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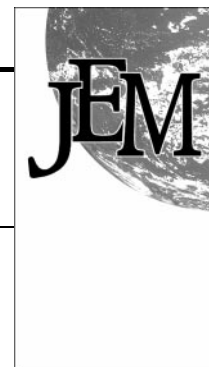
A new method for the determination of trichloroacetic acid in spruce foliage and other environmental media

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Trichloroacetic acid (TCAA) is a phytotoxic chemical, present throughout the environment. The majority of methods for analysis of TCAA require chemical derivatisation and multiple extraction steps prior to analysis by gas-chromatography. Here, a new analytical method for TCAA determination in environmental matrices is reported. The method is based on a modified Nielsen-Kryger steam distillation that combines into one 1 h reflux the thermal decarboxylation of TCAA to $CHCl_3$ and the partitioning and concentration of the $CHCl_3$ into 5 ml of hexane, which is analysed by GC. Sample preparation is minimal and no matrix standard additions are required. The background $CHCl_3$ in the sample is removed prior to extraction by degassing the solution for 1 h with nitrogen. Optimisation of the method gave recoveries from three separate solutions of 0.31 ppb aqueous TCAA standards of $93 \pm 15\%$ ($n=9$), $110 \pm 9\%$ ($n=9$) and $105 \pm 11\%$ ($n=6$). The extraction method has been compared with a decarboxylation and headspace GC method for determination of TCAA in Sitka spruce needles. No significant difference in TCAA concentration or replicate precision between the two methods was observed.

Introduction

In the last few years there has been a resurgence in interest regarding the sources and fluxes of trichloroacetic acid (TCAA) within the environment.[1] Historically, TCAA was used as an agricultural herbicide, but it remains ubiquitous at $ng\,g^{-1}$ (parts per billion) levels in surface waters, soil and foliage, where its known toxic properties are a cause for concern.[2] An important unresolved question is the modern-day source of TCAA in tree foliage, in particular, the relative contributions from atmospheric photochemical oxidation of anthropogenic C_2-chlorocarbon solvents (e.g. 1,1,1-trichloroethane and tetra-chloroethene) and uptake via wet or dry deposition,[3] or natural production in soils via chloroperoxidase enzymes acting on Cl^- and organic matter substrates[4] and uptake through the root system.[5] TCAA also arises from the action of chlorine and other disinfectants on natural organic matter in waters.

The first step towards resolving this question is the development of a sensitive and reliable technique for quantifying TCAA at trace levels in a range of environmental media. Since TCAA is too involatile to be analysed directly by gas chromatography, its analysis is invariably complex. Previous methods have utilised the thermal decarboxylation of TCAA to chloroform ($CHCl_3$) and headspace GC (HSGC) analysis of the $CHCl_3$,[6] or the derivatisation of TCAA to the methyl ester,[3] the difluoroanilide[7] or the pentafluorobenzyl ester[8] and subsequent GC determination of the derivative. Recently, it has been reported that TCAA can not be reliably analysed via the latter method because of the instability of the PFB derivative.[9]

The derivatisation techniques are multistep and time-consuming, and are consequently potentially subject to losses in recovery. Derivatisation via the methyl ester also has the disadvantage of using potentially explosive and highly toxic diazomethane, although the reagent can be avoided by methylating with acidified methanol.[10] The headspace de-carboxylation technique is more direct, but gas sample injection is more awkward, and the method is difficult to calibrate because of the variable impact of each (non-zero TCAA) matrix on headspace partitioning and interference from background $CHCl_3$.

To overcome these difficulties, a new method for the analysis of TCAA has been developed, which uses a modified Nielsen-Kryger steam-distillation apparatus (referred to by the acronym MONKS throughout the remainder of this paper). This method combines into a single process the extraction of TCAA from the matrix, its decarboxylation to $CHCl_3$, and sample concentration. This paper presents details of a thorough evaluation of the new MONKS method, including a direct comparison between the MONKS and HSGC techniques for determination of TCAA concentrations in Sitka spruce (Picea sitchensis) needle foliage.

Experimental

Details of MONKS method

Sample preparation and extraction. The steam-distillation apparatus follows the design of Veith and Kiwus[11] shown in Fig. 1. A 5.0 g sample is added to 100 ml of deionised water in a flask (aqueous samples are used directly). The solution is degassed with oxygen-free nitrogen for 2 h at room temperature prior to reflux in order to remove contaminant $CHCl_3$ from the deionised water, and to expel background $CHCl_3$ from the sample which would otherwise interfere with detection of $CHCl_3$ arising solely from TCAA decarboxylation. As an additional check, degassed deionised water is extracted along-side sample extractions, and hexane blanks are analysed directly for $CHCl_3$.

