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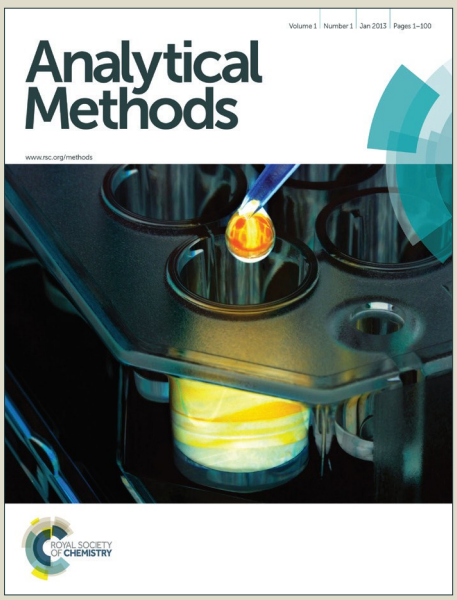


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# Multivariate optimization of a simple and sensitive method for the determination of secondary biogenic organic compounds in airborne particles

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## Abstract

In this study, a simple and sensitive method for the determination of biogenic secondary organic aerosol (SOA) in airborne particles, has been optimized and validated. An one step derivatization protocol, with N-methyl-N-trimethyl-silyltrifluoroacetamide (MSTFA), trimethyl-chlorosilane (TMCS) and pyridine, followed by gas chromatography – mass spectrometry (GC/MS) has been implemented. The method was optimized using a multivariate strategy including the application of a central composite design. The proposed method provided low limits of detection (0.10-0.19 μg $mL^{-1}$) and good precision (relative standard deviations below 5.2%). The method was performed to the analysis of SOA in PM10 particles from a semi-rural area.

Keywords: secondary organic aerosol, MSTFA, trimethylsilylation, gas chromatography-mass spectrometry, experimental design

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## 1. Introduction

Organic aerosols are emitted into the atmosphere due to either anthropogenic or biogenic sources and contribute to the atmospheric chemistry mechanisms [1-4], climate change and human health [5-16]. They can affect global climate by scattering and absorbing solar radiation, or by causing changes to the cloud properties by acting as Cloud Condensation Nuclei (CCN) [17,18]. Furthermore, they can adversely affect human health, causing infections to the respiratory system that in some cases can lead even to premature death [19, 20].

The unintended consequences of organic aerosols on the environment and human health depend on multitude of factors including the origin, the size, the chemical composition and the concentration of the aerosol particles [21]. Aerosols are generally consisted by saturated and unsaturated aliphatic compounds, aromatic compounds, alcohols, ketones, aldehydes, carboxylic acids, amines, sugars, polyols and organic sulfur compounds [15-22].

Secondary organic aerosol (SOA), a portion of the organic component of particulate matter in ambient atmospheres, is produced by ozone or radical-initiated reactions of hydrocarbon precursors, generating nonvolatile and semivolatile organic products, which undergo nucleation reactions to form new particles or condense onto pre-existing particulate matter [18,22-24]. Such biogenic precursors are isoprene, monoterpenes, and sesquiterpenes, which play a significant role in atmospheric chemistry due to some of their properties (i.e high emission rates, volatility, and reactivity) [25-28]. Their main oxidation products found in SOA are polar organic compounds containing oxygenated functional groups, namely hydroxyl, carbonyl and carboxyl, and their concentration can vary from ng $m^{-3}$ to μg $m^{-3}$ [3, 15, 25, 28, 30, 31].

So far, secondary biogenic organic aerosol composition studies have been performed using gas chromatography-mass spectrometry (GC/MS) [7,8,24,32-36]. Generally, these techniques are the most powerful tool for identifying the broad spectrum of compounds in aerosol samples. Of course, the interpretation of mass spectra is complicated by the fragmentation of productions and the formation of cluster ions. In order to achieve better results, the separation step is crucial to this analysis. Due to the polar functional groups of the oxidized compounds, both samples and available standards need to be derivatized.

Although different derivatization studies have been performed, to the best of our knowledge there is not a common sample preparation procedure for this determination.

Current procedures for analyzing SOA are based on single-step or multiple step derivatization techniques prior to GC/MS analysis. The most common single step derivatization technique is based on the simultaneous trimethylsilylation of both carboxyl and hydroxyl groups using N-O-(bis-trimethylsilyl) trifluorosilane (BSTFA) containing 1% or 10% trimethylchlorosilane (TMCS), which acts as a catalyst [24,32-36]. A multiple step derivatization technique based , in the first step, on the esterification of the carboxylic groups with the use of boron trifluoride (BF3)/Methanol, and on the second step, on the silylation of the ester compounds using N-O-(bis-trimethylsilyl) trifluorosilane (BSTFA) as the derivatization reagent, has also been described [37]. Finally, there are just a few studies using MSTFA as derivatization reagent [38-41]. The main differences between the already existed methods and the proposed one, is that the complicated acidification step is not included prior to the extraction and derivatization, and an increased number of SOA tracers is determined by a simple procedure.

The aim of this work is to develop and optimize a simple, rapid and sensitive gas chromatography-mass spectrometry method for the determination of SOA tracers for isoprene and α/β-pinene. Pinonic and pinic acids are selected as tracers for α/β-pinene and 2-C-methyl-D-erythritol as tracer for isoprene products, while MSTFA/TMCS/pyridine is used as derivatization agent after initial experiments. To the best of our knowledge no previous works dealing with optimization and validation of such a method have been reported. Variables, such as derivatization agents volume, pyridine volume, heating temperature and derivatization time, were optimized univariately, since their role and significance on method performance are well known. The optimization study was performed using fractional factorial and central composite design. Using the optimized conditions, precision, linearity, limits of detection (LOD) and quantification (LOQ), and trueness of the method were further evaluated. Finally, the proposed method was performed on PM10 samples from a semi-rural area.

