

# Validation of a multistep derivatization method for the determination of polar species in organic aerosol.

Kanellopoulos P.G.<sup>1</sup>, Chrisohou E.<sup>1</sup>, Koukoulakis K.<sup>1</sup>, Papadakis I.<sup>1</sup>, Bakeas E.<sup>1,\*</sup>

<sup>1</sup>National and Kapodistrian University of Athens, Greece, Laboratory of Analytical Chemistry, Department of Chemistry, Zografou GR-15784

\*corresponding author: Bakeas Evangelos, e-mail: bakeas@chem.uoa.gr

## Abstract

Secondary organic aerosol (SOA) is a significant constituent of airborne fine particles. A multistep derivatization method was developed, optimized, and validated for the determination of organic compounds in the atmospheric aerosol with mono- and multi-functional species, with GC/MS. The first step includes the alkylation of the carbonyl (C=O) moieties using *o*-methylhydroxylamine hydrochloride as the derivatization reagent. The second step refers to the conversion of carboxylic acids to methyl ester derivatives using TMSD/methanol or BF<sub>3</sub>/methanol. Finally, alcohols are converted to trimethylsilyl ethers with BSTFA+ 1% TMCS. The use of TMSD/methanol as the derivatization reagent in step 2 is preferred for the procedure, achieving higher sensitivity. Additionally, BF<sub>3</sub>/MeOH was ineffective at converting some mono/dicarboxylic acids and species with more than 2-OH moieties. The derivatization procedure in stage 3 was optimized yielding the appropriate reaction temperature and time, while in stage 2 yielding the appropriate amounts of the derivatization reagents. The method was validated for twenty-two compounds which are possible SOA tracers. Detection limits ranged from 0.31 to 0.98 ng m<sup>-3</sup> and recoveries from 73.5% to 108%. The method was applied to field samples from rural and industrial areas with concentration levels ranging from below limits of detection to 42 ng m<sup>-3</sup>.

**Keywords:** Secondary Organic Aerosol, Multistep derivatization, GC-MS, Oxygenated compounds

## 1. Introduction

Atmospheric aerosol is a significant component of the atmosphere with various effects on human health, climate and atmospheric chemistry (Seinfeld and Pandis, 2006; Kanakidou et al., 2005). Organic aerosols can be directly emitted to the atmosphere (Primary Organic Aerosol - POA) from both natural and anthropogenic sources, or can be the result of atmospheric reactions that occur in the gas and particle phase (Secondary Organic aerosol - SOA) (Pöschl, 2005). SOA consists of a large number of organic species with varying polarities. The wide polarity range is attributed to the nature and the number of the oxygenated functional groups [e.g carboxyl (-COOH), hydroxyl (-OH), carbonyl (C=O) and multivariate combinations of them] of the SOA compounds (Borras

and Tortajada-Genaro, 2011). Analysis of the latter compounds is based on single/multistep derivatization techniques prior to GC/MS injection (Jaoui et al., 2004). A one-step derivatization method has been optimized and validated in our previous study (Mologousi and Bakeas, 2016). Meanwhile, few 3-step derivatization methods have been reported (Flores and Doskey, 2015).

The scope of this study is to develop and compare two 3-step derivatization methods (hereafter method A and method B) and optimize the procedure with better precision and higher sensitivity. Further evaluation steps were performed for the chosen method. Finally, the proposed method was applied to PM<sub>10</sub> samples from rural and industrial areas.

## 2. Experimental

### 2.1. Sample preparation

Analytical procedures, for method A and B, have step 1 in common. This step refers to the conversion of the C=O moieties to oxime derivatives (R-C=N-OCH<sub>2</sub>) using *o*-methylhydroxylamine hydrochloride (MHA) as the derivatization reagent. In step 2 of method A carboxylic acids are converted to methyl esters (O=C-R-OCH<sub>3</sub>) with the use of BF<sub>3</sub>/methanol as the derivatization reagent, whereas in method B trimethylsilyldiazomethane (TMSD)/methanol is used for the same purpose. Step 3 includes the derivatization of alcohols into trimethylsilyl ethers [R-OSi(CH<sub>3</sub>)<sub>3</sub>] using N,O-Bis(trimethylsilyl) trifluoroacetamide with 1% TMCS (BSTFA) as a catalyst. A total of 25 compounds (internal and surrogate standards included) were selected for the comparison and optimization of both methods. Briefly, 200 µL of the mix solution was evaporated to dryness. Common step 1 starts by adding 60 µL of heated MHA (diluted in ACN). The reaction takes place in a water bath for 1 hour. In step 2 of method A, carboxylic acids were derivatized to methyl esters with 50 µL of BF<sub>3</sub>/MeOH at 65°C for 20 min. After that, the acid was neutralized with 0.5 mL of a saturated aqueous solution of sodium chloride. After a three-time extraction (with 1 mL of 1:1 (v/v) Hex/DCM) the organic layer was filtered through a Pasteur pipette containing 500 mg of anhydrous sodium sulfate and reduced to 100 µL with a gentle stream of nitrogen. Step 2 of method

B includes the conversion of carboxylic acids by adding 12  $\mu\text{L}$  of TMSD and 3.4-8  $\mu\text{L}$  of MeOH in an ultrasonic bath for 20 minutes. Alcohols were transformed to trimethylsilyl ethers in step 3 with 180  $\mu\text{L}$  of 1% TMCS

6- in BSTFA and 50  $\mu\text{L}$  of pyridine at 70°C for 60 min in method A and with 180-245  $\mu\text{L}$  of 1% TMCS in BSTFA and 50  $\mu\text{L}$  of pyridine at 65°C for 60 min in method B.