A precise aliquot of 25 ml degassed deionised water and 5 ml HPLC grade hexane is added to the solvent collection region in the condenser, which is connected to the main reflux apparatus by an overflow tube. The water fills the collection region above the level of the return tube and prevents subsequent loss of
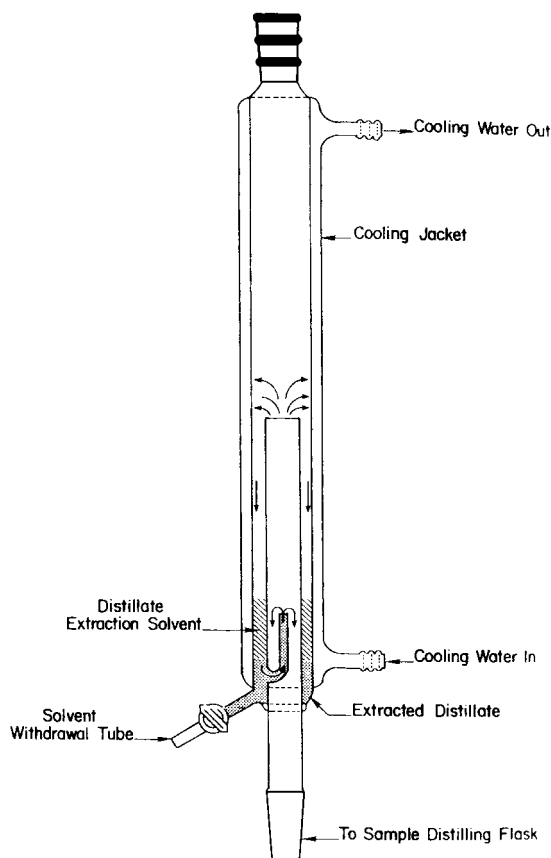
Fig. 1 The modified Nielson-Kryger steam-distillation (MONKS) apparatus.

Labels in figure: Cooling Water Out; Cooling Jacket; Distillate Extraction Solvent; Solvent Withdrawal Tube; Cooling Water In; Extracted Distillate; To Sample Distilling Flask

hexane to the reflux flask. The solution is refluxed at 100 °C for 1 h during which time $CHCl_3$ produced by TCAA decarboxylation condenses in the upper part of the apparatus and partitions into the hexane. The condensed steam passes through the hexane layer and is recycled *via* the return tube. A second Liebig condenser was used to eliminate evaporative loss of hexane or chloroform. After reflux the apparatus is cooled and the hexane extract removed and stored in crimp-sealed glass vials at 4 °C. It is not necessary to recover the entire hexane extract since the initial volume of hexane is accurately known.

GC analysis. The concentration of $CHCl_3$ in the hexane extract is quantified by GC and electron-capture detection (ECD). The GC parameters are given in Table 1. The concentration of TCAA in the sample is calculated from the $CHCl_3$ concentration in the extract. Corrections are applied for any background $CHCl_3$ determined in the water or hexane blanks.

Calibration. The preparation of accurate low concentration $CHCl_3$ standards in hexane is difficult because of the volatility of $CHCl_3$. To ensure consistent analytical determinations between analyses a $CHCl_3$ standard (30 ppb) was prepared in

Table 1 GC-ECD conditions used in the determination of $CHCl_3$

Gas chromatograph	Perkin-Elmer Autosystem
Capillary column	$50 \times 0.32 \times 1.8$ μm BP624
Injector	Split, 200 °C
Carrier gas	Helium, 12.6 psi
Detector	ECD, 375 °C
Make-up gas	N_2, 35 ml min^{-1}
Temperatur programme	5.5 min at 50 °C, 20 °C min^{-1}, 3 min at 200 °C
Integration	PC with Hewlett-Packard software

duplicate and analysed. If agreement was within 10% a standard and blank were stored and analysed against a fresh standard prepared for the following analyses. The samples were analysed against a five-point $CHCl_3$ calibration standard in the range 1–30 ppb. A standard and blank were analysed every 6 samples.

