software, version 1.4. The oven temperature program was similar as in Section 2.3.1.

2.4. Method validation

The analytical method was validated as per the single laboratory validation approach of Thompson, Ellison, and Wood (2002). The quantification was based on seven-point external calibration graph obtained using matrix-matched standards. The matrix extracts were at first analysed by GC–MS to confirm the absence of 2,4,6-TCA in them before spiking for method development studies. The quantification ion was the base peak of the MS spectra obtained by GC–TOFMS analysis. The limit of detection (LOD) of the target compound was set at signal to noise (S/N) of 3, whereas, the limits of quantification (LOQ) was set to a signal with S/N of 10.

2.4.1. Sample volume and extraction solvent

To determine influence of sample volume on sensitivity and precision of analysis, two different sample sizes viz. 30 and 60 ml of wine were compared. Wine sample (1 l) was spiked with 2,4,6-TCA to achieve 10 μg l^{-1} concentration. From this, 30 and 60 ml sample was drawn in separate sets of six replicates, extracted with 2 ml toluene and analysed. The data of the two sets was statistically assessed by Student's *t*-test. The relative standard deviations (RSDs) and method detection limit (MDL) for each dataset were compared to identify any influence of the sample size on the precision and sensitivity of analysis. Different solvents viz. toluene, ethyl acetate, hexane, dichloromethane and acetonitrile were evaluated in terms of the sensitivity (signal to noise ratio, S/N) and chromatographic peak shape of 2,4,6-TCA.

2.4.2. Sample preparation technique

Wine samples (2 l) were filtered through Whatman-1 filter paper under ambient conditions and from this 60 ml sample was drawn for final analysis in 125 ml polypropylene screw-capped centrifuge tube. The samples were extracted with 2 ml toluene, in presence of 24 g anhydrous $MgSO_4$ and 6 g NaCl. The mixture was vortexed for 3 min and centrifuged for 10 min (6000 rpm, −20 °C). The upper organic layer along with a portion of the matrix was collected in 15 ml centrifuge tube and further centrifuged for 3 min as above. The supernatant was collected in 2 ml Eppendorf tube and the tube was cooled for 15 min at −20 °C. The extract was immediately cleaned by dispersive solid phase extraction (DSPE) with mixture of anhydrous $CaCl_2$ (100 mg), PSA (25 mg) and anhydrous $MgSO_4$ (50 mg) and further vortexed for 1 min followed by centrifugation at 10,000 rpm for 3 min. The supernatant was filtered through 0.2 μm polytetrafluoroethylene membrane filter and 2 μl was injected in splitless mode into the GC–TOFMS and GC–MS/MS for identification and quantification of 2,4,6-TCA.

2.5. Method performance

2.5.1. Recovery

The recovery experiment was carried out in red and white wines at concentration levels of 0.04, 0.2 and 0.8 ng ml^{-1} ($n = 6$). For this, 60 ml wine was fortified with 24 and 120 μl of 100 ng ml^{-1} 2,4,6-TCA solution to achieve concentration of 0.04 and 0.2 ng ml^{-1}, respectively. To achieve the concentration of 0.8 ng ml^{-1}, 96 μl of 2,4,6-TCA solution of 500 ng ml^{-1} was fortified into 60 ml wine. The samples were extracted by the method described in Section 2.4.2. Quantification was performed by external calibration using matrix-matched standards.

2.5.2. Enhancement factor (EF)

EF was calculated as the ratio between the initial wine sample volume and the resulting toluene phase, considering the obtained recovery of each type of wine sample (red or white).

2.5.3. Precision

The precision in terms of repeatability and intermediate precision was determined at 0.04, 0.2 and 0.8 ng ml^{-1}. Horwitz ratio (HorRat) pertaining to intra-laboratory precision, which indicates the acceptability of a method with respect to precision (Horwitz & Albert, 2006; Horwitz, Kamps, & Boyer, 1980) was calculated by the following way:

$$\text{HorRat} = \frac{\text{RSD}}{\text{Prsd}}$$

where RSD stands for relative standard deviation and Prsd is the predicted relative standard deviation. Prsd = $2C^{-0.15}$; where C is the concentration expressed as mass fraction (40 ng l^{-1} = 4×10^{-11}).

