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Center for Analytical Chemistry
Environmental Analysis Section
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Title: Determination of Pheromones in Air Trapped on XAD-4 By Gas Chromatography-Mass Spectrometry

1. Scope:

This section method (SM) is applicable to the analysis of Pheromones in air trapped on XAD-4 resins. The reporting limit for all three chemicals: E-11-tetradecenyl acetate, Z-11-tetradecenyl acetate and 9,11-tetradecadienyl acetate are 0.5 μg per sample.

2. Principle:

A SKC XAD-4 resin tube is used to collect the analytes from air samples. The tube is clamped on a rack. The chemicals are eluted with hexanes. The eluant is then concentrated using N-evaporator and analyzed by GC/MS.

3. Safety:

All general laboratory safety rules for sample preparation and analysis shall be followed.

4. Interferences:

There were no matrix interferences that caused quantitative problems during method development and validation

5. Apparatus and Equipment:

5.1 Nitrogen Evaporator (Meyer N-EVAP Organomation Model #112 or equivalent)
5.2 Vortex-vibrating mixer
5.4 XAD-4 tube, SKC part number 225-30-02
5.5 Laboratory rack and clamps. The clamp should be able to clamp the 1 cm diameter tube vertically and firmly.
5.6 Pasture pipette
5.6 Gas Chromatograph equipped with mass spectrometer.

6. Reagents and Supplies:

6.1	E-11-tetradecenyl acetate	CAS# 33189-72-9
6.2	Z-11-tetradecenyl acetate	CAS# 20711-10-8
6.3	9,11-tetradecadienyl acetate	CAS# 50767-79-8
6.4	Hexanes, pesticide residue grade	

6.5 GCMS Columns:
Analytical column: HP-5, 30m x 0.25mm x 0.25 µm (part # 19091S-433) or equivalent
Guard column: No pre-column is used

7. Standards Preparation:

7.1 All individual stock standards of 1.0 mg/mL were obtained from the CDFA/CAC Standards Repository.

7.2 A combination standard of 10 µg/mL was prepared with hexanes from the individual 1.0 mg/mL standard. The combination working standard was diluted to the following concentrations: 0.1, 0.2, 0.5, and 1 µg/mL in hexanes for instrument calibration.

7.3 Keep all standards in the designated refrigerator for storage.

7.4 The expiration date of each standard is six months from the preparation date or the expiration date of the stock standards which ever comes first.

8. **Sample Preservation and Storage**:

Store all samples waiting for extraction in the sample refrigerator (32-40 °F).

9. **Test Sample Preparation**:

9.1 Preparation of blank and QC spike

9.1.1 Use a file or an electric cutting device to break off the tips of the sample tube. Use one as QC blank.

9.1.2 Spike one mL of 10 µg/mL combination standard on top of the QC tube resin bed and let set for 30 minutes.

9.2 Test Sample Extraction

9.2.1 Remove sample from freezer and allow them to come to ambient temperature.

9.2.2 Clamp the sample tube on the rack vertically, Place a calibrated 15 mL test tube below the sample tube. The exit end of the sample tube is just inside the test tube.

9.2.3 Using a pasture pipette, add hexanes to the top of the resin bed of the tube. Allow the solvent elute by gravity. If the flow is too fast (more than 1 mL/min), slow down the flow by placing a sample tube cap on the inlet end. This process allows the solvent and resins a longer and better contact.

9.2.4 Repeat the step 9.2.3 until 15 mL hexanes are eluted into the test tube

9.2.5 Evaporate the eluant to 2.0 mL in a water bath at 35-40°C under a gentle stream of nitrogen. Transfer the extract into two autosampler vials (one with inserts). The sample extract is analyzed by GC/MS.

10. **Instrument Calibration**:

10.1 The calibration standard curve consists of a minimum of four levels. The lowest level must be at or below the corresponding reporting limits.