## Experimental

### 2.1 Chemicals and standards

Pinonic acid and 2-C-methyl-D-erythritol (>98%) were purchased from Sigma-Aldrich (Steinheim, Germany) along with all the surrogate standards, ketopinic acid ,mesoerythritol, as well as the internal standard tetracosane d-50 (≥ 99%). Pinic acid (standard solution in methanol 0,1mg $mL^{-1}$) (99.1%) was purchased from Chiron (Trondheim, Norway).

All standards and chemicals used for the derivatization procedure were of the highest available purity. MSTFA (>98.5%), BSTFA+1%TMCS, TMCS (GC grade, ≥99%) and pyridine (anhydrous, >99.8%) were purchased from Sigma-Aldrich., while boron trifluoride (≥99.5%) and sodium chloride from Chem Service (West Chester, USA) and Panreac (Barcelona, Spain) respectively. All the organic solvents used were of GC grade (>99.5%) and were obtained from Sigma-Aldrich. Water used was purified using a Millipore Milli-Q UV plus and Ultra-Pure Water System (Tokyo, Japan).

Stock standard solutions in methanol were prepared for all the compounds and stored at -25$^{o}$ C. Working standard solutions at different concentrations (0.5-50 μg $mL^{-1}$) were prepared in methanol and used in method validation studies. Other working standard solutions were obtained by appropriate dilutions with the appropriate organic solvent.

### 2.2 $PM_{10}$ sampling

The $PM_{10}$ samples were collected according to EN 12341:1998. Samples were collected in a semi-rural area near to Athens basin. Quartz filters (47 mm, 99.98%) used for sampling were obtained from Umwelttechnik MCZ GmbH (Bad Nauheim, Germany) while An LVS16 sampler (Umwelttechnik MCZ GmbH) was also used. The sample flow rate was set to be approximately 2,3±2% $m^3h^{-1}$ and the sampling time for each filter was 24 hours. The filters, prior to the sampling were equilibrated at 20 °C±1 °C και 50±5 %RH until mass stabilization and weighed. After sampling, the equilibration and weighing steps were repeated. The filters were stored refrigerated (4 °C) until their analysis.

### 2.3 Proposed sample extraction and derivatization.

Filters aliquots were extracted three times with a dichloromethane/methanol mixture (1:1 v/v) in an ultrasonic bath for 45min. Prior to the extraction, ketopinic acid (KPA) and meso-erythritol were added as surrogate standards for the products of α-pinene and

isoprene (methyltetrols). The extract was transferred to a round-bottom tube and was concentrated on a rotary evaporator to approximately 3 mL. Purification on a column, filled with 1g anhydrous sodium sulfate followed, using 10 ml of dichloromethane. After purification was completed, the eluant was dried under a gentle stream of ultrapure nitrogen and then 250 µL MSTFA, 2.5 µL TMCS and 50 µL pyridine were added. The tube was sealed with a Teflon coated cap and allowed to react at 70 $^{o}$C for 2 h. Finally GC/MS determination followed.

### 2.4 Gas Chromatography

Analyses were performed on a GC/MS system (Electron Impact, EI mode) from Agilent (Agilent Technologies, USA) consisting of a 6890N gas chromatograph and a 5975B mass spectrometer system. The GC was equipped with a 30m *L* × 250 µm *ID* HP-5MS ultra inert capillary column coated with 5% diphenyl and 95% dimethylpolysiloxane (Agilent J&W GC columns) with a film thickness of 0.25 µm. The chromatographic temperature program was: 84$^{o}$C for 1 min, raised to 200$^{o}$C (4 $^{o}$C min$^{-1}$) and held for 2 min; then raised to 300$^{o}$C (10 $^{o}$C min$^{-1}$) and held for 15 min. The carrier gas (helium 99.999%) flow rate was set in constant flow mode at 1.5 ml min$^{-1}$. Splitless injection of a 1 µL volume was carried out at 280 $^{o}$C. The transfer line and ion source temperatures were maintained at 300 and 230 $^{o}$C, respectively. Analyses were performed in the full-scan mode, in the mass range 45-450m/z at electron energy of 70 eV.

The identification of the compounds was performed using both retention times and mass spectra with the help of the NIST library. GC/MS analysis for the tracer compounds was conducted using the total ion chromatogram. The use of 6 major ions for identification and 3 of them for quantification provides more consistent estimates than those with a single ion.

Characteristic mass fragments, classified according to their abundance, and retention times used for the determination of each analyte are shown in Table 1.

**Insert Table 1**

## 3. Results and discussion

3.1. Optimization of extraction and derivatization parameters

3.1.1. Extraction solvent

The selection of an appropriate extraction solvent is one of the most important factors of the proposed method. In order to optimize the method performance in this study, organic solvents and mixtures such as methanol, dichloromethane, dichloromethane/methanol (1:1, v/v) and dichloromethane/methanol (2:1, v/v), which were previously reported, were tested thoroughly [7,24,37]. Additionally to the solvent selection, solvent volume and extraction time were tested as well, using ultrasonic extraction. The entire extraction procedure was evaluated using two standard solutions (5 and 25 μg $mL^{-1}$) in six replicates. Peak areas were obtained for each set of experimental variables. The studied analytes were better extracted by a triplicate extraction using 30 mL of dichloromethane/methanol (1:1, v/v) mixture for 15 min each time . The afore described conditions were selected throughout our next experiments.

