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Determination of Natural Pyrethrins in Medical Marijuana Products Using Gas Chromatography-Tandem Mass Spectrometry
NYS-DOH MML-308

Table of Contents

1.0 **Scope and Application**

1.1. This method (NYS ELAP Method ID 9940) is used in the analysis of Medical Marijuana (MM) products for the determination of residual concentrations of six natural pyrethrins of the pyrethrin groups I and II (**Table 1**). To be in accordance with Title 10 (Health), Chapter XIII, Part 1004 of the official Compilation of Codes, Rules, and Regulations of the State of New York, this method will be employed when the use of natural pyrethrins in MM cultivation is disclosed by the Registered Organizations (ROs).

Table 1. Analyte List

Analyte**	CAS Number	LOD in Matrix Match STD (ng/mL)	LOQ in Matrix Match STD (ng/mL)	LOD in Matrix Match (ng/g)	LOQ in Matrix Match (ng/g)
Pyrethrin Group I:					
Cinerin I	97-12-1	0.03	0.08	25	80
Jasmolin I	4466-14-2	0.01	0.04	13	40
Pyrethrin I***	121-21-1	0.32	0.97	321	970
Pyrethrin Group II:					
Cinerin II	121-20-0	0.01	0.04	10	40
Jasmolin II	1172-63-0	0.01	0.04	12	40
Pyrethrin II***	8003-34-7	0.13	0.40	133	400
Internal Standard (IS):					
Bifenthrin-(biphenyl-2′,3′,4′,5′,6′-*d*5	*82657-04-3	N/A	N/A	N/A	N/A

*The CAS number is for unlabeled Bifenthrin.

** See Appendix A for structures and chromatograms in analyte list.

*** Major analytes (all others are minor analytes).

1.2. This method is restricted to use by or under the supervision of analysts experienced in the use gas chromatography-tandem mass spectrometry (GC-MS/MS). The analyst must demonstrate the ability to generate acceptable results when using this method following the procedures described in **Section 14.0**. LOQs referenced within **Table 1** are subject to change based on LOD/LOQ determinations detailed within **Section 14.2.**

2.0 **Summary of the Method**

2.1 Samples are extracted and diluted in acetonitrile (ACN), and the extracts are analyzed using a GC-MS/MS system operating in the negative chemical ionization (NCI) mode. Multiple reaction monitoring (MRM) is used with specific MS/MS transitions for detection of six natural pyrethrins and the bifenthrin-d_5 (**IS**). Chromatographic separation of the analytes is achieved on a 10-m HP-5 column with a total time of 30.5 minutes. The **LODs** for the natural pyrethrins, Cinerin I, Jasomolin I, Pyrethrin I, Cinerin II, Jasmolin II and Pyrethrin II, in matrix are 25 ng/g, 13 ng/g, 320 ng/g, 10 ng/g, 12 ng/g and 133 ng/g, respectively. The **LOQs** for Cinerin I, Jasmolin I, Pyrethrin I, Cinerin II, Jasomolin II and Pyrethrin II in Matrix are 80 ng/g, 40 ng/g, 970 ng/g, 40 ng/g, 40 ng/g and 400 ng/g, respectively.

3.0 **Definitions**

3.1 Internal Standard **(IS)** –A pure compound that is not found in any sample. The IS is a compound added at a specified concentration to unknown samples, calibration standards, and quality control (QC) samples, including method blanks, laboratory fortified blanks, matrix spikes, and duplicates, to provide a basis for peak area ratios used in quantitation. The instrument performance is assessed by monitoring the IS response in each analysis.

3.2 Internal Standard Stock Diluent (**ISD**) – A concentrated solution of IS that is prepared in extraction solvent. This stock diluent is used to prepare the IS working diluent (**IWD**).

3.3 Internal Standard Working Diluent (**IWD**) – A solution of IS that is prepared from the **ISD** by dilution in extraction solvent and is added to all samples, standards and QC samples to give the same final **IS** concentration in each.

3.4 System Blank **(SBLK)** – A portion of an appropriate pure solvent that is analyzed to verify that the instrument is free from background contamination.

3.5 Laboratory Reagent Blank (**LRB)** – An aliquot of extraction solvent that is treated exactly as if it were a sample, including exposure to all glassware, equipment, solvents, internal standards, surrogate standards, and reagents that are used with the samples. The **LRB** is used to determine whether method analytes or other interferences are present in the laboratory environment, reagents or apparatus. Synonym: Method Blank.

3.6 Laboratory Fortified Blank (**LFB**)– An aliquot of extraction solvent that is spiked with known quantities of target analytes and prepared and analyzed as if it were a sample. The **LFB** is used to evaluate the accuracy of the methodology. Synonyms: Method Blank Spike (**MBS**) and Laboratory Control Sample (**LCS**).

3.7 Representative Matrix – A "representative" matrix is used when sample is not suitable due to interferences. The "representative" matrix is "clean" and free of interferences. The laboratory can use a matrix which is "representative" of the real test material, homogeneous and stable over time.

3.8 Matrix Blank (**MB**) – An aliquot of matrix that is treated exactly as if it were a sample, including exposure to all glassware, equipment, solvents, internal standards, and reagents that are used with the samples. The **MB** is analyzed to verify that there are no interfering compounds arising from the matrix.

3.9 Matrix Spike (**MS**)– An aliquot of matrix that is spiked with known quantities of target analytes and extracted/analyzed as a quantifiable sample. The matrix from which the portion to be spiked is taken must be analyzed separately to determine the levels of background analyte concentrations. The **MS** is used to correct for background concentrations of the analyte and to determine whether the sample matrix contributes bias to the sample results. The

MS is also used to determine the accuracy of the methodology in a manner like that of the **LFB**. Synonym: Laboratory Fortified Sample Matrix (**LFM**).