2.5.4. Matrix effect

The slope of the calibration graph based on the matrix-matched standards of red and white wines was compared with the slope of the solvent based calibration graph by Student's *t*-test. A higher slope of the matrix calibration indicates matrix induced signal enhancement; whereas, a lower slope represents signal suppressions.

2.5.5. Inter-laboratory precision and measurement uncertainty

The reproducibility of the method developed by the National Research Centre for Grapes, India was evaluated in Argentina by QUIANID laboratory, who validated the method in the same way as described above in typical Argentinean wines. The global uncertainty in the determination of the 2,4,6-TCA was calculated at 0.04 ng ml^{-1} as per the EURACHEM/CITAC Guide CG 4 (EURACHEM CITAC guide, 2000). Five individual sources of uncertainty were considered for the assessment of global uncertainty as described earlier (Banerjee et al., 2007) and reported as expanded uncertainty which is twice the value of the global uncertainty. The uncertainties obtained at 0.04 ng ml^{-1} in the two laboratories of India and Argentina were compared for evaluation of the ruggedness of method.

2.6. Testing the method for analysis of incurred and commercial wines

The validated method was applied for the determination of 2,4,6-TCA in 5 incurred (spoiled wine with mouldy-musty aroma) and 50 commercial wine samples obtained from major wineries of India. The method was also tested on 4 incurred wine samples in Argentina. The wine varieties tested from India were Cabernet Sauvignon, Merlot, Zinfandel, Shiraz, Classic French Red, Gamay, Chenin Blanc, Sauvignon Blanc, Ugni Blanc, Sahyadri, Classic French White, and Chardonnay. The varieties from Argentina included Malbec, Tempranillo, Merlot and Torrontes.

3. Results and discussion

3.1. Extraction solvent

The efficiency of 2,4,6-TCA analysis was affected by several factors, including extraction solvent type, its volume, and the variables that govern sample preparation technique. The study and optimisation of these variables were performed by modifying one at a time while keeping the remaining factors unchanged. The extraction solvent was selected on the basis of the solubility of 2,4,6-TCA, selectivity (lower co-extraction of matrix components) and GC compatibility. Among the tested extraction solvents, viz. toluene, hexane, ethyl acetate, dichloromethane and acetonitrile, 2,4,6-TCA in toluene showed highest relative response (Fig. 1) in terms of S/N and also enhanced peak shape. The recovery of organic extract layer (1.6–1.7 ml out of 2 ml) was also highest for toluene followed by ethyl acetate and hexane (1.0–1.1 ml). For acetonitrile and dichloromethane, no phase separation could be achieved, which could be due to their high degree of miscibility with wine matrix.

The clearly separated organic phase for different solvents was collected and measured for colour intensity by UV–Vis spectrophotometry at 520 nm. The absorbance was highest in ethyl acetate (0.593 absorbance units, when diluted by 1000 times); whereas, lower absorbance was recorded in toluene (0.080 absorbance units) and hexane (0.032 absorbance units). The extraction efficiency in terms of recovery of 2,4,6-TCA was highest for toluene. Furthermore, the transfer efficiency of 2,4,6-TCA from the injector to the detector was also highest for toluene as indicated by narrower and more uniform peak shape with superior injection repeatability in comparison to other solvents. Therefore, toluene was selected as the extraction solvent for further studies. In order to get better phase separation and higher organic solvent recovery, 24 g $MgSO_4$ and 6 g NaCl were added during extraction in the similar way as for the QuEChERS technique of pesticide residue analysis (Anastassiades & Lehotay, 2003).

3.2. Sample to solvent ratio

The volumetric recovery of toluene phase was in the range of 1.7–1.8 ml and 1.6–1.7 ml for 30:2 and 60:2 ratios, respectively.

