Deliverable D3.15:
Standard Operating Procedures (SOPs) for the targeted set of OA Tracers

This document was prepared by Erik Swietlicki, Hafiz Abdul Azeem and Margareta Sandahl (ULUND), Anke Mutzel, Martin Brüggemann and Hartmut Herrmann (TROPOS), after consultation also with other relevant ACTRIS partners.

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Comments	*Delayed due to:* *The collaboration between the EUROCHAMP-2020 project and ACTRIS-2 resulted in a division of work also for the organic tracers. The ILC on BSOA marker compounds was conducted within EUROCHAMP WP3, by the OGTAC CC (Organic Tracers and Aerosol Constituents Calibration Centre, TROPOS, Germany).*

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1 Background – Task 3.1 subtask on organic aerosol tracers

This deliverable deals with Task 3.1 of ACTRIS-2 WP3 ("Improvement of instrumentation, standardization and quality assessment of essential climate and air quality variables"). The task includes, among other variables, also selected Organic Aerosol (OA) tracer compounds. The OA tracer subtask aims to establish a set of common European Standard Operating Procedures (SOP) for sampling and analysis of a selection of organic tracers, in order to facilitate their subsequent implementation at several ACTRIS sites for the purpose of organic aerosol (OA) source apportionment across Europe. The work on OA tracers in ACTRIS-2 Task 3.1 is a continuation of that performed within the EU FP7 Infrastructure Project ACTRIS (Task 3.3b). See also the previous ACTRIS-2 WP3 Task 3.1 deliverable related to the work on Organic tracers (D3.1, "Expert workshop to determine the targeted set of OA tracers", public report, M6).

According to the ACTRIS-2 Grant Agreement, Task 3.1 including the OA tracer part aims:

- to increase the amount and quality of delivered data,
- to control implementation of existing Standard Operation Procedures (SOP), and
- to eventually propose revisions.

This will be achieved (here only that relevant for OA tracers) via:

- inter-laboratory comparison exercises (round-robin),
- use of ACTRIS TNA (WP8).

This means that within ACTRIS-2, inter-laboratory comparison (ILC) studies are performed for the anhydrous sugars as suitable tracers for biomass burning (see Deliverable D3.10).

As motivated in Deliverable D3.1, ACTRIS-2 selected 3-methyl-1,2,3-butanetricarboxylic acid (3-MBTCA) in order to establish this compound as a new OA tracer for biogenic (monoterpene) secondary organic aerosol (BSOA) in Europe.

The main reasons for selecting MBTCA as a BSOA tracer for ACTRIS-2 are:

- MBTCA is a unique oxidation product of monoterpenes (pinenes), that are major BVOCs;
- MBTCA shows near-complete partitioning to the particle phase (low volatility);
- MBTCA has a long atmospheric lifetime (~10 days) compared to other BSOA compounds;
- Several analytical techniques exist for MBTCA that can form the basis for SOPs;
- The required analytical instrumentation is already available in most well-equipped analytical chemistry laboratories, also within ACTRIS and EUROCHAMP;
- Extraction techniques are available for pre-concentration of MBTCA in aerosol samples;
- Standards (reference material) of MBTCA are available, both in the pure form and on filters (NIST);
- It has been shown, also at ACTRIS sites, that it is possible to sample and analyze MBTCA with reasonable accuracy, precision and cost.
- Other OA tracers can be quantified simultaneously with MBTCA in many cases.

The chain of events towards establishing an OA tracer for ACTRIS is:

i. Selection of a candidate OA tracers;
ii. Performing ILC for the selected candidate OA tracers to estimate their applicability within ACTRIS and EUROCHAMP;

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iii. Establishing new SOPs for the suitable OA tracers for subsequent implementation in ACTRIS;
iv. Establishing control procedures for the suitable OA tracers (merging SOPs and ILC procedures);
v. Implementing the control procedures for the OA tracers at regular time intervals to ensure data quality and operability at ACTRIS sites.

For MBTCA, D3.1 completed the first step (Selection), while this Deliverable D3.15 describes the ILC and the drafting of SOPs. Establishing and implementing control procedures for MBTCA and other BSOA compounds will not be performed within ACTRIS-2, but is rather the task for the ACTRIS ERIC.