3.10 Matrix Spike Duplicate (**MSD**) – Prepared identically to the **MS**, the **MSD** is a second portion of actual sample. It is spiked and processed in an identical manner to that of the **MS**. The **MS** and **MSD** are used together to evaluate the precision of the methodology.

3.11 Primary Stock Standard (**PS**) – A concentrated solution of method analyte(s) prepared in the laboratory from referenced and certified analyte standards, or a concentrated solution of method analyte(s) purchased directly from a referenced and certified source.

3.12 Primary Working Standard (**PWS**) – A solution of the method analyte(s) prepared from stock standard solutions that is diluted as necessary to prepare calibration standards or other necessary analyte solutions. Synonym: Primary Dilution Standard Solution (**PDS**).

3.13 Secondary Stock Standard (**SS**) – A solution of the method analyte(s) prepared from stock standard solutions that is diluted as necessary to prepare calibration standards or other necessary analyte solutions. Synonym: Primary Dilution Standard Solution (**PDS**).

3.14 Calibration Standard Intermediate (**CalSI**) – A solution of method analytes at intermediate levels prepared from stock or working standard solutions that is used to prepare the final calibration standards. These standards are prepared using only the analytes.

3.15 Calibration Standard (**CalS**) – The final calibration standard solution of method analytes prepared from stock or working standard solutions containing the analytes that is used to calibrate the instrument response with respect to analyte concentration. These standards are prepared using the matrix, analytes and internal standards.

3.16 Continuing Calibration Verification Intermediate Standard (**CCVI**) – A solution of method analytes at an intermediate concentration, prepared from one of the calibration standards, is used to verify the acceptability of an existing calibration.

3.17 Continuing Calibration Verification Standard (**CCV**) – One of the calibration standards used to verify the acceptability of an existing calibration. This standard is prepared using the matrix, analytes and internal standards. Synonyms: Continuing Calibration Check Standard (CCC).

3.18 Cross Check Reference Standard (**CCR**) – A solution of method standards prepared from a stock standard solution with matrix that is obtained from a source that is independent of that used to prepare the calibration standards (*e.g.*, independent vendor, independent lot, or independent preparation). This standard is prepared using the matrix, analytes and internal standards. The **CCR** is used to verify that the original calibration source is acceptable.

3.19 Limit of Detection **(LOD)** – The statistically calculated minimum concentration of an analyte that can be measured with 99% confidence that the value is greater than zero. Synonym: Method Detection Limit (**MDL**).

3.20 Limit of Quantitation (**LOQ)** – The minimum concentration that can be quantitatively reported for a target analyte. The lowest calibration standard must be at or below the **LOQ** for the analyte. The **LOQ** is typically 3-5 times the **LOD**. Synonym: Method Reporting Limit (MRL).

3.21 Upper Limit of Quantitation (**ULOQ**) – The **ULOQ** corresponds to the maximum concentration that is an acceptable point on the calibration curve. The **ULOQ** is the concentration of the highest **CalS** standard.

3.22 Demonstration of Capability (**DOC**) – The procedure used to demonstrate that an analyst can perform the method with acceptable precision, accuracy, sensitivity and specificity.

3.23 Preparation Batch - Samples that are prepared and/or analyzed together with the same process and personnel, using the same lot(s) of reagents. A preparation batch consists of one to twenty samples (not including method blanks, LCS, matrix spikes and matrix duplicates) of the same matrix with a maximum processing time of twenty-four (24) hours between the first and last sample.

3.24 Analytical Batch – An analytical batch consists of prepared samples which are analyzed together as a group. An analytical batch can include prepared samples originating from different matrices and can exceed twenty samples. .

3.25 Major Analyte – The analytes which have the highest ratio in the standard mix.

3.26 Minor Analyte – The analytes which have a ratio less than the major analyte in the standard mix.

4.0 **Health and Safety**

4.1 The toxicity and carcinogenicity of each chemical used in this method have not been thoroughly investigated. Therefore, each chemical must be treated as a potential health hazard, and exposures must be limited to the lowest possible levels.

4.2 Always follow guidelines listed in safety data sheets (SDS) for proper storage, handling, and disposal of solvents, reagents, and standards. SDSs are located within the laboratory in labeled, yellow binders and are available online. These guidelines must be made available to all personnel involved in the chemical analysis.

4.3 Lab coats, safety glasses and gloves must be worn when performing standard or sample preparations, working with instrumentation, disposing of waste, and cleaning glassware.

4.4 The fume hood must be used when using or preparing standards, reagents, or samples that require proper ventilation.

5.0 **Interferences**

5.1 Method interferences are caused by contaminants in solvents, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts observed as chromatographic peaks or elevated baselines in chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running **SBLK** and **LRB**s as described in **Section 15.0.**

5.1.1 Glassware and syringes used in the medical marijuana laboratory must be thoroughly cleaned to prevent contamination. After use, rinse with the last solvent used, then rinse three times with dichloromethane, three times with acetone, and three times with methanol.

5.1.2 The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in the laboratory is not performed, nor required.

5.1.3 After cleaning, glassware is stored away from standards and syringes to prevent and cross-contamination.

5.2 When interferences and/or contamination are evident in samples, the re-preparation of the original sample is recommended after the source of contamination has been identified.

5.3 Interfering contamination known as “carryover” can occur when a sample containing low concentrations of analytes is analyzed immediately following a sample containing relatively high concentrations of analytes. Rinsing of the sample syringe and associated equipment between samples with solvents such as dichloromethane (DCM) and acetonitrile (ACN) can minimize this sample cross contamination. After analysis of a sample containing high concentrations of analytes, one or more injections of the **SBLK** should be made to ensure that accurate results are obtained for the next sample.

