1. INTRODUCTION

Background and Objective:

The objective of this study was to independently validate an analytical method[2] for the determination of chloropicrin in air using XAD-4 (1400 mg/250 mg) sorption cartridges for a 48 hours air sampling period, followed by extraction with chilled ethyl acetate by shaking and final GC-MS/MS determination/confirmation of the analyte, achieving a target limit of quantitation (LOQ) of 0.1 µg/m^3.

Method Principles:

The air method procedure involved trapping of chloropicrin from the vapor phase using XAD-4 (1400 mg/250 mg) sorption cartridges, extraction from the sorbent with chilled ethyl acetate by shaking and final GC-MS/MS determination/confirmation of the analyte, monitoring 2 MS/MS mass transitions for the analyte. Evaluation was performed using multi point calibrations obtained with calibration solutions in solvent.

The total air sampling volume for efficient sorption of air on sampling cartridges was set to ≈ 100 mL/min, ≈ 6 L/h or ≈ 300 L/48 h resulting in a total air sampling volume of ≈ 0.3 m³. The total amount of chloropicrin to be trapped for the target LOQ was about 0.03 µg per trap. After air sampling the samples were extracted with 10 mL of ethyl acetate, therefore, the instrumental detection limit by GC-MS/MS was targeted at 0.5 ng/mL.

2. MATERIALS AND METHODS

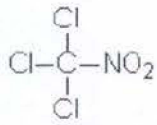
2.1 Test System

Ambient air (average temperature 25 °C, relative humidity: 42 % - 51 % on average, for details see Appendix 3)

2.2 Analytical Test / Reference Item

The following standard obtained commercially from Dr. Ehrenstorfer was used as test / reference item:

Common name: Chloropicrin



IUPAC Name: Trichloronitromethane

Chemical Formula: CCl_3NO_2 Molecular Mass: 164.37 g/mol

See Appendix 2 for information on the test/reference item used.

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[2] Arndt, T. and C. Warling, 17-Feb-15, amended 22-Jan-16. Method Validation of an Analytical Method for Monitoring Chloropicrin in Air. PTRL West Study No. 2540W.

2.3 Analytical Method

2.3.1 Apparatus

Analytical balances:

Mettler-Toledo XP205DR

Miscellaneous equipment:

Membrane pump N840.3 FT.18, KNF Neuberger

Vortex mixer REAX top, Heidolph

Horizontal shaker HS 260 B, IKA

SPE station Baker SPE-12G, Macherey & Nagel

SPE station equipped with vacuum gauge and flow control valve.

Glass column used as soap bubble flow meter (500 mm length, 50 mm i.d., calibrated with 100 mL portions of water).

Air sampling cartridges:

Media IA Tube/XAD-4 (1400 mg/250 mg) CPM032416-001, SKC/Analyt-MTC-GmbH (Prod.No.: 55556)

Humidity / temperature determination:

Thermohygrometer TH3121, Airflow Lufttechnik.

Flexible tubing and adapters for distribution of air.

Common laboratory glass or plastic ware. All the glass or plastic ware was cleaned in a laboratory dishwasher and air-dried before use.

GC-MS/MS System:

A Thermo Trace 1310 GC equipped with TriPlus RSH Base liquid + HS autosampler, split/splitless injector, helium as carrier gas, which was coupled to a Thermo TSQ 8000 Evo (triple-quad. mass spectrometer), operated via Thermo Xcalibur Software.

Column: Agilent DB-624 (length: 30 m, i.d.: 0.25 mm, 1.4 µm film thickness)

2.3.2 Reagents and Chemicals

Ethyl acetate, Promochem, for pesticide residue analysis

2.3.3 Preparation of Standard Solutions

2.3.3.1 Stock and Fortification Solutions

A stock solution of chloropicrin was prepared in ethyl acetate as follows:

Substance name	Amount [mg]	Dissolve in [mL]	Obtain* [mg/mL]
Chloropicrin (purity 99.0 %)	90.2	10	8.93

*The concentration was corrected for purity.