10.2 The GCMS calibration curves were obtained using quadratic regression.

11. **Analysis:**

11.1 GC-MS

11.1.1 GC-MSD instrument: Agilent Model 6890N Gas Chromatography, 7683 Auto-sampler, 5973 Mass Selective Detector.

11.1.2 Column: HP-5MS, 30m x 0.25 mm, 0.25µm

11.1.3 Operating parameter:

11.1.3.1 GC conditions

Initial temperature:	70 C
Initial time:	1 min

Ramp #	Rate	Final temp	Final time
1	10.00	250	1.0
2	10.00	270	10.0

Run time: 32 minutes

Inlet:	Splitless
Inlet temperature:	250
Inlet pressure:	7.65 psi
Inlet purge flow:	45.5 mL/min
Inlet purge time:	0.50 min
Total Flow:	48.1 mL/min
Gas type:	Helium

11.1.3.2 MSD conditions:

Transfer line temp:	280 C
Acquistion mode:	Scan
Solvent delay:	6 min
Scan low mass:	50
Scan high mass:	450
Scan threshold:	150

11.1.4 Retention Time:

11.1.4.1	peak1	E-11-tetradecenyl acetate	14.74 min
11.1.4.2	peak 2	Z-11-tetradecenyl acetate	14.79 min
11.1.4.3	peak3	9,11-tetradecadienyl acetate	15.36 min

11.2 Data analysis

11.2.1 Peak 1

Target ion:	194.20
Qualifier1	82.10
Qualifier 2	96.10

11.2.3 Peak2	Target ion	194.20
	Qualifier1	82.10
	Qualifier 2	96.10
11.2.3 Peak 3	Target ion	252.20
	Qualifier 1	67.00
	Qualifier 2	95.10

12. **Quality Control**:

12.1 Method Detection Limits (MDL)

Method Detection Limit (MDL) refers to the lowest concentration of the analyte that a method can detect reliably. To determine the MDL, 7 sample tubes were spiked at 0.500 µg and processed through the entire method along with a blank. The standard deviation derived from the spiked sample recoveries was used to calculate the MDL for each analyte using the following equation:

MDL = tS

Where t is the Student t test value for the 99% confidence level with n-1 degrees of freedom and S denotes the standard deviation obtained from n replicate analyses. For the n=7 replicates used to determine the MDL, t=3.143.

The results for the standard deviations and MDL are in Appendix 1.

12.2 Reporting Limit (RL)

Reporting limit (RL) refers to a level at which reliable quantitative results may be obtained. The MDL is used as a guide to determine the RL. The RL is chosen in a range 1-5 times the MDL, as per client agreement. The reporting limit for this method is 0.5 µg/sample.

12.3 Method Validation

The method validation consisted of five sample sets. Each set included four levels of fortification and a method blank. All spikes and method blanks were

processed through the entire analytical method. Spike levels and recoveries for the target compounds are shown in Appendix 2.

12.4 Control Charts and Limits

Control charts were generated using the data from the method validation for each analyte. The upper and lower warning and control limits are set at ± 2 and 3 standard deviations of the % recovery, respectively, shown in Appendix 2.

12.5 Acceptance Criteria

12.5.1 Each set of samples will have a matrix blank and a spiked matrix sample.

12.5.2 The retention time should be within ± 2 per cent of that of the standards.

12.5.3 The recoveries of the matrix spikes shall be within the control limits.

12.5.4 The sample shall be diluted if results fall outside of the calibration curve.

13. **Calculations:**

Quantitation is based on an external standard (ESTD) calculation using either the peak area or height. We choose the quadratic curve fit, with all levels weighted equally. Alternatively, at the chemist's discretion, concentrations may be calculated using the response factor for the standard whose value is < 30% to the level in the sample.

$$\mu g = \frac{(\text{sample peak area or ht}) \times (\text{std conc } \mu g/mL) \times (\text{std vol. Injected}) \times (\text{final vol of sample mL})}{(\text{std.peak area or ht}) \times (\text{sample vol injected})}$$

14. **Reporting Procedure:**

Sample results are reported out according to the client's analytical laboratory specification sheets or consent.