5.4 Matrix interferences may occur because of excipients present in the sample. If a matrix interference is believed to be present, the sample may be spiked with target analytes and analyzed together with the nonspiked sample to verify the results. If these analyses verify the original results, report only the results from the original nonspiked sample. This may not always be possible if a limited amount of sample is received for analysis. If additional sample is not available for reanalysis, the original results must be qualified on the final report.

5.5 Samples and standards must be prepared in the same final solvent to allow for comparable chromatography of samples and standards.

6.0 **Equipment and Supplies**

6.1 Standard and Sample Preparation Equipment **(**References to specific vendors and products are for illustrative purpose**s** only and do not imply product endorsement).

6.1.1 Syringes, various sizes.

6.1.2 Class "A" volumetric flasks with stoppers, various sizes.

6.1.3 Disposable glass pipettes and bulbs.

6.1.4 Positive displacement pipet, Handy Step S or equivalent.

6.1.5 2-mL autosampler vials with Teflon lined screw caps or vials with crimp-top caps.

6.1.6 Analytical balance, Mettler-Toledo Model # 205DU or equivalent.

6.1.7 Vortex, Maxi Mix 11 Model #37615 or equivalent.

6.1.8 Centrifuge, Model # 5415D or equivalent.

6.2 Instrumentation.

6.2.1 A GC-MS/MS system composed of a GC and a tandem mass spectrometer, equipped with a column oven that is suitable for use with a variety of columns, as well as all the required accessories including: syringes, autosampler, columns, gases, and a data system for instrument control and data analysis/processing. The system used in the development of this method is described below:

6.2.1.1 Agilent GC, Model 7890B.

6.2.1.2 Agilent triple-quadrupole MS, Model 7010.

6.2.1.3 Agilent Autosampler, Model G4567A.

6.2.1.4 Agilent MassHunter Software, Version B.07.01 SP1.

6.2.2 Ultra High Purity Carrier and Collision Gases (Helium, Nitrogen, Methane)

7.0 Reagents and Stock Standards

7.1 **Solvents (HPLC Grade) and reagents** – All solvents and reagents must have records to demonstrate traceability to origins and preparations, including certificates of analysis and preparation records.

7.1.1 ACN, GC/MS grade, Macron, catalog # 0043-08.

7.1.2 DCM, pesticide residue analysis, Burdick and Jackson, catalog # 299-4.

7.2 **"Representative" Matrix**

7.2.1 A "representative" marijuana extract prepared from plant material obtained from the National Institute on Drug Abuse (NIDA), National Institutes of Health (NIH), or equivalent.

7.2.2 The "representative" matrix using a marijuana extract that has been prepared as in **Section 7.2.3** with endogenous analytes less than **LOQ**. If the ratio of marijuana extract to solvent remains constant, the matrix can be prepared in alternative volumes to accommodate sample and standard preparation.

7.2.3 A "representative" matrix at 1 mg/ml is prepared by weighing 100 mg of the marijuana extract in a tared vial. The material is then dissolved in 10 mL of ACN. Aliquots (100 µL) of this matrix are then used to prepare matrix match standards and matrix match quality control samples including **LFB, MS, MSD** and **CalS** (See **Section 15.0**). Store "representative" matrix at ≤ -20 °C for up to 1 year, in a sealed vial.

Table 2. Matrices, NIH

Matrices	Manufacturer
Marijuana Extract	**NIDA, NIH or equivalent**

7.3 **Stock Standards**

7.3.1 Stock standards may be purchased from any vendor. Standards must be NIST traceable (if available) and are preferably ISO Guide 31 and 35 traceable, when possible.

7.3.2 The commercial standards listed (**Table 3**) are examples of those currently used in the laboratory. Commercial standards are stored according to the manufacturers' recommendations, and expiration dates of commercially prepared standards are as indicated by the vendors on their Certificates of

Analysis. Substitutions may be made provided that the criteria described in **Section 7.3.1** are met. See **Table 7** for an example of the ratios of each analyte in a standard curve.

Table 3. Analytical Standard

Standard	Manufacturer	Catalog #	Concentration	Form
Pyrethrin Mix	Ultra-Scientific	CPST00399	100 µg/mL	ACN Solution
Pyrethrin Mix	LGC	DRE-L16620000AL	10 µg/mL	ACN Solution
Bifenthrin-(biphenyl-2′,3′,4′,5′,6′-d_5)	Fluka	16582	N/A	Oil

8.0 Preparation of Reagents, Solutions and Stock Standards

8.1 General Preparation Information

8.1.1 All reagents, solutions and standards must be traceable to stocks and, if possible, have NIST-traceable documentation. The preparation method, date of preparation, expiration date and analyst must also be traceable in laboratory documentation. Storage specifications for both prepared and stock standards are detailed within **Sections 8, 11 and 12.** To prevent degradation, protect standards from light.

8.1.2 Standard preparation steps are for guidance only. In addition, different concentrations or alternate stock mixtures may be prepared as necessary.

8.1.3 A syringe or positive-displacement pipet is used for the transfer of any volume of sample or standard that will be quantified in the analysis. Pipet tips must be changed with each solution change.

8.2 IS

8.2.1 If stocks of the internal standard cannot be purchased in solution, it is prepared from the neat form. An internal standard stock solution of bifenthrin-d_5 is prepared in separate 1-mL (certified "A" class) volumetric flask. If necessary, the **IS** is diluted further to prepare the **ISD** and **IWD**.