3.1.2. Initial evaluation of the derivatization reagent

Derivatization techniques are affected mainly by the derivatization reagent and the experimental conditions (reagent volumes, pH, derivatization temperature and time). In preliminary studies for the selection of the derivatization reagent, two single and one multiple step derivatization techniques were evaluated using BSTFA, MSTFA and $BF_3/CH_3OH$. In every case, a standard solution (1 μg $mL^{-1}$) of the studied compounds was used. Both reagents have the exact performance, by derivatizing hydroxyl/carboxylic groups simultaneously to trimethylsilyl ethers and esters, respectively. Briefly, the first one-step technique includes the following steps: addition of the KPA to the standard solution; evaporation until dryness using nitrogen; addition of 250 μL BSTFA+1% TMCS and 100 μL of pyridine; heating at 70 $^{o}$C for 3 h; addition of the internal standard and GC/MS analysis. On the contrary, the second one-step technique uses MSTFA instead of BSTFA. In addition to the previous one-step techniques, a double derivatization method described by Jaoui et al. [37] was also investigated. In this method the addition of KPA to the standard solution is followed by evaporation until dryness under a gentle stream of nitrogen. Subsequently, the solution is treated with 0.5mL of $BF_3/CH_3OH$ and heated at 65$^{o}$C for 20min. After the mixture is allowed to cool to room temperature, 1.0 mL of ultrapure water saturated with sodium chloride is added. Then extraction with 2.0mL of solvent follows (in triplicate) and the organic layer is transferred into a tube containing 1.0g of anhydrous sodium sulfate. The extract is filtered (PTFE filter disks, 0.22 μm) and then dried using nitrogen. After this, 250 μL BSTFA+1% TMCS and 100 μL of pyridine are added and solution is heated at 70 $^{o}$C for 2 h and GC/MS analysis is finally performed.

During the multiple step technique dichloromethane, hexane, petroleum ether and a mixture of dichlorometane/hexane (2:1 v/v) were tested as extraction solvents after the first derivatization. Peak areas were obtained for each set of experimental parameters. The best results were obtained when MSTFA was used. Moreover, the chromatograms obtained using MSTFA as derivatization reagent, did not exhibit so many artifacts as in the case of BSTFA.

3.1.3. Chemometric optimization study

The optimization study was performed using spiked filters with the studied compounds at the concentration level of 1 μg $mL^{-1}$.

3.1.3.1. Screening design

Apart from the derivatization reagent, MSTFA volume, MSTFA/TMCS volume ratio, pyridine volume, derivatization temperature and time are also expected to induce significant impact on the method performance. In order to evaluate the significance of these factors, a factorial experimental design was used. The variables investigated were evaluated at two levels, low (denoted as -1) and high (denoted as +1). A three replicate centre point (level 0) was included in the design to estimate the experimental variance and check the loss of linearity between the levels chosen for each variable. A $2^5$ factorial design was applied to evaluate the main effects. In total, the design matrix had 35 runs, three of them at the central point. The experiments were run randomly in order to minimize the effect of the uncontrolled parameters [43] while each run included the performance of the entire method.

The levels of the experimental design are summarized in Table 2. The data was processed using the SPSS 21.0 statistical program. For the determination of significance of the main effects the response area was used. The data obtained for these variables were evaluated through analysis of variance (ANOVA). The results of the ANOVA, expressed in terms of *F*-ratios and *p*-values, showed that only the volume of pyridine (*F*: 8.64, *p*:0.02), derivatization temperature (*F*: 7.54, *p*: 0.01) and time (*F*: 7.39, *p*: 0.02) were found to significantly affect the method performance. The results of this first step led to the elimination of two variables: MSTFA and TMCS volume. Hence, the fixed values of 250 μL and 2.5 μL were chosen for the following step.

**Insert Table 2**

3.1.3.2. Optimization design

In order to optimize the variables that had significant influence, a central composite design (CCD), was carried out, including a fractional factorial part with three variables, namely pyridine volume, derivatization temperature and time. The CCD consisted of the points of factorial design ($2^3$) augmented by (2×3) star points. The star points were located at –α and +α from the centre of the experimental domain. An axial distance α was selected with a value of $2^{3/4}$ (1,682) in order to establish the rotatability condition. With the inclusion of this condition, the design generates information equally in all directions, i.e. a rotation of design about the origin does not alter the variance contours. The runs at the centre of the experimental field were performed in triplicate. Therefore, the matrix of CCD design involved 16 experiments. The values corresponding to every factor in each experiment are shown in Table 3. The experiments were randomly carried out, and each run was performed twice.

**Insert Table 3**

The CCD data were evaluated using ANOVA and the coefficient of determination ($R^2$) for the model was calculated to be 0.9643. The value of the coefficient indicated that 96.43% of the total variation about the average is explained by the regression. The lack of fit of the model to the observed values was checked performing the *F*-test. Fig. 1 shows the response surface developed by the model of the design. For the presentation of the surfaces, the variable not shown is kept at the centre point value. Maximum was reached when the pyridine volume was 50 µL, and the temperature and time were 70 ºC and 2 h respectively. These parameter values were used for the validation of the method as they were also verified by the model used. A new design using this as a centre point was made and the procedure was repeated. The optimal conditions were found inside the experimental conditions.

**Insert Figure 1**

3.2 Validation of the method

The developed method was validated for linearity, specificity, precision, trueness, and limits of determination (LOD) and quantitation (LOQ). Additionally, uncertainties of the results were calculated for the studied compounds. Main validation data of the optimized analytical method are shown in Table 4.

Linearity was evaluated using calibration standards at six concentration levels, ranged from 0.5 to 50 μg $mL^{-1}$, while the number of the performed iterations for each level was 3. Coefficients of determination values ($r^2$) are given in Table 4.

The specificity was evaluated by analyzing blank quartz filters in triplicate. The obtained chromatograms showed that there was no interference from the matrix in the areas of retention time the compounds were eluted.

The precision of the method was determined in terms of repeatability (intra-day) and intermediate precision (inter-day) at two different concentration levels (5 and 25 μg $mL^{-1}$). The experiments were performed in five (intra-day) and ten (inter-day) replicates respectively for each level. These results are shown in Table 4. The method presicion expressed in relative standard deviation values (RSD %) for inter-day study were ranged from 2.3 % to 5.2 % for all the compounds in both concetration levels.

The trueness of the method was evaluated performing recovery experiments using spiked filters at the same concentration levels (5 and 25 μg $mL^{-1}$). The recovery values are shown in Table 4 and ranged from 78.8% to 82.1% for all the compounds at the low level and from 79.2% to 87.1 at the high level, showing the good efficiency of the proposed method in terms of extraction recovery and precision. The results for both precision and trueness indicate that the method is accurate for the intended scope of analysis.