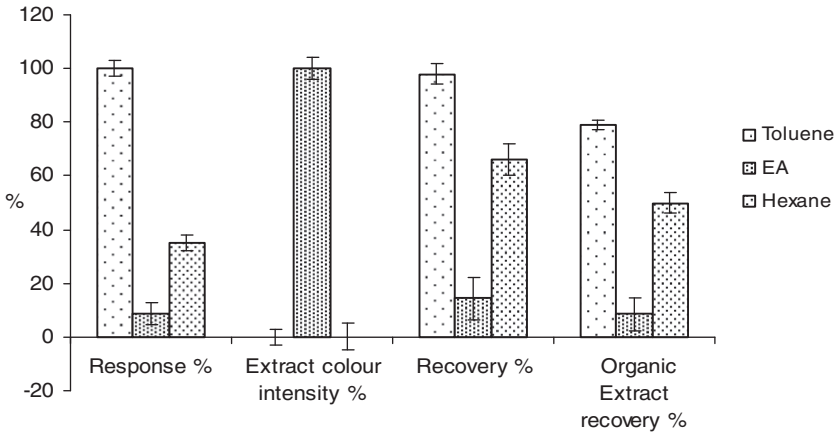

Fig. 1. Comparison of different solvents for the selection of extraction solvent.

Thus, nearly 90% of the solvent phase could be recovered. For sample volume above 60 ml, the volumetric recovery of the toluene phase was lower owing to inefficient phase separation. Recoveries of 2,4,6-TCA at sample size 30 and 60 ml were statistically similar and within 80–110% with RSDs below 8%. Taking into consideration the enhancement factor, sample size of 60 ml was preferred, as this provided 30 times enrichment of residues resulting in lower method detection limits.

3.3. Centrifugation time and temperature

To determine the effect of centrifugation time, 4, 7, 10 and 15 min were considered at 8000 rpm at −20 °C. The maximum recovery of toluene phase (90%) was achieved at 10 min and it was comparable to that of 15 min. A 40% reduction of the toluene phase volume was observed when centrifugation was carried out at room temperature. Thus, −20 °C temperature and 10 min centrifugation time were chosen as optimum centrifugation conditions to ensure highest and most repeatable recovery.

3.4. Cleanup

The matrix interference by co-extracted fatty acids originated from wine makes identification of target compounds ambiguous (Patil et al., 2009). Earlier methods employing cleanup using tandem GCB (graphitized carbon black)-PSA solid phase extraction cartridges reported removal of fatty acids and coloured interfering compounds (Soleas et al., 2002). But, we did not find such procedure effective in generating sufficiently clean extract for unequivocal quantification at trace levels (<1 ppb) and thus, the implementation of a cleanup stage was essential. Dispersive solid phase extraction (DSPE) is a novel alternative for such purpose. In toluene extract, addition of anhydrous $CaCl_2$ for DSPE minimised the solubility of esters and fatty acids (decanoic acid, tetradecanoic acid, palmitic acid, etc. and their alkyl esters), and retained them in the lower polar phase separated out on centrifugation at −20 °C. Removal of fatty acids by $CaCl_2$ was to the extent of ~97% (Fig. 2), which considerably reduced the matrix effect and background noise. Different amount of anhydrous $CaCl_2$ were assayed for 1.2 ml toluene extract with DSPE. Anhydrous $CaCl_2$ (50, 100 and 200 mg) in combination with 50 mg anhydrous $MgSO_4$ was evaluated. The S/N of 2,4,6-TCA was 20% higher for 100 mg $CaCl_2$ as compared to 50 mg; and this was comparable to the effect obtained with 200 mg. Therefore, combination of 100 mg $CaCl_2$ and 50 mg $MgSO_4$ was selected for optimised cleanup.

Although the above cleanup was effective for removing fatty acids, traces of other co-extracted matrix compounds still remained in the toluene phase, which affected the instrumental sensitivity and selectivity for 2,4,6-TCA determination at trace concentrations. Consequently, peak-find option of the software could not identify 2,4,6-TCA at less than 0.5 ppb. Further cleanup was therefore carried out by adding 25 mg PSA into the above DSPE mixture comprising of 100 mg $CaCl_2$ + 50 mg $MgSO_4$ in the toluene phase. This resulted in significant reduction (>90%) in the peak area of the remaining fatty acid (m/z 143) and other co-extractives (Fig. 3). Through such cleanup, the peak purity improved by at least 20% in the concentration range of 0.25–0.5 ppb with simultaneous increase in NIST library spectral match. Thus, it was possible to achieve almost clean extract before final analysis by GC–MS, with limit of quantification near about 0.25 ppb.