8.2.2 Fill a volumetric 1 mL flask 1/3 full of ACN.

8.2.3 Weigh 10 mg (± 0.02 mg) of neat bifenthrin-d_5 and add to a labeled volumetric flask.

Equation 1. (10 mg) (1.0 mL) = (X mg/mL)

X = 10 mg/mL

8.2.4 Fill to volumetric flask to volume with ACN and invert 3x to mix.

8.2.5 Portions of the **IS** solution are transferred to 2.0-mL crimp-cap amber vial and labeled.

8.2.6 Store stock solutions at ≤ -80 °C for up to 2 years, in a sealed vial.

8.3 **ISD**

8.3.1 Preparation of **ISD** bifenthrin-d_5 solution @ 0.1 mg/mL is as follows:

8.3.2 Fill a volumetric 2 mL flask 1/3 full of ACN.

8.3.3 Dispense 20 µL of bifenthrin-d_5 **@** 10 mg/mL **(See Section 8.2)** to a labeled volumetric flask.

8.3.4 Fill to volumetric flask to volume with ACN and invert 3x to mix.

Equation 2.

$$(10 \text{ mg/mL})\ (0.020 \text{ mL}) = (X \text{ mg/mL})\ (2.0 \text{ mL})$$

$$X = 0.1 \text{ mg/mL or } 100 \text{ µg/mL}$$

8.3.5 The **ISD** solution is transferred to 2.0-mL crimp-cap amber vial and labeled.

8.3.6 Store stock solutions at ≤ -80 °C for up to 2 year, in a sealed vial.

8.4 **IWD**

8.4.1 Preparation of **IWD** for use in the final preparation of standards and samples. An example of the **IWD** prepared @ 2.0 µg/mL is as follows:

8.4.2 Fill a 25-mL volumetric flask 1/3 full of ACN.

8.4.3 Dispense 50 µL of bifenthrin-d_5 **ISD** as prepared in **Section 8.3** to the 25.0-mL volumetric flask.

8.4.4 Dilute to volume with ACN and invert 3x to mix well.

Equation 3.

$$(100\ \mu g/mL)\ (0.50\ mL) = (X\ \mu g/mL)\ (25.0\ mL)$$

$$X = 2.0\ \mu g/mL$$

8.4.5 Portions (1.5 mL) of the solution are transferred to the appropriate number of 2.0-mL crimp-cap vials and labeled and sealed.

8.4.6 Store vials at ≤ -80 °C for up to 1 year, in a sealed vial.

8.5 **PS** – @ 100.0 µg/mL (Ultra-Scientific). Standard contains all six pyrethrin analytes at various ratios. See **Table 7** for example of ratios of pyrethrin in standard mix.

8.5.1 Store at ≤ -80 °C for up to 1 year in a sealed vial.

8.6 **PWS** – @ 1000 ng/mL

8.6.1 Fill a 1-mL volumetric flask 2/3 full of ACN.

8.6.2 Dispense 10 µL of the 100 µg/mL standard mix into the volumetric flask.

8.6.3 Fill to volume invert 3 x to mix.

Equation 4. $(100\ \mu g/mL)\ (0.01\ mL) = (X\ \mu g/mL)\ (1\ mL)$

$$X = 1\ \mu g/mL \text{ or } 1000\ ng/mL$$

8.6.4 The **PWS** solution is transferred to 2.0-mL crimp-cap amber vial and labeled**.**

8.6.5 Store at ≤ -80 °C for up to 1 year in a sealed vial.

8.7 **CCR Stock**– @ 10.0 µg/mL (LGC). If available, an alternative preparation of pyrethrin mix in ACN or a separately prepared stock at the same concentration.

8.7.1 Store at ≤ -80 °C for as per vendor recommendations or 2 years, in a sealed vial.

9.0 Sample Transport, Receipt, Preservation, Handling, and Storage

9.1 Sample transport conditions:

9.1.1 The MM products from the ROs are shipped as per the manufacturer's specifications and must adhere to all regulatory requirements.

9.2 Sample receipt:

9.2.1 Medical marijuana products from the RO are received, verified and documented ensuring that method, regulatory and Accreditation Body requirements are met.

9.2.2 All medical marijuana products must be stored under the conditions recommended by the manufacturer. The storage is documented.

9.2.3 All MM products must be stored under the conditions recommended by the manufacturer(s) prior to analysis.

9.3 Sample Preservation and Storage:

9.3.1 Prepared samples (**see Section 15.0**) are stored in a freezer at ≤ -20 °C until analysis, which must be completed within 10 days of preparation. The samples are warmed to room temperature and vortexed to ensure homogeneity before analysis. After analysis is complete, the remaining extract is stored at ≤ -80 °C for one month, if necessary.

10.0 Instrument Parameters

10.1 GC/MS

The MS/MS parameters are optimized using the Auto tune program on the instrument. The current GC and MS/MS conditions are described in **Tables 4, Tables 5** and **Table 6.**

Table 4: MS/MS Analyte specific parameters.

Analytes	Retention Time	Polarity	MS/MS Transition	Collision energy (eV)	Dwell Time (ms)
Pyrethrin Group I	10.68	Negative	*m/z* 167→167	4	70
Pyrethrin Group II	17.61	Negative	*m/z* 211→211	16	400
Pyrethrin Group II	17.61	Negative	*m/z* 211→135	16	400
Bifenthrin-d_5	12.74	Negative	*m/z* 241→35	4	70

10.2 Using the MS/MS parameters referenced in **Table 4** and the GC operating parameters described in **Tables 5** and **6**, analyze a mid-level calibration standard to obtain retention times for the target analyte using an MRM instrumental method, or equivalent. For optimal MS/MS precision, there must be a minimum of 10 scans across each peak.