2 The first inter-laboratory comparison on BSOA marker compounds

Background

The collaboration between the EUROCHAMP and ACTRIS communities dates back several years, with many partners in common to both infrastructure projects (FP6 EUROCHAMP and EUSAAR, FP7 EUROCHAMP-2 and ACTRIS, H2020 EUROCHAMP-2020 and ACTRIS-2). It is the ambition that EUROCHAMP and ACTRIS will join to form the ACTRIS ERIC, and extensive efforts are made towards this aim, for instance within the H2020 ACTRIS Preparatory Phase Project.

EUROCHAMP-2020 established a separate unit for organic tracers, the OGTAC CC (Organic Tracers and Aerosol Constituents Calibration Centre, TROPOS, Leipzig, Germany). The OGTAC CC is part of their WP3 on "Standard Protocols, Instrumentation, Quality Assurance and Data provision", and WP8 on "Transnational Access to Calibration Facilities". OGTAC CC is a calibration centre for the analysis of atmospheric organic tracers and particle-phase constituents. This is the first of its kind world-wide and focuses on tracers and main SOA constituents of biogenic and anthropogenic origin. It offers inter-laboratory comparisons (ILCs) and training of users. Such a coordinated and dedicated calibration centre for organic tracers has not been part of ACTRIS-2.

Considering this, it is logical that the work on organic tracers outlined in the ACTRIS-2 Workplan will be continued and extended within EUROCHAMP-2020, and later in the ACTRIS ERIC.

Also, ACTRIS-2 WP3 failed to find volunteers among the ACTRIS-2 partners for hosting the ILC on BSOA tracer compounds, including MBTCA. Note that no dedicated funding for this ILC was earmarked in the ACTRIS-2 budget.

TROPOS, through the OGTAC CC of EUROCHAMP-2020, graciously volunteered to take on the BSOA ILC, to a large extent since this is in the interest of EUROCHAMP community and in accordance with the EUROCHAMP WP8. This also avoided duplication of work by the merging EUROCHAMP-ACTRIS communities.

Here, in the ACTRIS-2 Deliverable D3.15, only a summary of the BSOA ILC are presented. The full results will be reported on the EUROCHAMP-2020 webpage.

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Summary of results from the BSOA ILC

The invitation for participation in the BSOA ILC was launched by the OGTAC CC of EUROCHAMP-2020 (TROPOS) in October 2017 via the E2020 and ACTRIS-2 mailing list, later followed by a reminder. The ILC activity was also announced at the ACTRIS-2 WP3 meeting in Madrid in October 2017. Altogether thirteen laboratories responded to the call and participated in the ILC. These were mainly from Europe (France, Spain, Germany, Poland, Denmark, Switzerland, Slovenia and UK) but also from the US. Unfortunately, there were no participants from the ACTRIS-2 community, despite a late reminder sent by the ACTRIS-2 Project office shortly prior to the end of deadline.

Each of the thirteen participants received a set of 7 samples:

3 collected from PM10 filters in Melpitz, Germany in March 2018 (operated by TROPOS)
3 from α-pinene oxidation collected in the new twin chamber at the atmospheric chemistry department at TROPOS (ACD-C)
1 blank filter

In total, 98 filter aliquotes were prepared. The analysis of the aliquotes had to be repeated three times. Target compounds were five α-pinene oxidation products: Terebic acid, Terpenylic acid, Pinonic acid, Pinic acid and 3-Methyl- 1,2,3-tricarboxylic acid (MBTCA), as also suggested in the review by Nozière et al[1] (2015, section 3.3).

Only seven of the thirteen participants reported results. The participants were free to use their preferred analytical technique and procedure, and included LC/MS (4), LC/HRMS (1) and GC/MS (2). Each participant received a questionnaire asking them to describe: Storage, extraction procedure, type of analysis, column for separation, detector, eluent gradient, type of standards used for quantification (purchased from supplier, surrogate, synthesized standard) and detection limit.