## Results

The relative MS responses of the derivatized compounds in each method are presented in (Fig.1). As it is clear, Method B presented higher sensitivity than Method A with higher MS responses for every compound studied. Method A, was even ineffective at converting some species such as: benzoic acid, meso-erythritol, trimellitic acid and 2-methylerythritol.

Step 2 and 3 of Method B were optimized regarding the volumes of each derivatization reagent and the reaction time respectively (Fig 2; Fig 3). Preferable conditions include: a) 12 $\mu\text{L}$  TMSD- 8 $\mu\text{L}$  MeOH- 245 $\mu\text{L}$  BSTFA and b) 1hour reaction time.

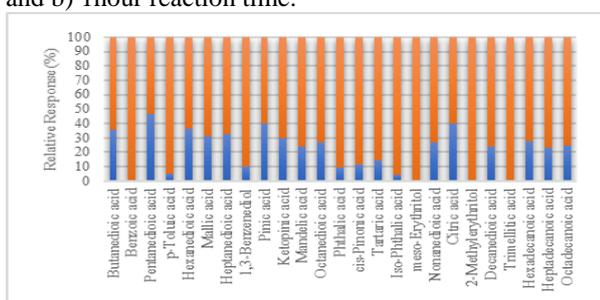


Figure 1. Relative response (%), of the two methods. (Blue = Method A; Orange = Method B), Concentration of the mix solution was 25  $\text{ng mL}^{-1}$ .

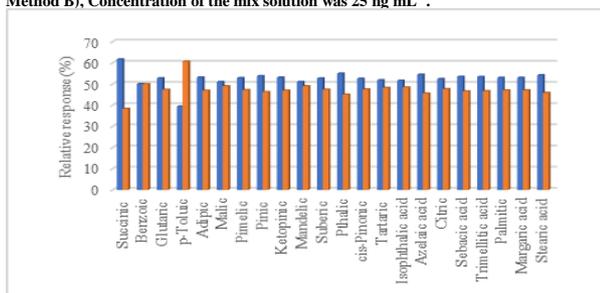


Figure 1. Relative response (%) of derivatized carboxylic acids using two different ratios of the derivatization reagents (Blue= 12 $\mu\text{L}$  TMSD- 8 $\mu\text{L}$  MeOH- 245 $\mu\text{L}$  BSTFA / Orange= 6 $\mu\text{L}$  TMSD- 3.4 $\mu\text{L}$  MeOH- 180 $\mu\text{L}$  BSTFA)

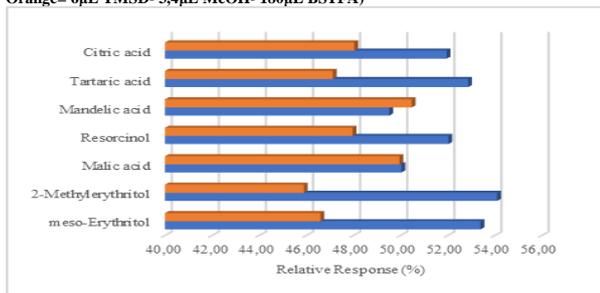


Figure 2. Relative Response (%) of -OH containing derivatized species. (Blue = 1 hour reaction time / Orange = 2 hour reaction time)

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Using the optimized conditions, the method was validated for 22 compounds (+3 surrogate/internal standards). A 4-point calibration curve in the range of 0.5 to 10  $\mu\text{g mL}^{-1}$  was constructed by injecting each concentration level 6 times. Limits of detection (LODs) ranged from 18 to 54  $\text{ng mL}^{-1}$  i.e. 0.31-0.98  $\text{ng m}^{-3}$  for approximately 55  $\text{m}^3$  typical volume collected over a 24hour period. Regarding the trueness of the method, recovery experiments were performed using spiked filters at the same concentration level (1  $\mu\text{g mL}^{-1}$ ) with recoveries ranging from 73.5 to 108%. Precision of the derivatization procedure was determined in terms of repeatability and reproducibility at the concentration level of 5  $\mu\text{g mL}^{-1}$  with RSD% ranging from 1.85 to 8.00%.

The optimized method was applied to 20 field samples from rural (n=10) and industrial sites (n=10) of Cyprus during the winter period. Concentration levels ranged from <LOD to 42  $\text{ng m}^{-3}$ . Pinic and malic acids were the most abundant species in the rural area while phthalic acid was dominant in the industrial area. Apart from the compounds that have been used for the development of the method, we identified approximately about 30 other compounds. Identification was based on the mass spectra with the help on NIST library with a match greater than 90% (Mologousi and Bakeas, 2016). Among the identified compounds the most abundant were sugars, fatty alcohols, fatty acids and biomass burning tracers.

The method is considered suitable for the determination of polar organic species in the atmospheric aerosol and can be applied for more compounds in future studies.

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