Table 5: GC parameters

Column	**Value**
Column:	Agilent HP-5MS, 10.0 x 0.25 mm x 0.25 µm, # 19091S-433UF
Transfer line	250 °C
Mass Spec temperature	150 °C
Carrier gas:	Helium
Collision gas:	Methane
Autosampler	**Value**
Injection Volume:	2.0 µL
Solvent A # Washes:	6
Solvent B # Washes	6
Rinse Solvent A:	Methylene Chloride
Rinse Solvent B:	ACN
# Sample Pumps:	3
# Sample Washes:	2
Max Sample Wash volume:	4 µL
Autosampler Viscosity Delay	3
Inlet Temperature	200 °C
Inlet Pressure	1.0 psi
Column Flow	1.25 mL/min
Column PSI	1.0 psi
	Constant Flow
Septum Purge Flow	mL/min

Table 6: Temperature Program

Temperature Program Rate	Oven Temperature	Time	Total Run Time
Start	80	1.0	1.0
15 °C/min	200	10.0	19.0
20 °C/min	300	6.0	30.0
-	80	0.5	30.5

11.0 Intermediate Calibration Curve Standards (CalS1*I* through CalS6*I*)

11.1 **Intermediate Calibration-** Serial dilutions are made from the **PWS** natural pyrethrin mixture @ 1000 ng/mL (**Section 8.6**). The intermediate concentration range for the six Pyrethrins is 12.35 to 1000 ng/mL (**see Table 8**). See **Table 7** for individual concentrations of each analyte which is based on the % of each analyte in the mixture. This information is provided on the certificate of analysis from each calibration standard provider.

11.1.1 Store at ≤ -20 °C for up to 1 year in a sealed vial.

Table 7. Examples of final concentrations of individual pyrethrin analytes based on % in certificate of analysis.

Ratio of Analyte in STD *	Individual Analytes		STD1	STD 2	STD 3	STD 4	STD5	STD 6**
		Total Final Pyrethrin Conc. with IWD **See Section 12.0** ng/mL	1.24	3.70	11.11	33.33	50.00	100.0
0.04*	Cinerin I	ng/mL	0.049	0.148	0.444	1.33	2.00	4.00
0.02*	Jasmolin I	ng/mL	0.025	0.074	0.222	0.667	1.00	2.00
0.65*	Pyrethrin I	ng/mL	0.803	2.41	7.22	21.7	32.5	65.0
0.03*	Cinerin II	ng/mL	0.037	0.111	0.333	1.00	1.50	3.00
0.02*	Jasmolin II	ng/mL	0.025	0.074	0.222	0.667	1.00	2.00
0.24*	Pyrethrin II	ng/mL	0.296	0.889	2.67	7.99	12.0	24.0

*Ratio of individual analytes as per certificate of analysis. The ratio of individual analytes may change from vendor to vendor and from lot to lot.
**STD6 = prepared from PWS 1000 ng/ml (see section 8.6)

11.1.2 ***CalS5I (500 ng/mL)***

11.1.2.1 Dispense 500 µL of **ACN** into a 2-mL vial.

11.1.2.2 Dispense 500 µL of **PWS @ 1000 ng/mL (Section 8.6)** into the vial.

11.1.2.3 Vortex for 10 seconds at least 3 times to mix well.

11.1.2.4 Label the vial as ***CalSI5.***

Equation 5. (1000 ng/mL) (500 µL) = (X ng/mL) (1000 µL)

X = 500 ng/mL

11.1.3 ***CalS4I (333.33 ng/mL)***

11.1.3.1 Dispense 500 µL of **ACN** into a 2-mL vial.

11.1.3.2 Dispense 250 µL of ***CalS5I @ 1000 ng/mL*** **(Section 8.6)** into the vial.

11.1.3.3 Vortex for 10 seconds at least 3 times to mix well.

11.1.3.4 Label the vial as ***CalS4I***.

Equation 6. (1000 ng/mL) (250 µL) = (X ng/mL) (750 µL)

X =333.33 ng/mL

11.1.4 ***CalS3I (111.11 ng/mL)***

11.1.4.1 Dispense 500 µL of **ACN** into a 2-mLvial.

11.1.4.2 Dispense 250 µL of ***CalS4I*** @ **333.33 ng/mL (Section 11.1.3)** into the vial.

11.1.4.3 Vortex for 10 seconds at least 3 times to mix well.

11.1.4.4 Label the vial as ***CalS3I.***

Equation 7. (333.33 ng/mL) (250 µL) = (X ng/mL) (750 µL)

X= 111.11 ng/mL

11.1.5 ***CalS2I (37.04 ng/mL)***

11.1.5.1 Dispense 500 µL of **ACN** into a 2-mLvial.

11.1.5.2 Dispense 250 µL of ***CalS3I @ 111.11 ng/mL*****(Section 11.1.4)** into the vial.

11.1.5.3 Vortex for 10 seconds at least 3 times to mix well.

11.1.5.4 Label the vial as ***CalS2I.***

Equation 8. (111.11 ng/mL) (250 µL) = (X ng/mL) (750 µL)

X= 37.04 ng/mL

11.1.6 ***CalS1I (12.35 ng/mL)***

11.1.6.1 Dispense 500 µL of **ACN** into a 2-mL vial.

11.1.6.2 Dispense 250 µL of ***CalS2I*** @ **37.04 ng/mL (Section 11.1.5)** into the vial.

11.1.6.3 Vortex for 10 seconds at least 3 times to mix well.

11.1.6.4 Label the vial as ***CalS1I.***

Equation 9. (37.04 ng/mL) (250 µL) = (X ng/mL) (750 µL)

X= 12.35 ng/mL

Table 8. – Preparation of Intermediate Calibration Curve

CalS STD	Initial Pyrethrin Mix STD Concentration (ng/mL)	Pyrethrin Mix Ref Section ID	Volume of Pyrethrin Mix Std (µL)	Volume of ACN (µL)	Total Volume (µL)	Pyrethrin Mix CalS Intermediate Ref Section ID	Pyrethrin Mix CalSI Intermediate Concentration (ng/mL)
CalS5I	1000	8.6	500	500	1000	11.1.2	500
CalS4I	1000	8.6	250	500	750	11.1.3	333.33
CalS3I	333.33	11.1.3	250	500	750	11.1.4	111.11
CalS2I	111.11	11.1.4	250	500	750	11.1.5	37.04
CalS1I	37.04	11.1.5	250	500	750	11.1.6	12.35