3.5. Instrumental parameters

3.5.1. Optimisation of GC oven program

The optimum initial column oven temperature was found to be 100 °C. Starting with higher initial temperature (e.g. 110 °C) and higher ramp resulted in early elution of the analyte; but S/N and peak purity were affected. The 2,4,6-TCA peak was also affected by coeluted solvent. Similarly, with too low initial temperature (e.g. 40 °C), there was broadening of peak width (27%) and it also lowered the peak height (33%) as compared to the optimised condition. A hold of 1 min at 100 °C with ramping at the rate of 10 °C min^{-1} to 110 °C with a hold for 7 min could remove entire solvent traces from the column before elution of 2,4,6-TCA. The temperature was then ramped at the rate of 15 °C min^{-1} up to 150 °C resulting in elution of 2,4,6-TCA with minimum peak width of 4 s and higher response (S/N). Finally, a higher temperature ramp at 20 °C min^{-1} up to 270 °C reduced the chromatographic run time and also ensured removal of the high boiling matrix co-extractives. The temperature was held for 4 min at 270 °C in order

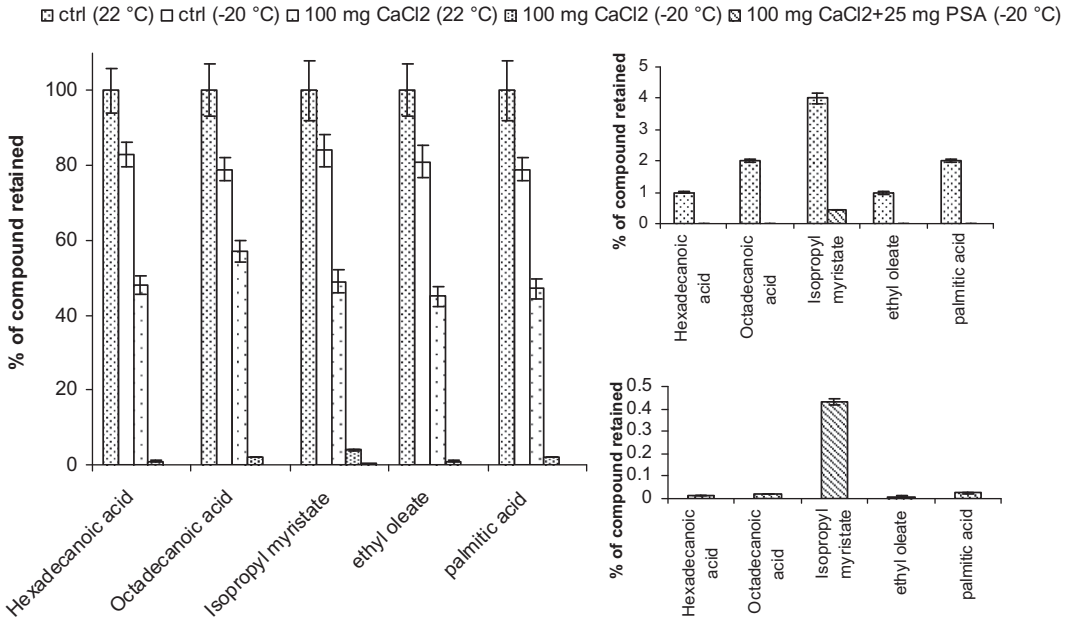

Fig. 2. Effect of $CaCl_2$ on removal of long chain organic acids and esters.