LOD and LOQ values are also presented in Table 4. Both LOD and LOQ were calculated experimentally from a signal-to-noise-ratio of 3.3 and 10, respectively, by using blank filters. The analysis was performed in ten replicates. The calculated values were verified by analyzing filters at the LOQ levels.

Finally, the uncertainty values for two concentration levels (5 and 25 μg $mL^{-1}$) were calculated according to the procedure described in EURACHEM/CITAC Guide [42] and taking into consideration the contribution of bias (from trueness), precision (from precision experiments) and purity of the standards. The relative combined uncertainty (*U%*) for both levels ~~was~~ ranged from 4.8 to 21.4 at the 95% confidence level (Table 4). The highest values, observed at the low concentration levels, can be considered as satisfactory. To the best of our knowledge this is the first time that the analysis results of SOA tracers in $PM_{10}$ are accompanied with their uncertainty.

**Insert Table 4**

3.3 Performance in $PM_{10}$ samples.

The developed method was performed in $PM_{10}$ samples collected in a semi-rural area near ~~to~~ Athens basin. Totally 10 samples were collected and analyzed for the SOA content during winter and summer. A representative chromatogram of one of those samples is shown in Fig.2. The results are shown in Table 5. In the vast majority of samples, pinic acid, pinonic acid and 2-C-methyl-D-erythritol had an average value of 15.1, 38.6 and 14.7 ng $m^{-3}$ ,respectively.

Additionally to the studied compounds, a large number of others were identified and quantified as well. Their identification was based on their mass spectrum which was compared to the spectra of compounds in NIST library with a match greater than 90%, whereas their quantification was based on their precursor hydrocarbon; mesoerythritol and KPA were used for the quantification of isoprene and the a-pinene products respectively. Moreover, tetracosane d-50 was used for the quantification of fatty acids, sugars, and others. [8,24]

**Insert Figure 2**

**Insert Table 5**

**Insert Figure 3**

According to the results a difference of the oxidation products concentration in airborne particles is clear. During the warm period, when both ambient temperature and solar radiation intensity are increased, volatile biogenic compounds remain in the gas phase for a short time, rapidly decomposing into other derivatives with the main product being the photochemical ozone. On the contrary, during the cold period, the photo dissociation of these compounds is limited resulting in their reaction towards the formation of secondary particles. No significant differences were found between the total concentration of isoprene and a-pinene products, while the a-pinene products showing a greater variation in concentration depending in the sampling area and the season. The above findings obviously require a greater number of samples, which is also a subject to further research work.

## 4. Conclusions

In this study a derivatization GC/MS method using MSTFA/TMCS/pyridine for the determination of SOA tracers in $PM_{10}$ was optimized by the use of a multivariate strategy.

Among the experimental parameters studied pyridine volume, derivatization temperature and time were found to significantly affect the method application in SOA tracer analysis in air particles. The validation results indicated that the MSTFA/TMCS/pyridine-GC/MS method can be applied successfully for the determination of pinic and pinonic acids and 2-C-methyl-D-erythritol in $PM_{10}$ samples as an alternative method to the existing ones. The method also seems to be promising for the determination of other SOA tracers.

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**Figure captions**

Figure 1. Response surface for chromatographic peak area estimated from the central composite design as obtained by plotting the peak response versus the experimental variables. A: pinic acid, B: pinoninc acid, C: 2-C-methyl-D-erythritol

Figure 2. Chromatogram obtained from the analysis of the $PM_{10}$. Peak codes are given in Table 5.

Figure 3. Seasonal variation of SOA in $PM_{10}$ samples

Table 1. Characteristic mass fragments and retention time used for the determination by GC/MS

| Compound | $t_R$ (± SD*), min | MW | MWder. | Major Ions (m/z) |
|---|---|---|---|---|
| **Ketopinic acid** | 15.59 (± 2.9 $10^{-3}$) | 182 | 254 | 239, 73,75,197,226,137 |
| **Pinonic Acid** | 16.38 (± 9.0 $10^{-3}$) | 184 | 256 | 73, 171,75,83,43,98 |
| **Mesoerythritol** | 16.57 (± 2.2 $10^{-3}$) | 122 | 194 | 73, 217,147,205,103,204 |
| **2-C-methyl-D-erythritol** | 17.74 (± 7.0 $10^{-3}$) | 136 | 424 | 219, 73,117,147,220,129 |
| **Pinic Acid** | 20.46 (± 7.9 $10^{-3}$) | 186 | 330 | 73, 129,75,171,172,157 |
| **Tetracosane-D50** | 35.94 (± 1.7 $10^{-3}$) | 389 | | 66, 82,50,98,46,62 |

* SD: standard deviation under reproducibility conditions (n=18)

Table 2: Experimental variables, levels and design matrix of the factorial design