Extraction procedures used were ultrasonic bath (2) and orbital shaker (5), with extraction times between 20–90 min in the orbital shaker and 60 min for the ultrasonic bath. Extraction solvents were MeOH (3), ACN/MeOH (1), ACN/H_2O (1), MeOH/Methylenechloride (1) and ACN (1). Internal standards (ISTD) used were sebacic acid, 1,2,3,4-cyclobutane-tetra-carboxylic acid, and keto-pinic acid. Five of the participants chose not to use any ISTD. It was noted that there was a strong variability in the extraction procedure used.

For terebic acid, very good agreement was seen between all seven reporting laboratories for ambient SOA (at a terebic acid concentration of ~0.4 μg/m^3). For the same compound and the ACD-C generated SOA, one laboratory appears to be an outlier (high value). All groups used the same supplier for the terebic acid standard.

For Pinonic acid, a stronger variability was reported compared to Terebic acid. This may partly be due to different standards being used (synthesized, Sigma-Aldrich, Toronto Research Chemicals). Laboratories reporting similar values used the same supplier for the pinonic acid standard.

Since standards may well prove to be a cause of the reported variability, OGTAC initiated work to identify all suppliers for BSOA compounds and to compile all available information (CAS, purity, isomer, price etc.).

[1] Nozière B., M. Kalberer, M. Claeys, J. Allan, B. D'Anna, S. Decesari, E. Finessi, M. Glasius, I. Grgić, J. F. Hamilton, T. Hoffmann, Y. Iinuma, M. Jaoui, A. Kahnt, C. J. Kampf, I. Kourtchev, W. Maenhaut, N. Marsden, S. Saarikoski, J. Schnelle-Kreis, J.D. Surratt, S. Szidat, R. Szmigielski, and A. Wisthaler (2015) The Molecular Identification of Organic Compounds in the Atmosphere: State of the Art and Challenges, Chem. Rev., 2015, 115 (10), pp 3919–3983, DOI: 10.1021/cr5003485.

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For 3-Methyl- 1,2,3-tricarboxylic acid (MBTCA), only five laboratories were reporting data. The variability was observed to be much larger than for the other BSOA compounds. For instance, there was as much as a factor of 3 difference between the lowest and highest reported value for the ambient Melpitz PM10 sample (at ~3 µg/m^3). There was no apparent dependency on technique (2 x LC/MS, 3 x GC/MS), use of ISTD (3 x Yes, 2 x No), or extraction procedure (3 x orbital shaker, 2 x ultrasonic bath).

Parallel work at ULUND, Sweden, on MBTCA led to a possible explanation to the apparent underestimation of MBTCA by some laboratories, due to the formation of MBTCA iron(III) complexes. The work is described in more detail in Azeem et al (2019)[2].

A steady complexation behavior of the analyte was observed in experiments performed using 0.5-250 µg/mL standard solutions of MBTCA. Furthermore, the role of both positive and negative electrospray ionization in the formation of iron(III) complexes was investigated. Regardless of different combinations of capillary and cone voltages, the complexes were observed in all runs. It was, however, observed that fine-tuning the voltages influences the relative abundance of the ions. Therefore, it is recommended that ESI–MS signals of both MBTCA and its subsequent complexes should be considered by UHPLC–ESI–QToF users for qualitative as well as quantitative analysis of MBTCA in aerosol samples, especially when dealing with samples of low concentrations. It was further noted that MBTCA iron(III) complexes may be avoided using EDTA in direct infusion mode.

It is also worth noting that dispersive liquid-liquid micro-extraction (DLLME) may be used as an extraction technique for MBTCA analysis at small concentrations. Additive assisted DLLME for extraction of polar compounds such as MBTCA provided an enrichment factor of 16.6. The optimized DLLME shows a limit of detection of MBTCA using GC–MS (0.12 pg/m^3) that is significantly lower than previously reported.

Remaining work on BSOA tracers

The remaining steps for the BSOA ILC includes:

1) Data evaluation according to ISO 13528 and ISO 5725-2
2) Recommendations (SOPs) for:
    - Extraction procedure
    - Quantification procedure
    - Authentic standards (trustful suppliers, synthesis, characterization of synthesized standards)
    - Suitable Surrogate (if possible)
3) Start compilation of MS fragmentation pattern in positive and negative mode

This work will be done within EUROCHAMP-2020 OGTAC CC (WP8), with the assistance also of ACTRIS-2 partners.