Method optimisation and evaluation. The following factors were optimised for the MONKS procedure: prior degassing time to remove background $CHCl_3$, extraction time, the stability of the stored extracts under various conditions, and TCAA concentrations determined using either whole or homogenised needle samples. The recovery and limits of detection of the method were evaluated.

Details of HSGC method

The headspace gas chromatography method used in this study as an independent method of TCAA determination is similar to that of Plumacher and Renner.[6] Samples are sealed into a headspace vial (in triplicate) and heated at 100 °C for 1 h to decarboxylate TCAA. Vials are re-equilibrated at 60 °C for a further 1 h prior to headspace sampling for $CHCl_3$ analysis by GC-ECD. Background $CHCl_3$ is determined from a parallel sample equilibrated at 60 °C only. Calibration is complex because the extent of partitioning of $CHCl_3$ into the headspace depends on the type of sample matrix and the headspace to sample volume ratio. TCAA is most accurately quantified by standard additions of known amounts of TCAA to a series of vials of each sample, but this requires many analyses for a single sample determination. Instead, constant "calibration factor ratios" are determined for the response of TCAA standard additions to a particular environmental sample matrix relative to TCAA aqueous standards. It is then only necessary to determine the daily GC response factor to an aqueous standard.[6]

Sample site details

A sampling programme was initiated in autumn 1998 at Glentress Forest in the Southern Uplands of Scotland. The forest is predominantly Sitka spruce, growing at elevations between 325 and 602 m a.s.l. (above sea level) and is well-isolated from areas of industry or urbanisation. Several year classes of Sitka spruce needles are sampled monthly at the two elevations. Rainwater and cloudwater are collected weekly by passive samplers. Soil samples are also collected. Air is actively sampled through a Na_2CO_3-impregnated open-face Whatman filter to capture weekly total gaseous and particle-bound TCAA. Full details and results will be published at the end of the sampling programme.

Results

MONKS method optimisation

Degassing prior to extraction. Background $CHCl_3$ must be eliminated prior to the MONKS extraction because it cannot be distinguished from $CHCl_3$ arising from TCAA decarboxylation. Relatively large concentrations of $CHCl_3$ (0.30 ppb) spiked in 100 ml samples of water and degassed for 1 or 2 h were reduced to undetectable levels after MONKS extraction (Table 2). In addition, there was 95% recovery of TCAA standards spiked with $CHCl_3$ and degassed for 2 h, indicating little or no loss of TCAA itself in the degassing process, although there was some inexplicable loss of TCAA in samples degassed for 1 h only.

As an ongoing check on levels of background $CHCl_3$ and TCAA, degassed deionised water was always extracted alongside sample extractions, and hexane blanks were analysed directly for $CHCl_3$.

Table 2 Effect of nitrogen degassing on removal of $CHCl_3$ prior to MONKS extraction. The concentration of $CHCl_3$ spike added to both the water and TCAA standard solution was 0.30 ppb. The concentration of the TCAA standard solution was 1.24 ppb. Error ranges are 95% confidence intervals for triplicate measurements

Degassing time/h	[CHCl₃] determined from spiked water (ppb)	[CHCl₃] determined from spikedTCAA solution (ppb)
No degassing	0.22 ± 0.10 (Recovery $CHCl_3 = 73 \pm 33\%$)	1.47 ± 0.15 (Recovery $CHCl_3 + TCAA = 95 \pm 10\%$)
1	ND[a]	0.79 ± 0.25 (Recovery TCAA $= 64 \pm 19\%$)
2	ND[a]	1.18 ± 0.37 (Recovery TCAA $= 95 \pm 29\%$)

[a]ND = not detected.

Extraction time. The impact of MONKS extraction time on TCAA recovery was evaluated by extraction of 0.31 ppb TCAA standards for 1 or 2 h. Recoveries, with 95% confidence intervals, were not significantly different, $105 \pm 11\%$ ($n = 6$) and $111 \pm 9\%$ ($n = 4$), respectively, indicating that 1 h extraction time is sufficient.

Volume of hexane extractant. The standard MONKS method developed uses 5 ml volume of hexane. Environmental samples with low TCAA concentrations may require an enhanced concentration factor which can be achieved using a smaller volume of hexane. Extractions using 2 ml of hexane and 100 ml solutions of 8.1 ppb or 0.31 ppb TCAA standards gave TCAA recoveries of $97 \pm 14\%$ ($n = 3$) or $111 \pm 9\%$ ($n = 4$), respectively, where error ranges are 95% confidence intervals. Enhanced sensitivity can also be achieved by utilising a greater mass of sample.