| Run | Factors* | | | | | Total response | Run | Factors* | | | | | Total response |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | | | 1 | 2 | 3 | 4 | 5 | |
| **27** | 150 | 3% | 50 | 80 | 3 | 12046599 | **4** | 300 | 3% | 50 | 60 | 1 | 13120595 |
| **23** | 150 | 3% | 150 | 60 | 3 | 15805169 | **14** | 300 | 1% | 150 | 80 | 1 | 10481287 |
| **17** | 150 | 1% | 50 | 60 | 3 | 13023491 | **26** | 300 | 1% | 50 | 80 | 3 | 12961625 |
| **19** | 150 | 3% | 50 | 60 | 3 | 14273447 | **8** | 300 | 3% | 150 | 60 | 1 | 13002291 |
| **20** | 300 | 3% | 50 | 60 | 3 | 15257287 | **18** | 300 | 1% | 50 | 60 | 3 | 13128760 |
| **15** | 150 | 3% | 150 | 80 | 1 | 12360547 | **32** | 300 | 3% | 150 | 80 | 3 | 12293156 |
| **3** | 150 | 3% | 50 | 60 | 1 | 13900832 | **2** | 300 | 1% | 50 | 60 | 1 | 15034298 |
| **5** | 150 | 1% | 150 | 60 | 1 | 15392071 | **10** | 300 | 1% | 50 | 80 | 1 | 11550480 |
| **21** | 150 | 1% | 150 | 60 | 3 | 14546059 | **16** | 300 | 3% | 150 | 80 | 1 | 13024444 |
| **7** | 150 | 3% | 150 | 60 | 1 | 12946625 | **25** | 150 | 1% | 50 | 80 | 3 | 13839670 |
| **31** | 150 | 3% | 150 | 80 | 3 | 11075404 | **28** | 300 | 3% | 50 | 80 | 3 | 14678846 |
| **11** | 150 | 3% | 50 | 80 | 1 | 12386247 | **13** | 150 | 1% | 150 | 80 | -1 | 12371203 |
| **6** | 300 | 1% | 150 | 60 | 1 | 14466188 | **12** | 300 | 3% | 50 | 80 | -1 | 13839142 |
| **1** | 150 | 1% | 50 | 60 | 1 | 13369478 | **29** | 150 | 1% | 150 | 80 | 3 | 14715731 |
| **30** | 300 | 1% | 150 | 80 | 3 | 14411599 | **34 (C)** | 225 | 2% | 100 | 70 | 2 | 15939145 |
| **33 (C)** | 225 | 2% | 100 | 70 | 2 | 15588914 | **24** | 300 | 3% | 150 | 60 | 3 | 15069012 |
| **9** | 150 | 1% | 50 | 80 | 1 | 10738539 | **22** | 300 | 1% | 150 | 60 | 3 | 12164712 |
| **35 (C)** | 225 | 2% | 100 | 70 | 2 | 14930806 | | | | | | | |

* 1: MSTFA volume (150-300 µL), 2: TMCS % v/v (1-3%), 3: pyridine volume (50-150 µL), 4: derivatization temperature (60-80 $^{o}$C), 5: derivatization time (1-3) h

Table 3: Experimental variables, levels and design matrix for the central composite design (CCD)

| Test | Factors | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | Total response |
| **4** | 145 | 75 | 1,10 | 12794203 |
| **8** | 145 | 75 | 2,89 | 13125979 |
| **14** | 100 | 70 | 3 | 11741385 |
| **15(c)** | 100 | 70 | 2 | 12044940 |
| **11** | 100 | 60 | 2 | 2904754 |
| **6** | 145 | 65 | 2,89 | 5003835 |
| **3** | 55 | 75 | 1,10 | 13092740 |
| **2** | 145 | 65 | 1,10 | 5224390 |
| **5** | 55 | 65 | 2,89 | 4812621 |
| **10** | 150 | 60 | 2 | 2437928 |
| **12** | 100 | 80 | 2 | 3915797 |
| **16(c)** | 100 | 70 | 2 | 13196115 |
| **1** | 55 | 65 | 1,10 | 4401923 |
| **7** | 55 | 75 | 2,89 | 13259801 |
| **9** | 50 | 70 | 2 | 14077685 |
| **13** | 100 | 70 | 1 | 11405776 |

* 1: pyridine volume (50-150 µL), 2: derivatization temperature (60-80 $^{o}$C), 3: derivatization time (1-3) h

Table 4: Validation data for the MSTFA/TMCS/pyridine-GC/MS method optimized for the determination of SOA tracers.

| Method parameter | | Pinonic acid | 2-C-Methyl-D-erythritol | Pinic acid |
|---|---|---|---|---|
| **Linearity** (0.5 to 50 µg $mL^{-1}$) | | y = 0,6962x -0,0218 ($r^2$ = 0,9991) | y = 2,2945x + 0,0091 ($r^2$ =0,998) | y = 0,8139x - 0,0167 ($r^2$ =0,998) |
| **Precision,** %RSD[a] | 5 µg $µL^{-1}$ | 5.2 | 3.4 | 4.8 |
| | 25 µg $µL^{-1}$ | 2.8 | 2.3 | 3.1 |
| **Trueness** | 5 µg $µL^{-1}$ | 78.8 ± 2.9 | 82.1 ± 1.7 | 80.1 ± 2.5 |
| | 25 µg $µL^{-1}$ | 81.9 ± 1.2 | 87.1 ± 1.3 | 79.2 ± 1.6 |
| **LOD, µg $mL^{-1}$** | | 0.19 | 0.10 | 0.12 |
| **LOQ, µg $mL^{-1}$** | | 0.6 | 0.3 | 0.4 |
| ***U(%)***[b] | 5 µg $µL^{-1}$ | 18.2 | 12.3 | 21.4 |
| | 25 µg $µL^{-1}$ | 4.8 | 5.4 | 6.6 |

[a] %RSD: relative standard deviation (intra-day)

[b] *U %*: relative expanded uncertainty at 95% confidence level (k=2)

Table 5: SOA tracers concentration in $PM_{10}$ samples.