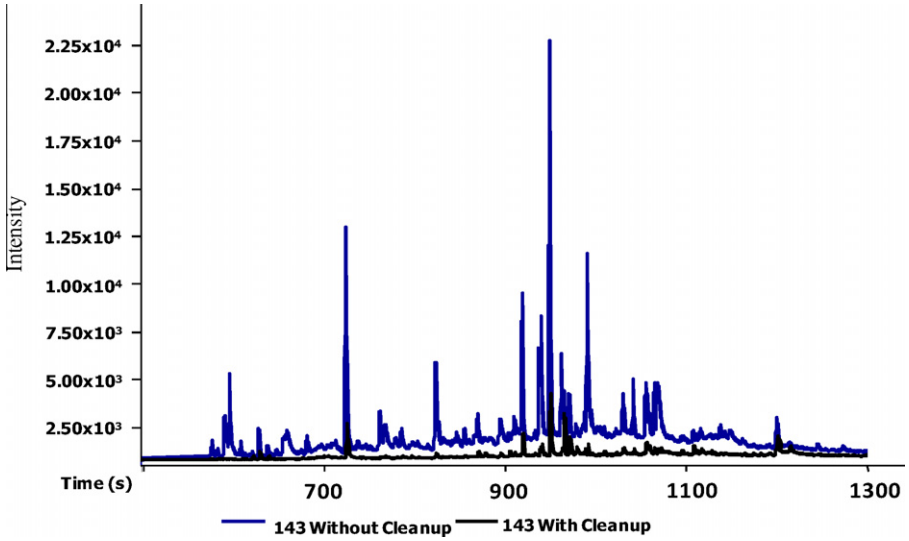

Fig. 3. Effect of $CaCl_2$ and PSA cleanup on entire chromatogram for removal of fatty acids.

to remove all the high boiling matrix compounds which otherwise would result in higher noise in subsequent chromatographic runs. Under the optimised conditions, 2,4,6-TCA eluted at 591.4 s on GC–TOFMS and at 10.6 min on GC–MS/MS.

3.5.2. Optimisation of GC–TOFMS parameters

To achieve the highest S/N, optimisation of the ion source temperature was done by step-wise increment in ion source temperature from 150 to 250 °C. Increase in S/N from 612 to 1213 was observed at 240 °C, which is in agreement with earlier experiences for pesticides (Banerjee et al., 2008). S/N increased from 405 to 1290 when the acquisition rate decreased from 100 to 10 spectra/s. Further decrease in acquisition rate to 5 spectra/s improved sensitivity by 24% with sufficient resolution at 0.25 ng ml^{-1} of 2,4,6-TCA (>90% NIST based mass spectral confirmation) in matrix. Fig. 4 presents the full scan spectra of 2,4,6-TCA at 5 ppb.

3.5.3. Optimisation of GC–MS/MS parameters

Collision energy and dwell time were optimised to attain highest sensitivity in GC–MS/MS. At first 2,4,6-TCA was analysed in full scan MS mode to identify the parent ion for MRM (multiple reaction monitoring) scan. From the full scan spectra, m/z 195 (base peak) and 210 were selected as the parent ions. The collision energy was then increased by increment in steps of 1 V to obtain the daughter ions corresponding to the parent m/z 195 and 210. The objective was to achieve the highest sensitivity for the daughter ion keeping the relative abundance of the parent ion at 10–20% level for individual MRM transitions. The optimum collision energy for 195 > 167 (quantifier MRM), 210 > 195 and 210 > 167 (qualifier MRMs) were 14, 15 and 22 V, respectively. After the collision energies were set, the dwell times for individual MRMs were optimised to obtain highest response (S/N) and better peak shape. The optimum dwell time was set at 0.05 min. (Fig. 5).

3.6. Analytical performance

3.6.1. Accuracy and precision

The recovery of 2,4,6-TCA at three concentration levels, i.e. 0.04, 0.2, and 0.8 ng ml^{-1} at NRCG were within the range of 80–110% with the RSDs ($n = 6$) below 8%. Similar results (80–110% recovery, RSD < 14%) were obtained from QUAINID laboratory (Table 1) in Argentina. The coefficient of regression for calibration curves was $r^2 > 0.999$ on both GC–TOFMS and GC–MS/MS. Since 60 ml sample volume was extracted in 2 ml toluene with the average recovery of the method for both white and red wines nearly 100%, the enhancement factor obtained was 30. Therefore, the method detection limit achieved was 0.0083 ng ml^{-1} [0.025 (LOQ, ng ml^{-1})/30]. HorRat of 2,4,6-TCA calculated at the concentrations mentioned above were 0.08, 0.07, 0.074 in white wine and 0.11, 0.12, 0.10 in red wine, respectively, for NRCG and 0.15, 0.16 and 0.09 in white wine and 0.19, 0.14, and 0.20 in red wine for QUIANID, indicating satisfactory intra-laboratory precision.