For the preparation of the stock solution of chloropicrin about 3 mL of ethyl acetate were transferred into a 10 mL volumetric flask, the flask sealed with a glass stopper and subsequently placed on an analytical balanced and tared. The ampoule containing the analyte was opened and the whole content was transferred by means of a Pasteur pipette into the volumetric flask which was immediately sealed again with the glass stopper. The amount of weighed chloropicrin was then read off the balance display and subsequently the volume was adjusted to 10 mL with ethyl acetate.

Fortification solutions were prepared by volumetric dilution of the stock solution in ethyl acetate. 1.12 mL of the stock solution were diluted in 10 mL of ethyl acetate to obtain a solution with 1.0 mg/mL to fortify at 500 x LOQ level. The 1.0 mg/mL fortification solution was further diluted to obtain solutions with 10 µg/mL to fortify at 10 x LOQ level and 1.0 µg/mL to fortify at the LOQ level

2.3.3.2 Calibration Standards in Solvent

Using the 10 µg/mL and 1.0 µg/ml fortification solutions, calibration solutions were prepared by accurate volumetric dilution in ethyl acetate as follows:

Fortification or Calibration solution used [µg/mL]	Volume Taken [µL]	Final Volume [mL]	Final Concentration [ng/mL]
10	100	10	100
10	50	10	50
10	40	10	40
10	30	10	30
1.0	200	10	20
1.0	100	10	10
1.0	50	10	5.0
0.10	200	10	2.0
0.10	100	10	1.0
0.10	50	10	0.50

2.3.3.3 Matrix Matched Calibration Standards

For the preparation of a matrix-matched standard the final volume of a control specimen (processed together with the validation specimens) was used. For the demonstration of the matrix effect a calibration solution in solvent (100 ng/mL) was added to an aliquot of the final volume of a control specimen resulting in a concentration of 30 ng/mL.

2.3.3.4 Storage of Standard Solutions

All solutions were stored in a freezer, in the dark, in 20 mL amber glass vials when not in use.

2.3.4. Fortification Procedure

Both ends of the air sampling cartridges were opened and the cartridge was attached to the air collection system (for details see 2.3.5. and Figure 1). The membrane pump was switched on and the air flow was adjusted to about 100 mL/min for each sampling cartridge. While the pump was running a known amount of the fortification solution was introduced, by means of a microliter syringe, into a trapping flask or impinger which was connected to the cartridge.

2.3.5 Air Sampling Procedure

Air sampling was performed by connecting a washing flask or impinger to the end of the cartridge with the front layer A (1400 mg sorbent beads) using silicon tubes. The rear end of the cartridge with the layer B (250 mg sorbent beads) was connected with silicon tubes to a SPE station with flow adjustment screws. A laboratory air pump was attached to the pressure gauge of the station with a tube, providing an air flow through the whole system. The surrounding air was drawn into the open end of the washing flasks and impingers (containing fortification solution of chloropicrin) from where it reached the sampling cartridges (the air being drawn over layer A and then over layer B) and subsequently the SPE station.

Scheme of the sampling cartridge (GW: glass wool plug; XAD: porous polymer):

GW	XAD A	GW	XAD B	GW

Direction of air flow ⇒

The air flow was determined every morning, noon and evening during the sampling period using a soap bubble meter calibrated with 100 mL portions of water.

For flow determination the bubble meter was attached to the open end of the washing flask or impinger in front of each sampling cartridge. The actual flow (in L/min) was calculated by dividing the volume travelled by the soap film (0.10 L) by the time measured using a stop watch.

The total air volume V_{Air} sampled through an individual cartridge was calculated by:

$$V_{Air} = \Sigma (t_i \times F_i) \times 0.001 \ m^3/L$$

with: V_{Air}: Total volume of air sampled, [m^3]

t_i: Time interval between two flow determinations [min].

F_i: Average flow of the two flow determinations of the time interval, [L/min].

The total sampling time, air temperature, and relative humidity of air were recorded. The experimental set up for air collection is shown in Figure 1.