| Code | Compound | Major Ions | C, ng $m^{-3}$ (average) | No of samples - detected | $C_{max}$, ng $m^{-3}$ | $C_{min}$, ng $m^{-3}$ |
|---|---|---|---|---|---|---|
| **Products of Isoprene** | | | | | | |
| I1 | Lactic acid | 147, 117, 73, 191, 90, 48 | 74.9 | 10 | 160 | 38.9 |
| I2 | Acetic acid | 73, 147, 66, 45, 148, 205 | 14.4 | 10 | 29.7 | 6.60 |
| I3 | Oxalic acid | 147, 40, 73, 148, 44, 45 | 8.91 | 9 | 11.6 | 5.00 |
| I4 | Malonic acid | 40, 147, 73, 44, 75, 148 | 9.49 | 9 | 14.6 | 4.82 |
| I5 | Fumaric acid | 245, 40, 73, 147, 75, 155 | 6.05 | 2 | 6.31 | 5.82 |
| I6 | Glycerol | 73,147, 205, 117, 103,218 | 88.5 | 10 | 234 | 47.7 |
| I7 | **2-C-Methyl-D-erythritol** | 219, 235, 73, 75, 117, 147 | 14.7 | 9 | 22.4 | 5.80 |
| I8 | Succinic acid | 147, 73, 75, 148, 247, 40 | 12.7 | 10 | 17.4 | 6.80 |
| I9 | 2-Methyl-acetoacetic acid | 73, 147, 204, 405, 75, 245 | 10.4 | 5 | 14.5 | 6.60 |
| I10 | Glyceric acid | 73,147, 40, 292, 189, 133 | 13.3 | 8 | 20.5 | 6.20 |
| I11 | Malic acid | 73, 147, 233, 245, 75, 133 | 19.0 | 8 | 39.5 | 9.00 |
| I12 | Acetoacetic acid | 73, 147, 75, 231, 207, 246 | 6.20 | 2 | 6.80 | 5.60 |
| **Products of a-pinene** | | | | | | |
| A1 | α-Hydroxy- glytaric acid | 73, 129, 147, 247, 75, 45 | 14.9 | 6 | 20.9 | 9.90 |
| A2 | Adipic acid | 73, 117, 75, 147, 116, 111 | 18.6 | 10 | 45.9 | 7.90 |
| A3 | **Pinonic acid** | 156, 73, 147, 157, 258, 230 | 38.6 | 10 | 82.1 | 17.8 |
| A4 | Pimelic acid | 73, 75, 147, 117, 45, 40 | 10.2 | 4 | 14.4 | 6.10 |
| A5 | β-Hydroxy-β-methyl-glytaric acid | 73, 147, 75, 247, 40, 231 | 9.95 | 4 | 14.3 | 6.10 |
| A6 | **Pinic acid** | 73, 75, 129, 147, 171, 217 | 15.1 | 9 | 38.6 | 4.00 |
| A7 | Phthalic acid | 147, 73, 75, 295, 148, 45 | 58.2 | 10 | 265 | 8.30 |
| A8 | Suberic acid | 73, 75, 147, 187, 129, 303, | 11.7 | 10 | 26.7 | 4.50 |
| A9 | Isocitric acid | 73, 273, 147, 285, 117, 75 | 84.7 | 10 | 126 | 39.3 |
| A10 | Tricarballylic acid | 73, 147, 75, 377,117,271 | 16.1 | 5 | 31.7 | 9.50 |
| A11 | Cis-aconitic acid | 73, 147, 75, 207, 229, 375 | 6.65 | 4 | 10.1 | 4.00 |
| A12 | Azelaic acid | 73, 75, 147, 317, 201, 117 | 18.3 | 10 | 37.6 | 7.90 |
| **Fatty acids** | | | | | | |
| B1 | n-tetradecanoic acid | 73, 75, 285, 117, 147, 129, | 12.7 | 6 | 18.0 | 6.80 |
| B2 | n-pentadecanoic acid | 299, 117, 73, 75, 132, 129 | 37.0 | 10 | 68.6 | 22.1 |
| B3 | Palmitoleic acid | 73, 117, 75, 293, 311, 129 | 39.4 | 10 | 77.6 | 26.3 |
| B4 | Palmitic acid | 313,117, 73, 75, 132, 129 | 339 | 9 | 779 | 185 |
| B5 | Heptadecanoic acid | 327,73, 117, 75, 132, 129 | 13.3 | 7 | 19.8 | 6.90 |
| B6 | Linoleic acid | 73, 75, 55, 81, 67, 337 | 17.9 | 10 | 34.3 | 10.7 |
| B7 | Oleic acid | 73, 339, 117, 75, 129, 55 | 31.7 | 10 | 66.3 | 20.9 |
| B8 | Stearic acid | 341, 117, 73, 75, 132, 129 | 140 | 10 | 278 | 73.5 |
| **Sugars and others** | | | | | | |
| C1 | Ribitol | 73, 217, 147, 307, 205, 103 | 33.8 | 9 | 95.4 | 11.9 |
| C2 | Glucitol | 129, 73, 205, 217, 147, 320 | 32.3 | 9 | 102 | 10.4 |
| C3 | Inositol | 73, 207, 147, 75, 217, 305 | 15.6 | 2 | 20.8 | 10.4 |
| D1 | Levoglucosan | 73, 204, 217, 147, 75, 333 | 554 | 10 | 2409 | 18.2 |