Draft SOP for MBTCA

A suggested protocol for analysis of MBTCA is given below, prepared by ULUND. Note that this is not officially recommended by ACTRIS as a SOP for MBTCA and other BSOA tracers. The Draft SOPs identified through the BSOA ILC to be candidate SOPs will be posted on the ACTRIS web pages for SOPs (see Aerosol in-situ variables): http://actris.nilu.no/Content/SOP .

[2] H.A. Azeem, T. Tolcha, P. Ekman Hyberg, S. Essén, K. Stenström, E. Swietlicki and Margareta Sandahl, 2019. Extending the scope of dispersive liquid-liquid microextraction for trace analysis of 3-methyl-1,2,3-butanetricarboxylic acid in atmospheric aerosols leading to the discovery of iron(III) complexes. Analytical & Bioanalytical Chemistry. DOI: 10.1007/s00216-019-01741-1.

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Protocol for analysis of 3-methyl-1,2,3-butane tricarboxylic acid using dispersive liquid-liquid microextraction followed by gas chromatography – mass spectrometry

Prepared as part of ACTRIS-2 WP3 under the lead of ULUND

Version 1, March 14, 2019

Objective

3-methyl-1,2,3-butane tricarboxylic acid (MBTCA) is a secondary organic aerosol compound originating from biogenic emissions of terpenes. After several complex atmospheric oxidation reactions, monoterpenes such as α- and β-pinene undergo a series of atmospheric reactions through several channels to produce MBTCA. MBTCA has been identified as a unique marker of monoterpene biogenic emissions.

Application

MBTCA can be extracted from aerosol samples and analysed by dispersive liquid-liquid microextraction (DLLME) followed by gas chromatography – mass spectrometry (GC–MS). The method provides low limits of detection and can be used to quantify MBTCA for the purpose of biogenic source apportionment.

Extraction protocols for aerosol filter samples

The filter sample is cut into small pieces. MBTCA from these pieces is then extracted in 5 mL milliQ water acidified to pH 2 by HNO_3 using a Branson 3200 sonicator (Branson, Danbury, CT, USA) for 1 hour. The extract is filtered using 0.45 µm polypropylene membrane syringe filter.

Dispersive liquid-liquid microextraction (DLLME)

- MBTCA extracts are saturated by dissolving 25% NaCl (w/v)
- extraction solvent (150 µL of 1-octanol containing 15% tri-*n*-octyl phosphineoxide, w/w) is mixed with dispersion solvent (500 µL of methanol) and injected into the extract.

  An emulsion of fine droplets of extraction solvent is produced.

  Critical step: *extraction and dispersion solvents are mixed in a GC vial before taking into syringe to ensure mixing.*

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- The emulsion is allowed to stay for 15 min and then another injection of dispersive solvent (500 µL of methanol) is made to break the emulsion.

  Extraction solvent separates as top layer.

- Extracting solvent is collected and used for further analysis.

  Critical step: *It is important to perform extractions in glassware with narrow neck to facilitiate collection of 150 µL of extraction solvent (as top layer).*