TCAA recovery. The combined conversion and recovery efficiencies of the optimised MONKS method for an aqueous TCAA standard solution of 0.31 ppb in three separate multi-replicate experiments were $93 \pm 15\%$ ($n = 9$), $110 \pm 9\%$ ($n = 9$) and $105 \pm 11\%$ ($n = 6$). Error ranges are 95% confidence intervals.

Limit of detection. The limit of detection of the MONKS method depends on a number of factors such as mass of sample, concentration of hexane extractant and variability of background TCAA in the water added to the reflux apparatus. Attainable limits of detection are 1 ng g^{-1} fresh needles for 5 g of Sitka spruce needles in 100 ml of water, and 0.05 ng ml^{-1} for 100 ml of rain- or cloudwater.

Sample storage. The potential for loss of $CHCl_3$ from the hexane extract prior to GC analysis was investigated by comparing 5.0 ppb $CHCl_3$ standards stored for 2 weeks in 2 ml crimped vials against equivalent standards analysed directly. A three-factor experimental design was used (two replicates in each): vials capped with PTFE or rubber septa; vials half or completely filled; vials stored at room temperature or refrigerated at 4 °C. No significant difference in $CHCl_3$ concentration after storage was observed for any of the factors.

Comparison of whole *versus* ground Sitka spruce needles. The effect of sample pre-treatment on the concentrations and precision of TCAA measured in Sitka spruce needles was investigated by parallel extractions of whole needles and needles homogenised to a powder under liquid nitrogen (Fig. 2). In most cases, homogenised needle samples yielded higher TCAA concentrations than the corresponding whole needle samples. These results suggest enhanced extraction efficiency for homogenised samples. All Sitka spruce needle data reported in this paper are for homogenised samples.

Replicate precision of TCAA determination in Sitka spruce needles. Batches of year C needles from 7 separate Sitka spruce trees selected at random on the same day from different areas and different altitudes were analysed in replicate ($n = 4$–7) by

the MONKS method. The results are shown in Fig. 3; error bars are 95% confidence intervals. The average relative standard deviation across all 7 sets of analyses was 16%. A one-way ANOVA showed that variability between the trees significantly exceeded analytical variability ($P < 0.002$).

Comparison between MONKS and HSGC methods

Seven different samples of Sitka spruce needles (collected from different trees and different sites) were each analysed in triplicate by both the MONKS extraction and HSGC methods to compare accuracy and precision. Results are shown in Fig. 4. (N.B., the needle samples are different to those shown in Fig. 3.) For each sample there is no significant difference in magnitude of TCAA concentration determined by the two methods. Furthermore, the relative precision for each triplicate (expressed as half the t-statistic 95% confidence interval divided by the mean) range from 6 to 42% (mean 24%) for the MONKS determinations and from 18 to 53% (mean 29%) for the HSGC determinations. There is therefore no evidence that the precision of the two methods differs significantly ($P = 0.45$).

Discussion

In contrast to previous multiple extraction and derivatisation techniques for the determination of TCAA the MONKS method requires zero, or minimal, sample preparation. Quantitative calibration is straight-forward since all TCAA present in the sample is extracted as $CHCl_3$ into hexane which is compared directly against standards of an equivalent matrix. No matrix standard additions are required. The extraction time of only 1 h, using standard glass apparatus, permits high sample throughput compared with derivatisation techniques. Although it is possible that $CHCl_3$ could also arise as a by-product of reactions between chlorine (if present in water) and
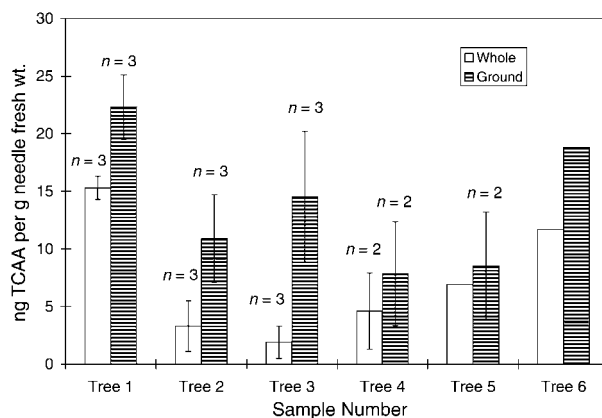