After sampling for 48 hours the cartridges were removed from the sampling apparatus and the ends sealed with plastic caps. Extraction was performed on the same or following day as the end of the trapping period.

2.3.6 Extraction Procedure

1. 20 mL amber glass vials were filled with 10 mL and 5 mL of ethyl acetate and chilled in a refrigerator.
2. The caps on the sorbent cartridge were removed and the front-end glass wool plug was transferred to a chilled 20 mL amber glass vial containing 10 mL of ethyl acetate followed by the front sorbent beads. The vial was designated as the "A" extract. Control samples also used the 20 mL vials from above and were extracted as above.
3. The back side glass wool was removed and discarded. The back side sorbent beads were transferred to the chilled 20 mL amber glass vial containing 5 mL of ethyl acetate. The vial was designated as the "B" extract.
4. The vials were immediately capped with Teflon lined caps and vortexed to mix.
5. The vials were shaken on a horizontal shaker for about 1 hour at 180 motions/min.
6. Supernatants were transferred to 20 mL amber glass storage vials and designated the "final extract" (A or B as appropriate). The final extracts were aliquoted to the GC autosampler vials for analysis directly (control, LOQ level fortified samples and all back sample extracts) or after dilution with ethyl acetate. Dilutions were as follows:

   Low level front extracts: no dilution required

   Mid level front extracts: 10 X (0.10 mL extract + 0.90 mL ethyl acetate)

   High level front extracts: 500 X (serial dilution; 0.10 mL diluted to 10 mL, followed by 0.20 mL extract + 0.80 mL ethyl acetate)
7. Sample "final extracts" were stored in the 20 mL amber glass storage vials in a freezer.

2.3.7 Method Validation

2.3.7.1 Storage Stability

Storage stability of the analyte on XAD-4 (for 28 days) as well as in calibration solutions, fortification solutions and final extracts (for 66 days) when stored frozen has already been demonstrated in the original method validation.

If the recoveries in the fortified samples were within the acceptable range of 70 % – 110 %, stability of the analyte in standard solutions and extracts was considered sufficiently proven.

2.3.7.2 Recovery and Breakthrough after Sampling of Air

For method validation sorption cartridges were fortified with chloropicrin by introducing a known amount of a fortification solution, using a microliter syringe, into a washing flask or impinger which was connected to the cartridge, while air was drawn through the system. To fortify at LOQ level 30 µL of a 1.0 µg/mL solutions was used, for 10 x LOQ level 30 µL of a 10 µg/mL solution and for 500 x LOQ level 15 µL of a 1000 µg/mL solution. Five replicates were analyzed per fortification level.

Two sorption tubes were kept untreated and processed according to fortified sorption tubes to give blank control samples.

One sorbent cartridge was kept untreated and was extracted as the fortified samples without former air sampling to give a reagent blank control sample.

After air sampling (approximately 48 hours) the XAD layer A was analyzed for recovery and the rear layer B was analyzed for breakthrough determination.

2.3.7.3. Matrix Effect

Matrix effects were evaluated by comparing the peak area of a matrix matched standard solution with the peak area of a corresponding standard solution in solvent. A matrix matched standard solution was prepared at 30 ng/mL by diluting a calibration solution in solvent with the final volume of a non-fortified blank control sample processed together with the fortified samples.

2.4 Instrumental Analysis

2.4.1 GC-MS/MS Method

The final volumes were analyzed by gas chromatography with tandem mass spectrometric detection (GC-MS/MS).

GC System	A Thermo Trace 1310 GC equipped with TriPlus RSH Base liquid + HS autosampler and split/splitless injector
GC Column	Agilent DB-624 (length: 30 m, i.d.: 0.25 mm, 1.4 µm film thickness)
GC Injection Volume	2 µL.
Injection Technique	S/SL injector used: splitless injection. Injector temperature: 150 °C Split flow: 50 mL/min Splitless time: 1 min Carrier flow program: 4 mL/min, 1 min hold, ramp with 1.75 mL/min to 2.25 mL/min, 7 min hold
GC Method	Carrier Gas: Helium Oven temperature program: 50 °C, 1 min hold, ramp with 15 °C/min to 140 °C, 0 min hold, ramp with 30 °C/min to 200 °C, 0 min hold.
Retention Time	chloropicrin ≈ 5 min
MS System	Thermo TSQ 8000 Evo (triple-quad. mass spectrometer)
Ion Source Conditions	Emission current: 50 µA, Electron Energy: 70 eV EI positive mode, EI ion volume installed
MS Conditions	Selected reaction monitoring (SRM) mode, monitoring the following transitions: 117 m/z → 82 m/z, collision energy: 25 V 119 m/z → 84 m/z, collision energy: 25 V