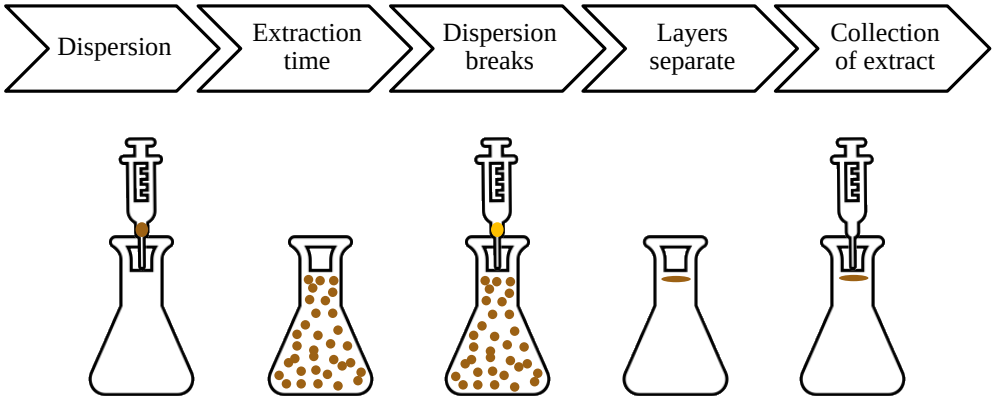

Figure 1. An illustration of dispersive liquid-liquid microextraction, brown dots represent mixture of extraction and dispersion solvents in step 1 forming an emulsion in steps 2 and 3. Yellow liquid in the syringe represents second injection of dispersion solvent that terminates extraction. In step 4, extraction solvent separates at top layer (brown) [1].

Derivatization

- The extracts are evaporated with 100 µL of acetone at 40 °C under a gentle stream of N_2. *Addition of a volatile aprotic solvent like acetone breaks intermolecular forces and facilitates evaporation.*
- MBTCA is derivatized with 15 µL of hexane containing internal standard and 10 µL of N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% Trimethylchlorosilane (TMCS) at 80 °C for 1 hr.
- Samples are injected in GC–MS immediately after derivatization.

Instrumental description

Agilent 6890 series gas chromatograph

Agilent 5973 network mass detector

Column: Varian hp-5ms (30m x 0.25mm ID 0.25)

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Analytical conditions and program

Injection: 2 µL splitless, injection temperature: 280 ^{o}C

Inlet temperature: 250 ^{o}C with constant gas flow mode, splitless time: 1 min.

Gas (He) flow: 1 mL min^{-1}

Column temperature: initial temperature is 60 ^{o}C for 3 min then it is raised to 170 ^{o}C at a rate of 15 ^{o}C min^{-1} & finally raised to 300 ^{o}C at a rate of 30 ^{o}C min^{-1} and held for 4 min.

MSD transfer line temperature 280 ^{o}C. The mass source temperature is 250 ^{o}C & MS quadruple temperature is 180 ^{o}C.

Data acquisition and quantification:

Samples are injected in duplicate. MBTCA is identified by comparison with mass spectrum and retention time of standards, and quantified using selected ion monitoring (SIM). Derivatized MBTCA and internal standard (1-phenyldodecane) are identified and quantified by ions m/z 405 and 246, respectively.

Chemicals and standards

a. 3-methyl-1,2,3- butanetricarboxylic acid (MBTCA) (Toronto Research Chemicals Inc, Toronto, Canada)
b. LCMS grade methanol (Honeywell, Seelze, Germany)
c. Octanol (HPLC- grade, 99%, Sigma-Aldrich, Munich, Germany)
d. HNO_3 (Merck, Darmstadt, Germany)
e. Tri-*n*-octyl phosphineoxide (TOPO, 99%, Acros, Geel, Belgium)
f. 1-phenyldodecane (internal standard, 97% Acros Organics 97% Acros Organics, New Jersey, USA) in *n*-hexane (50 µL dichloromethane containing 5 µg mL^{-1} 1-phenyldodecane).
g. N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylsilyl chloride (TMCS) (Sigma-Aldrich, Munich, Germany)

References

The protocol has been developed using the following literature.

1 H.A. Azeem, *Extraction and Chromatography of Targeted Emission Markers in Atmospheric Aerosols*. Doctoral Thesis, Lund University, Sweden (2018). http://lup.lub.lu.se/search/ws/files/54269282/Kappa.pdf

2 H.A. Azeem, T. Tolcha, P. Ekman Hyberg, S. Essén, K. Stenström, E. Swietlicki and M. Sandahl, *Extending the scope of dispersive liquid-liquid microextraction for trace analysis of 3-methyl-*

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1,2,3-butanetricarboxylic acid in atmospheric aerosols leading to the discovery of iron(III) complexes. DOI: 10.1007/s00216-019-01741-1

Contact persons

Dr. Hafiz Abdul Azeem (hafiz.abdul_azeem@chem.lu.se)

Dr. Margareta Sandahl (margareta.sandahl@chem.lu.se)

Center for Analysis & Synthesis, Department of Chemistry

Lund University LTH

PO Box 118, SE–22100 Lund, Sweden