Note 1: See ***Table 7*** for individual analyte concentrations. Note 2: ***CalS6I*** = PWS 1000 ng/ml (see section 8.6)

12.0 **Final Calibration Curve Standards**

12.1 Into 2-mL vial labeled **CalS6** through **CalS1**, dispense 750 µL of ACN into each vial (see **Table 9**).

12.2 Into each 2-mL vial, dispense 100 µL of "representative" matrix (see **Section 7.2**).

12.3 Into its respective vial, dispense 100 µL of each **CalS** intermediate standard ***CalS6I*** **(PWS, see section 8.6)** through ***CalS1I.*** See **Table 7** for individual analyte ratios in standards and **Table 9** for each standard final concentration**.**

12.4 Into each 2-mL vial**,** dispense 50 uL of **IWD** (See **Section 8.4**) into each ***CalSI*** standard. Final concentration of **IWD** is 100.0 ng/mL bifenthrin-d_5 in each standard.

Equation 10. (2000 ng/mL) (0.050 mL) = (X ng/mL) (1.0 mL)

X= 0.1 µg/mL or 100.0 ng/mL

12.5 Vortex all standards 10 seconds at least 3 times to mix well.

12.6 After at least one **SBLK** and **LRB** injection, start with the lowest standard concentration **(*CalS1*)** and analyze each calibration standard. Tabulate the response (peak area/IS peak area ratio) and use the result to prepare a calibration curve for each target analyte (weighted 1/C linear regression, see **Section 16.0).**

12.7 Store at ≤ -20 °C for up to 1 year in a sealed vial.

Table 9. – Final Calibration Curve with IWD (for analysis)

Cal STD w/IWD	Diluent (ACN) (µL)	"Representative" Matrix Ref ID section	Volume of NIH matrix (µL)	IStd Ref section (*CalSI*) ID	Volume of pyrethrin intermediate Std (µL)	Intermediate standard concentration (ng/mL)	Volume of IWD (µL)	IWD Ref ID	Final calibration curve with IWD (ng/mL)
CalS6	750	7.2	100	8.6	100	1000	50	8.4	100.00
CalS5	750	7.2	100	11.1.2	100	500	50	8.4	50.00
CalS4	750	7.2	100	11.1.3	100	333.33	50	8.4	33.33
CalS3	750	7.2	100	11.1.4	100	111.11	50	8.4	11.11
CalS2	750	7.2	100	11.1.5	100	37.04	50	8.4	3.70
CalS1	750	7.2	100	11.1.6	100	12.35	50	8.4	1.24

See ***Table 7*** for individual analyte concentrations.

13.0 Verification Standards

13.1 ***CCRI*** *@ 500 ng/mL* The ***CCRI*** is prepared as follows:

13.1.1 Into 2-mL vial labeled "***CCRI***", dispense 950 µL of **ACN**.

13.1.2 Into same 2-mL vial, dispense 50 µL of ***CCR-stock*** @10.0 µg/mL **(Section 8.7).**

13.1.3 Vortex for 10 seconds at least 3 times to mix well.

13.2 ***CCR*** *@ 50 ng/mL* - The ***CCR*** is prepared as follows:

13.2.1 Into 2-mL vial labeled "**CCR**", dispense 750 µL of **ACN.**

13.2.2 Into same 2-mL vial, dispense 100 µL of "representative" Matrix **(Section 7.2)**.

13.2.3 Into same 2-mL vial, dispense 100 µL of ***CCRI*** @ 500 ng/mL **(Section 13.1)**.

13.2.4 Into same 2-mL vial, dispense 50 µL of **IWD** See **Section 8.4**.

13.2.5 Vortex for 10 seconds at least 3 times to mix well and analyze on instrument.

13.3 **Initial Calibration Criteria**

13.3.1 A calibration curve with a minimum of 5 concentrations must be analyzed. The correlation coefficient, (R^2) of the calibration curve for each analyte must be ≥ **0.995** before analysis of samples can begin.

13.3.2 The absolute peak area of the IS in each chromatographic run must not deviate by more than **20%** from its initial calibration.

13.3.3 Each calibration standard, processed under the initial calibration, must be within 80-120% of the true values for the major analytes and 70-130 % for the minor analytes for the initial calibration to be considered valid. For calibration points at or below the **LOQ**, calculated values may be within 70-130% of the true values for the major analytes and 60-140 % for the minor analytes. Evaluation of each standard also serves as the measure of % Relative Error with the same acceptance criteria.

13.3.4 If all criteria are not met, a new calibration must be established.

13.4 Initial Verification of Calibration (**CCR)**

13.4.1 The initial calibration for natural pyrethrin mix must be verified by analyzing a mid-level concentration of **CCR** (*e.g.*, between 3.7 ng/mL and 33.33 ng/mL).

13.4.2 The measured recovery value for each analyte of the **CCR** must be within 80 – 120% of the known value for the major analytes and 70-130 % for the minor analytes.

13.5 Continuing Calibration Verification **(CCV)**

13.5.1 After verifying the initial calibration, a **CCV** that is ≤ 1/2 the highest calibration standard must be analyzed with each analytical batch (typically 20 samples). For external calibration, a **CCV** is required at the beginning and end of each analytical batch. For internal standard calibration, a **CCV** is only required at the beginning of the analytical batch.

13.5.1.1 The measured % recoveries for all analytes in a **LOQ CCV** must be within ± 30% of the true values for the major analytes and ±40% for the minor analytes. The measured % recoveries for all **CCVs>LOQ** must be within ± 20% of the true values for the major analytes and ±30% for the minor analytes for each analytical batch.

13.5.2 **CCVs** may also be interspersed throughout the analytical batch at varying concentrations provided that the **CCVs** analyzed at the beginning and end (for external calibration) of each analytical batch are equal to or less than half the highest calibration level. Additional **CCVs** may also be run at higher levels to evaluate the upper end of the calibration curve.