Fig. 2 TCAA concentrations in Sitka spruce needles determined by the MONKS method using whole or homogenised needles. $n =$ number of replicates. Error bars are 95% confidence intervals calculated as $t\,s/\sqrt{n}$, using the t-statistic for ($n-1$) degrees of freedom. Needle samples were taken at different times from different trees at different altitudes.
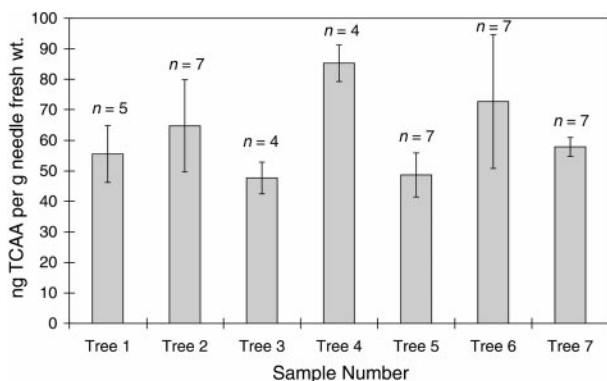
Fig. 3 Replicate analyses by MONKS of TCAA concentration in year C needles of seven separate spruce trees. n = number of replicates per tree. Error bars are 95% confidence intervals calculated as $t\,s/\sqrt{n}$, using the t-statistic for $(n-1)$ degrees of freedom. Needles samples were taken in June 1998 from different trees at different altitudes.

Fig. 4 Comparative concentrations of TCAA in different samples of Sitka spruce needles determined by the MONKS extraction and HSGC techniques. Error bars are 95% confidence intervals of triplicate analyses, calculated as $t\,s/\sqrt{n}$, where $t = 4.30$ for two degrees of freedom. Needle samples were taken in January 1999 from trees at different altitudes.

natural organic matter, the equivalent determinations of the MONKS and HSGC methods show that this is not a significant problem. (The addition of a reducing agent to limit $CHCl_3$ by-product formation may be feasible although this was not investigated in this work.) Furthermore, Frank *et al.*[3] have previously shown good agreement between the decarboxylation HSGC method and the diazomethane methyl derivatisation method, indicating that there is neither a major additional precursor present that decarboxylates to $CHCl_3$ nor any sample matrix effects. Both the MONKS and the HSGC methods have comparable limits of detection of ~ 1 ng g^{-1} for TCAA in needle foliage and it is straightforward to increase the sensitivity of the MONKS method by increasing the mass of sample analysed and/or decreasing the volume of hexane extractant.

An important additional finding from the work is the considerably better TCAA recovery from Sitka spruce needles that have been homogenised prior to analysis. This was true for both MONKS and HSGC methods.

The range of TCAA concentrations determined to date in Sikta spruce foliage at Glentress Forest (3–165 ng g^{-1} fresh wt.) is similar to the concentration ranges reported previously for TCAA in the foliage of Scots Pine and Norway Spruce in Germany, Finland and around the Caspian Sea.[3,12–14] The sampling programme at Glentress Forest is part of a wider study into the seasonal budget and fluxes of TCAA within this forest, and scientific conclusions based on the full data set with year class, altitude, season, *etc.* will be reported at a later date.

The MONKS method has also been applied to the analysis of TCAA in rainwater and cloudwater and in Na_2CO_3-impregnated filters used to sample atmospheric TCAA, and the method is therefore sufficiently versatile and robust for routine analysis of TCAA in a variety of environmental matrices.
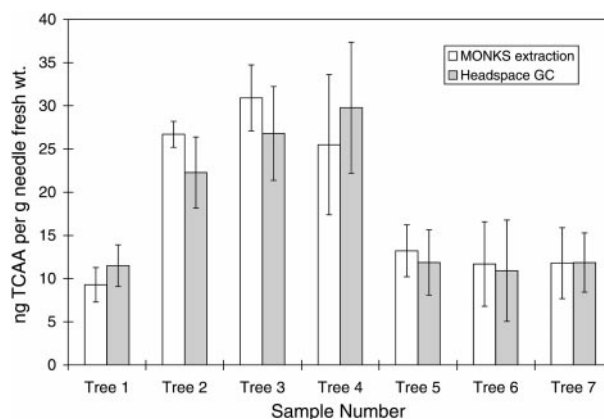
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