The fragment ion 117 m/z of chloropicrin was used as the precursor ion for MS/MS detection. The mass transition 117 m/z→82 m/z was used for quantification of the analyte and the mass transition 119 m/z→84 m/z was used for quantitative confirmation. For product ion mass spectra of chloropicrin see Figure 14.

Monitoring two ion transitions by GC-MS/MS is considered to be highly selective, thus it does not require further confirmation of detected residues.

Repeatability of GC-MS/MS determination was demonstrated by duplicate injection of selected fortified specimen extracts.

2.4.2 Calibration and Evaluation

Calculation of results was based on peak areas. Quadratic calibration functions were obtained by injections of standard solutions in solvent with 0.5 ng/mL to 30 ng/mL. The coefficients of determination (R^2) were > 0.99 in all cases. Typical calibration diagrams with functions and coefficients of determination R^2 are shown in Figure 2.

Evaluation of the calibration results showed that a quadratic regression was more suitable to describe the correlation between analyte concentrations and detected peak area than a linear

regression. The same observation had been made in the original method where recoveries were evaluated based on a quadratic calibration function. Therefore, the application of a quadratic calibration function in this study is considered justified.

2.5 Calculation

Quantification of the analyte was accomplished by the external standard method. The concentration of chloropicrin (C_{Air}, in $\mu g/m^3$) in the air specimen was calculated as follows:

C_{Air} fortified = Amount fortified / V_{Air}

C_{Air} found = Amount found / V_{Air}

= $(C_{End} \times V_{End} \times DF) / (1000\ ng/\mu g \times V_{Air})$

with:

C_{End}: Concentration determined by GC-MS/MS in the final extract, in ng/mL

V_{End}: Final extract volume, 10 mL (5 mL for breakthrough determination)

V_{Air}: Air sampling volume, in m^3

DF: Dilution factor, 10 for samples at 10 x LOQ level, 500 for samples at 500 x LOQ level and 1 for all other samples.

Recoveries (in %) were calculated as follows:

Rec. = (C_{Air} found / C_{Air} fortified) x 100 %

2.6 Example for Calculation

Prior to air sampling the sample cartridge labelled P3822-20 (see Table 1 for details of evaluation) was fortified with 0.030 µg of chloropicrin (LOQ). A total volume of 0.295 m^3 of ambient air was drawn through the cartridge, which simulates a concentration of chloropicrin in the air of 0.102 $\mu g/m^3$.

C_{Air} fortified = Amount fortified / V_{Air}
= 0.030 µg / 0.295 m^3
= 0.102 $\mu g/m^3$

By means of GC-MS/MS the concentration C_{End} of chloropicrin in the final extract P3822-20A was determined to be 2.67 ng/mL, using the mass transition 117 m/z → 82 m/z for quantitative evaluation (see Figure 8 for the respective chromatogram). Thus the concentration of chloropicrin in the air and the respective recovery is calculated as follows:

C_{Air} found = (C_{End} x V_{End} x DF) / (1000 ng/µg x V_{Air})
= (2.67 ng/mL x 10 mL x 1) / 0.295 m^3
= 0.0902 $\mu g/m^3$

Rec. = (C_{Air} found / C_{Air} fortified) x 100 %
= (0.0902 $\mu g/m^3$ / 0.102 $\mu g/m^3$) x 100 %
= 89 %

All calculations were performed with full precision, but were reported with rounding, thus minor discrepancies may occur when recalculated using the rounded values as displayed above.