13.5.3 The **LOQ** verification must be at a concentration at or below the **LOQ** as listed in (**Table 1**).

14.0 Quality Control/Assurance

14.1 Demonstration of Capability (DOC)

14.1.1 Initial DOC

14.1.1.1 Each analyst must perform an initial demonstration of capability using the procedures described in this method for the target analytes listed in ***Table 1***. The initial **DOC** must consist of the analysis of four or five matrix spike samples that have been fortified with the analyte(s) at a concentration of one (1) to four (4) times the LOQ (*e.g.*, concentration to total pyrethrins ~3.7- 50 ng/mL). The analyst must choose an

appropriate level for each analyte when preforming the DOC analysis, since the ratios of each analyte can vary depending on the standard source. The spiking solution used must be from a source that is independent of the standards used to prepare the calibration curves, if one is available.

14.1.1.2 Recovery values for major analytes must fall in the range of ± 20% and minor analytes must fall in the range of ± 30%. The precision of the replicate measurements, calculated as relative standard deviation (RSD), must be ≤ 20% for the major analytes and ≤ 25% for the minor analytes. When analyses fail to meet these criteria, this procedure must be repeated until satisfactory performance has been demonstrated.

14.1.2 Continuing DOC

14.1.2.1 Annually, each analyst must complete a continuing DOC for each target analyte using one of the procedures below.

14.1.2.1.1 Acceptable performance of the analysis of a blind sample, such as an external proficiency test, when available.

14.1.2.1.2 Acceptable performance of an initial DOC as described in **Section 14.1.1** at any concentration within the calibration range.

14.1.2.2 If major changes to the method or instrument are made, or the laboratory/analyst has not performed the method in a twelve (12) month (m) period, each analyst must complete an initial **DOC** as described in **Section 14.0.** Minor changes to the method are evaluated using the **LFB** per **Section 14.5**.

14.1.3 All initial and continuing **DOC**s must be documented.

14.2 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

14.2.1 An initial **LOD** study for each method must be completed and documented for all target analytes in each representative matrix (see **MML-301-SOP, Section 7.3**), on each instrument used to analyze sample extracts. If the laboratory intends to report results below the **LOQ**, an ongoing **LOD** verification is also required.

14.2.2 Based on the **LOD**, the laboratory shall select an **LOQ** that is greater than the **LOD** (typically 3-5x the **LOD**) and consistent with the needs of its client. An **LOQ** is required for each representative matrix, method and analyte

combination. For each method, the lowest calibration standard concentration must be at or below the corresponding **LOQ**.

14.2.3 An initial **LOQ** study for each method must be completed and documented for all target analytes in each representative matrix. The initial **LOD** samples may be used for this purpose as long as the concentration used is at or below the **LOQ**. The mean recovery shall be within 70-130% of the spiked value.

14.2.4 On an ongoing basis, the laboratory shall prepare and analyze a minimum of one **LOQ** verification sample spiked at the same concentration as the initial **LOQ** verification study on each instrument during each quarter in which samples are being analyzed for each representative matrix, method, and analyte combination. The recovery of the **LOQ** verification samples shall be within 70-130%.

14.2.5 The 2017 Method Update Rule finalized in the Environmental Protection Agency's (EPA's) Federal Register on August 28, 2017, prescribes a revised approach to Method Detection Limit (**MDL)/LOD** data collection and calculation per Part 136 Appendix B. The New York State (NYS) Environmental Laboratory Program (ELAP) requires that the revised procedure detailed within the EPA's document *Definition and Procedure for the Determination of the Method Detection Limit, Revision 2, December 2016* be implemented for all NYS ELAP accredited methods.

14.3 **SBLK**

14.3.1 Before processing samples, the analyst must demonstrate that the instrument is free from background interference by analyzing a **SBLK**.

14.3.2 For this analysis, a vial of ACN will be injected.

14.3.3 Background contamination, if it is observed and is such that it could interfere with the measurement of target analyte(s), must be < 1/3 of the **LOQ** for the target analyte.

14.4 **LRB**

14.4.1 Before processing samples, the analyst must demonstrate that all interferences arising from glassware and reagents are under control. An **LRB** must be analyzed with each preparation batch (one to twenty samples of the same matrix with a maximum processing time of twenty-four (24) hours between the first and last sample). If, within the relative retention time window of the target

analyte, the **LRB** produces a peak that would prevent the determination of the analyte, the source of the interference must be determined and eliminated before processing the samples.

14.4.2 Background contamination found must be <1/3 of the **LOQ** for the target analyte.

14.5 **LFB**

14.5.1 The laboratory must analyze at least one **LFB** with every preparation batch (one to twenty samples of the same matrix with a maximum processing time of twenty-four (24) hours between the first and last sample).

14.5.2 The **LFB** is fortified with the target analytes at a mid-level concentration (between 3.7 -33.33 ng/mL).

14.5.3 The accuracy is calculated as percent recovery. The recovery for the analyte must be 80 – 120% for the major analytes and 70 -130 % for the minor analytes of the fortified spike concentration.

14.5.4 The laboratory fortified blank contains only the standards, "representative" matrix, **IS** and solvent.

14.6 **IS**

14.6.1 The **IS** is spiked in all samples. The **IS** peak area in all the analyzed samples must be within **20 %** of the mean values of the initial calibration curves.

14.7 **MS** and **MSD**

14.7.1 An **MS** sample must be analyzed with each preparation batch (one to twenty samples of the same matrix with a maximum processing time of twenty-four (24) hours between the first and last sample).

14.7.2 The **MS** is fortified with the target analyte at a mid-level (between 3.7 - 33.33 ng/mL) concentration.

14.7.3 A separately prepared **MSD** must be prepared and compared against the original **MS** sample.

14.7.4 To determine the accuracy, the percent recoveries of the target analytes in the **MS** are calculated. Total recovery of the analytes must be within 80 – 120 % of the true value for the major analytes and 70 -130 % for the minor analytes.