3.6.2. Matrix effect

Matrix induced signal enhancements were up to 13% in red and 11% in white wine in case of GC–MS/MS. The signal enhancements were to the extent of 34% and 27% in red and white wine, respectively when analysed by GC–TOFMS. Hence, considering different extents of matrix effects, it is recommended to prepare separate matrix-matched standards for white and red wines. Relatively low matrix effect in GC–MS/MS could be due to higher selectivity offered by tandem mass spectrometry.

3.6.3. Inter-laboratory validation

In order to test the reproducibility, the developed method was thoroughly validated at NRCG and QUIANID laboratories in both white and red wines and the results were compared. The total uncertainty was evaluated assuming all the contributions independent of each other. A coverage factor of 2 was applied to evaluate the expanded uncertainty at a confidence level of 95%. The expanded uncertainty at 0.04 ng ml^{-1} of the 2,4,6-TCA was 10% for white wine and 14% for red wine for the NRCG and 17% and 21% for QUIANID laboratory, respectively which indicated ruggedness of the method. The uncertainties associated to precisions were low (below 3%) both on a day-to-day as well as analyst-to-analyst basis. In addition, 2,4,6-TCA also had low uncertainties associated with accuracy (below 6%). Thus, overall expanded uncertainties for 2,4,6-TCA in both white and red wines were below 21%. Although 2,4,6-TCA is volatile in nature, the low measurement uncertainty could be attributed to the stable nature of 2,4,6-TCA under the analytical conditions described above. It can therefore be assumed that the method selected for sample preparation and analysis is efficient and suitable for determination of 2,4,6-TCA from a wide range and types of wine samples.