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Appendix 1

The Determination of Method Detection Limit (MDL) and Reporting Limit (RL) in XAD-4

			E-11-tetradecenyl acetate		Z-11-tetradecenyl acetate		9,11-Tetradecadienyl acetate	
4/7/2008		Spiked	Found (ng/spl)	%recovery	Found (ng/spl)	%recovery	Found (ng/spl)	%recovery
	MDL 1	500ng	653	130.6%	498	99.6%	531	106.2%
MDL	MDL 2	500ng	728	145.6%	566	113.2%	554	110.8%
	MDL 3	500ng	666	133.2%	571	114.2%	614	122.8%
	MDL 4	500ng	688	137.6%	598	119.6%	642	128.4%
	MDL 5	500ng	707	141.4%	667	133.4%	668	133.6%
	MDL 6	500ng	620	124.0%	522	104.4%	585	117.0%
	MDL 7	500ng	659	131.8%	530	106.0%	606	121.2%
	Stdev		36.2		56.4		47.8	
	MDL		113.7 ng/spl		177.3 ng/spl		150.3 ng/spl	
	RL		500 ng/sample		500 ng/sample		500 ng/sample	

Appendix 2

Method Validation

Validation 1
4/10/2008

			E-11-tetradecenyl acetate		Z-11-tetradecenyl acetate		9,11-Tetradecadienyl acetate	
Validation 1		Spiked	Found (ng/spl)	% recovery	Found (ng/spl)	% recovery	Found (ng/spl)	% recovery
1st injection	Level 1	1000ng	1204	**120.4%**	1020	**102.0%**	1074	**107.4%**
	Level 2	2000ng	2227	**111.4%**	1922	**96.1%**	2050	**102.5%**
	Level 3	5000ng	5941	**118.8%**	4826	**96.5%**	5112	**102.2%**
	Level 4	10000ng	11464	**114.6%**	10415	**104.2%**	10960	**109.6%**

Validation 2
4/14/2008

			E-11-tetradecenyl acetate		Z-11-tetradecenyl acetate		9,11-Tetradecadienyl acetate	
Validation 2		Spiked	Found (ng/spl)	% recovery	Found (ng/spl)	% recovery	Found (ng/spl)	% recovery
1st injection	Level 1	1000ng	1126	**112.6%**	971	**97.1%**	1060	**106.0%**
	Level 2	2000ng	2668	**133.4%**	2174	**108.7%**	2460	**123.0%**
	Level 3	5000ng	6171	**123.4%**	5327	**106.5%**	5135	**102.7%**
	Level 4	10000ng	10333	**103.3%**	9575	**95.8%**	10805	**108.1%**

Validation 3
4/15/2008

			E-11-tetradecenyl acetate		Z-11-tetradecenyl acetate		9,11-Tetradecadienyl acetate	
Validation 3		Spiked	Found (ng/spl)	% recovery	Found (ng/spl)	% recovery	Found (ng/spl)	% recovery
1st injection	Level 1	1000ng	1196	**119.6%**	968	**96.8%**	938	**93.8%**
	Level 2	2000ng	2371	**118.6%**	2150	**107.5%**	2193	**109.7%**
	Level 3	5000ng	6000	**120.0%**	5290	**105.8%**	5155	**103.1%**
	Level 4	10000ng	10288	**102.9%**	10373	**103.7%**	10265	**102.7%**

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Validation 4
4/17/2008

			E-11-tetradecenyl acetate		Z-11-tetradecenyl acetate		9,11-Tetradecadienyl acetate	
Validation 4		Spiked	Found (ng/spl)	% recovery	Found (ng/spl)	% recovery	Found (ng/spl)	% recovery
1st injection	Level 1	1000ng	1231	**123.1%**	1059	**105.9%**	1048	**104.8%**
	Level 2	2000ng	2395	**119.8%**	2123	**106.2%**	2078	**103.9%**
	Level 3	5000ng	5679	**113.6%**	5138	**102.8%**	4840	**96.8%**
	Level 4	10000ng	10600	**106.0%**	10009	**100.1%**	10728	**107.3%**

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