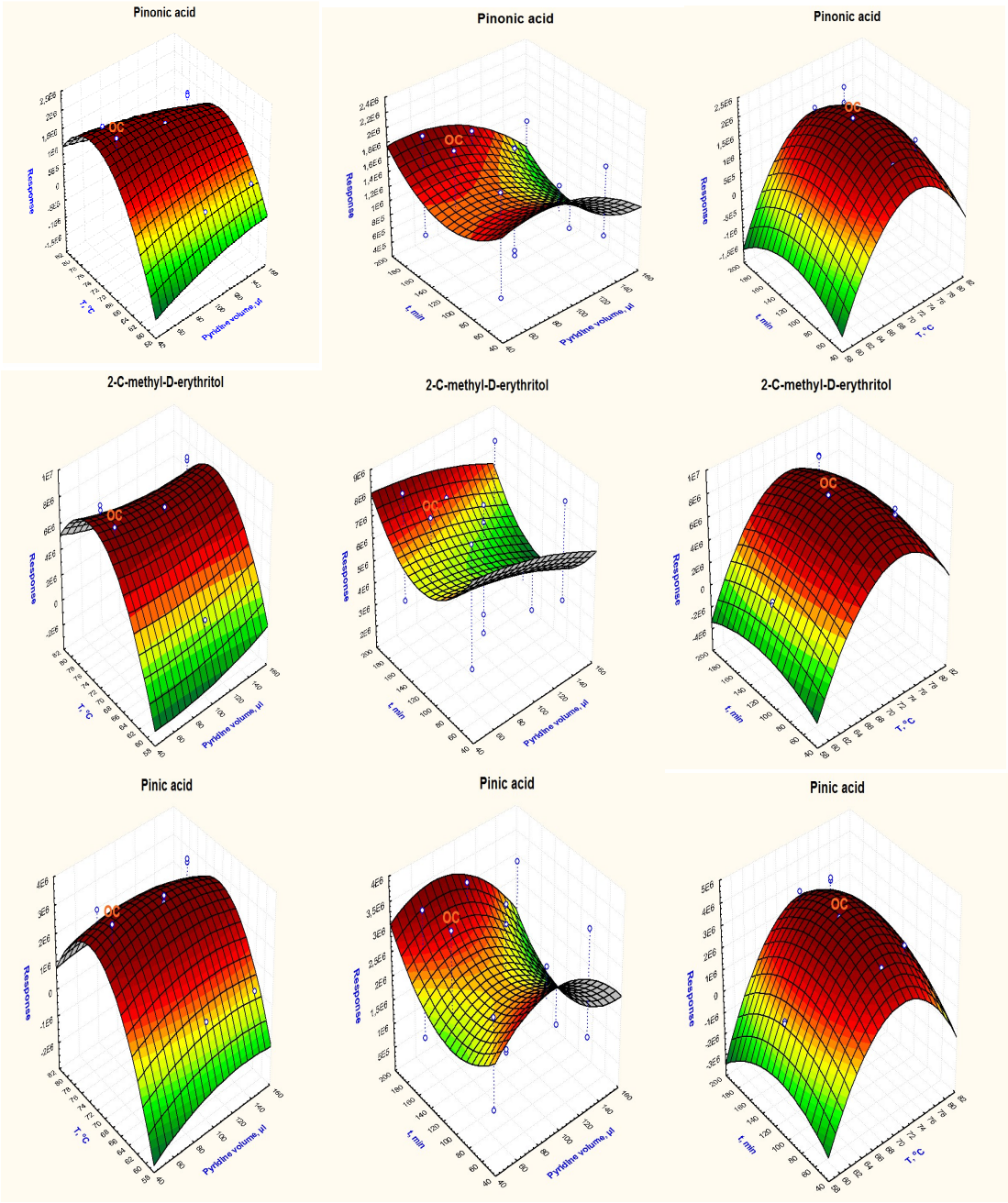



**Figure 1**

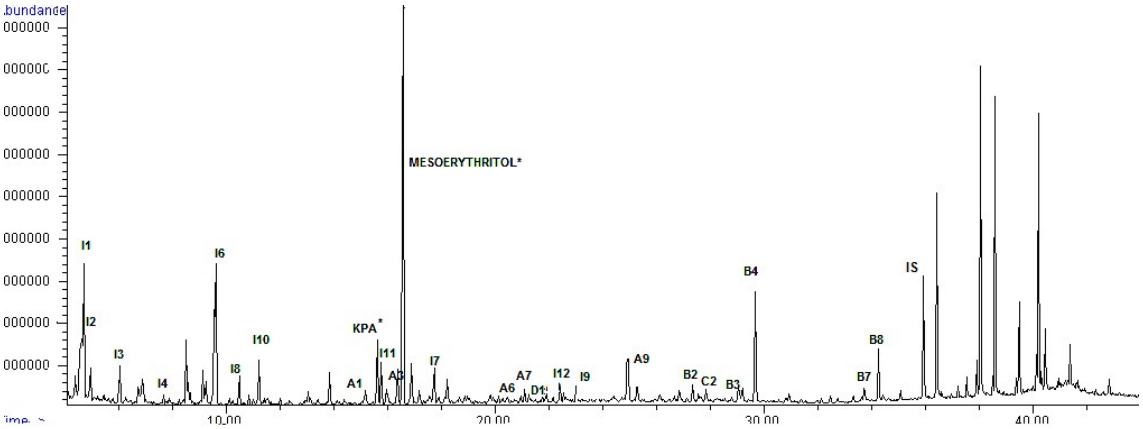
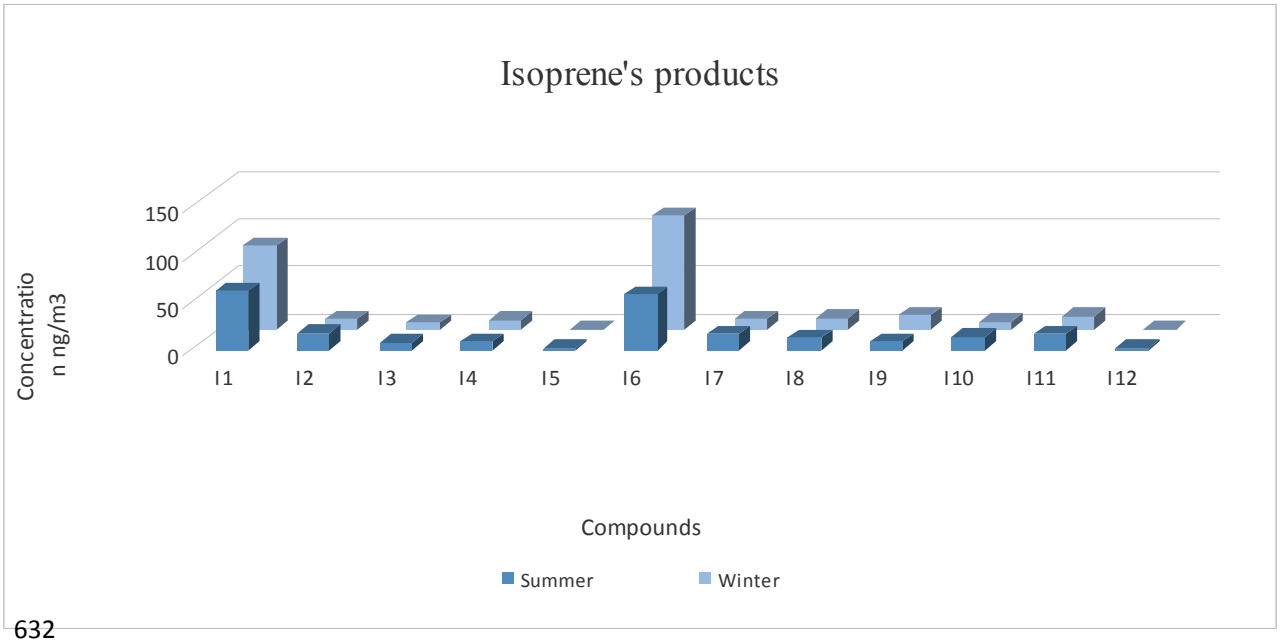
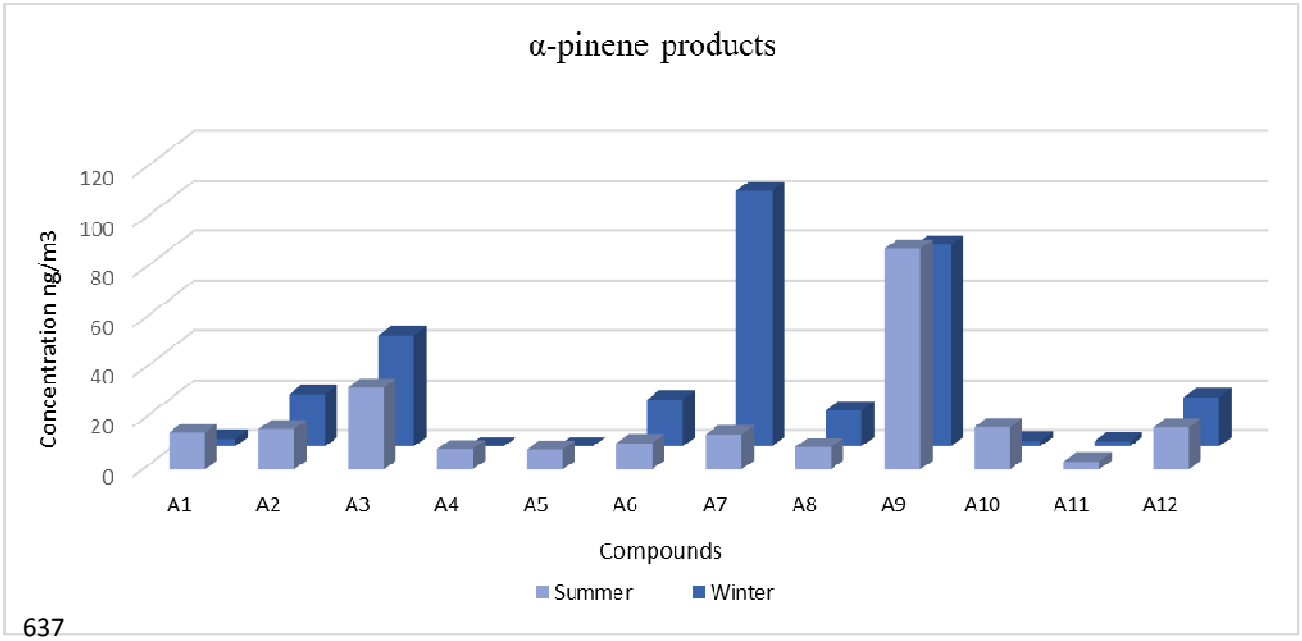