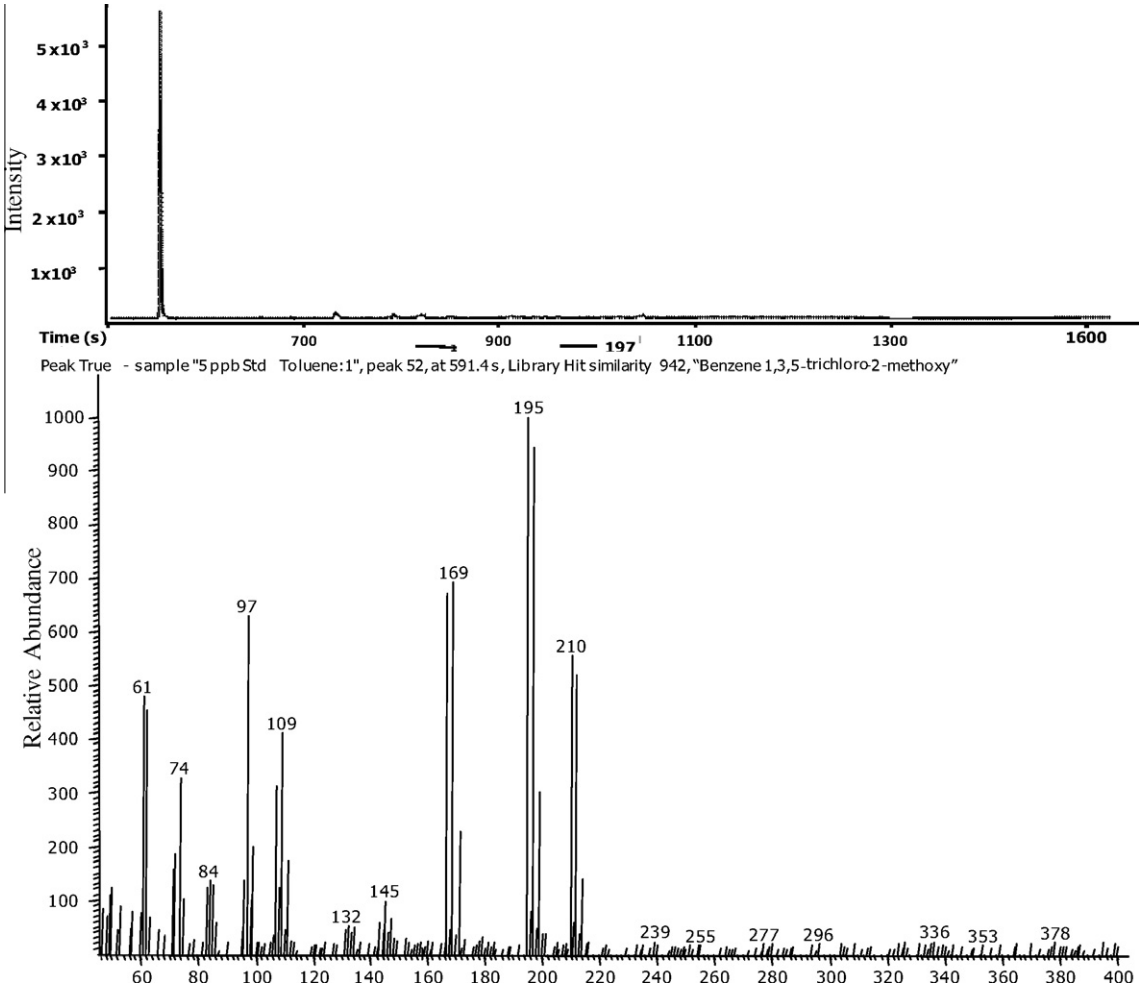

Fig. 4. XIC and full scan mass spectra of 5 ppb 2,4,6-TCA on GC–TOFMS.

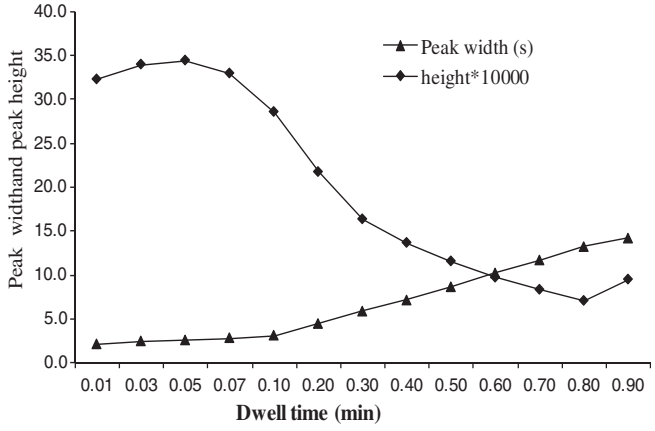

Fig. 5. Optimisation of the dwell time.

3.6.4. Economics of analysis

The input cost of sample processing for a single sample was below 1 USD, which is cheaper compared to the other available methods involving SPE, or HS-SPME. On an average a single chemist could prepare around 25 samples in 8-h working period and the GC–MS output was 30 samples in 24 h cycle. The solvent exposure to the chemists was also significantly low, which establishes operational safety.

3.7. Testing the method for analysis of incurred and commercial wines

2,4,6-TCA was found in all the 5 incurred (spoiled) wine samples from India with the concentration in the range of 40–100 ng l^{-1}. The 2,4,6-TCA concentration in 4 Argentinean incurred wines ranged within 180–280 ng l^{-1}. In case of the commercial samples, 2,4,6-TCA was detected only in 4 (3 red and 1 white) out of the 50 wine samples collected from Indian wineries with concentrations within 8–10 ng l^{-1}, which is less than the specified odour threshold of 40 ng l^{-1}.

Table 1
Validation results for 2,4,6-TCA in white and red wines.

	Recovery (%) ± RSD at 0.04 ng/ml		Recovery (%) ± RSD at 0.2 ng/ml		Recovery (%) ± RSD at 0.8 ng/ml	
	White wine	Red wine	White wine	Red wine	White wine	Red wine
NRCG	85 ± 4.7	82 ± 8.0	91 ± 3.8	95 ± 6.8	94 ± 3.1	93 ± 4.8
QUIANID	102 ± 10.6	108 ± 13.6	90 ± 9.0	98 ± 7.7	82 ± 4.4	102 ± 9.7

In conclusion, our method offers distinct advantages over the conventional sample preparation techniques. In comparison to the time-consuming and cumbersome SPE/tandem SPE, HS-SPME, SBSE techniques, the proposed method had definite advantages in terms of lower input cost and time of analysis. In addition, cleaner samples could be obtained that is expected to increase the life of GC liner and column, and we could achieve reproducible results even after analysing hundreds of samples. Method detection limit was significantly low with acceptable precision. 2,4,6-TCA could be quantified in MS/MS as well as in full scan mode within the run time of 21.67 min with low measurement uncertainties.

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