**Figure 2**



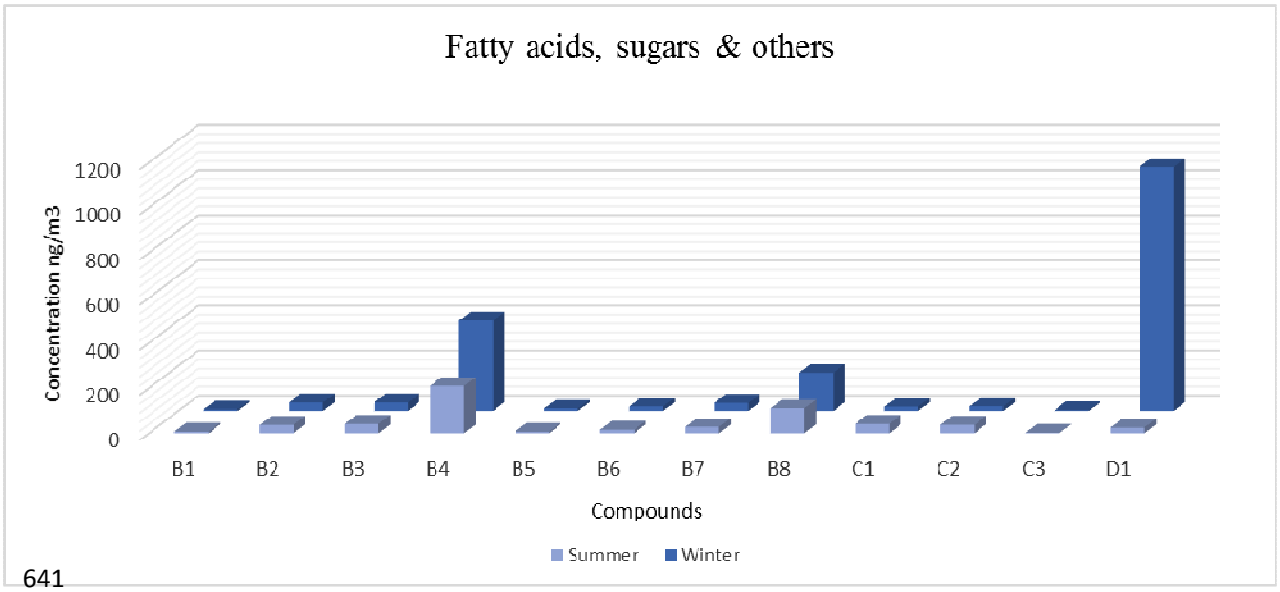



**Figure 3**