14.7.5 To determine the precision, the relative percent difference (**RPD**) of the **M**S and **MSD** is calculated. The **RPD** must be <20% for the major analytes and < 25 % for the minor analytes.

15.0 Sample Preparation Procedure

15.1 Sample Preparation

15.1.1 Intermediate Sample Preparation

15.1.1.1 Weigh 10 ± 0.5 mg of material into a 1-mL amber autosampler vial.

15.1.1.2 Add 990 uL of ACN.

15.1.1.3 Vortex for 10 seconds at least 3 times to mix well. Sample is retained a room temperature until final sample preparation.

15.1.2 Final Sample Preparation

15.1.2.1 Into a separate 2-mL vial labeled with sample identification number, dispense 850 µL of ACN.

15.1.2.2 Into same 2-mL vial, dispense 50 µL of **IWD** (**Section 8.4**).

15.1.2.3 Into same 2-mL vial, dispense 100 µL of intermediate sample (**Section 15.1.1**) or "representative" matrix for quality control purposes, where relevant **(Section 7.2)**.

15.1.2.4 Vortex the sample 10 seconds at least 3 times to mix well. Label with "Sample ID" and analyze on instrument.

15.2 **SBLK**

15.2.1 Into a 2-mL vial labeled "**SBLK**", dispense 1 mL of pure ACN.

15.3 **MB**

15.3.1 Into a 2-mL vial labeled "**MB**", dispense 850 µL of pure ACN.

15.3.2 Into same 2-mL vial, dispense 100 µL of "representative" matrix. **(Section 7.2)**.

15.3.3 Into same 2-mL vial, dispense 50 µL of **IWD** (**Section 8.4**).

15.3.4 Vortex for 10 seconds at least 3 times to mix well and analyze on instrument.

15.4 **LRB**

15.4.1 Into 2-mL vial labeled "**LRB**", dispense 850 µL of ACN.

15.4.2 Into same 2-mL vial, dispense 100 µL of "representative" matrix. **(See Section 7.2)**.

15.4.3 Into same 2-mL vial, dispense 50 µL of **IWD** (**Section 8.4**).

15.4.4 Vortex for 10 seconds at least 3 times to mix well and analyze on instrument.

15.5 **LFB**

15.5.1 Into a 2-mL vial labeled "**LFB"**, dispense 750 µL of ACN.

15.5.2 Into same 2-mL vial, dispense 100 µL of "representative" matrix. **(Section 7.2)**.

15.5.3 Into same 2-mL vial, dispense 50 µL of **IWD** (**Section 8.4**).

15.5.4 Into same 2-mL vial, dispense 100 µL of **CalSI3 (Section 11.1.4)**.

15.5.5 Vortex the sample 10 seconds at least 3 times to mix well and analyze on instrument.

15.6 **MS** and **MSD** Preparation:

15.6.1 Into 2-mL vials labeled "**MS**" and "**MSD**", dispense 800 µL of ACN.

15.6.2 Into each 2-mL vial, dispense 100 µL of "representative" matrix. **(Section 7.2)**.

15.6.3 Into each 2-mL vial, dispense 50 µL of **CalSI3** to each 2-mL vial **(Section 11.1.4)**.

15.6.4 Into each 2-mL vial, dispense 50 µL of **IWD** (**Section 8.4)**.

15.6.5 Vortex the vials for 10 seconds at least 3 times to mix well and analyze on instrument.

16.0 **Data Acquisition, Reduction, Analysis, and Calculations**

16.1 Other GC columns, chromatographic conditions, or detectors may be used if the requirements of **Table 1**. are met. Calibrate or verify the system calibration on each day of analysis as described in **Section 13.0**. For all analyses, the standards and sample extracts must be in ACN.

16.2 If the response for a target analyte exceeds the working range of the instrument, dilute the extract in diluent and reanalyze.

16.3 If concentrations above the calibration curve are expected, the sample extract may be diluted and analyzed to prevent detector saturation and/or negative impacts on the column and/or injector. Since the **IS** is added after the dilution of the extract, there is no impact on the IS.

16.4 When the software inadequately integrates peaks and manual integration becomes necessary, laboratory specific procedures must be used a guidance for any manual integration of peaks.

16.5 An **SBLK** and **LRB** should be analyzed prior to sample analyses to ensure that the total system (syringe, column and GC system) is free of contaminants. Additional preventive techniques prior to analysis include: rinsing the sample syringe between samples with methylene chloride and ACN, conditioning new columns prior to use and bake-out of currently in-use columns for a minimum of 1 h.

16.6 When contamination is observed, see **Section 5** and **Section 14.3**.

16.7 Use the instrument software and specified parameters to perform peak integration for all identified peaks.

16.8 The **IS** is used for response and retention time reference (**See Appendix B**).

16.9 Calculate the average retention time of the **IS** in the calibration curve. The retention time of each standard **IS** must be within **2 %** of the average retention time of the standards in the curve.

16.10 The **IS** is spiked in all samples. The **IS** peak area in all the analyzed samples must be within **20 %** of the mean values of the initial calibration curves.

16.11 Calculate the retention time of the **IS** in each sample. The retention time of the sample must be within **2%** of the average retention time of the standards in the curve.

16.12 Calculate the average **IS** peak area for the matrix. Evaluate the system stability by using the following equation on every injection and comparing to the criteria set in **Section 16.10 and 16.11**.

$$IS\ Peak\ Area\ Deviation\ (\%) = \frac{IS_I}{IS_A} * 100$$

Where: IS_I = IS peak area for individual injection
IS_A = IS peak area average for given matrix

16.13 Using the weighted 1/C linear regression curve for all calibration standards, check the curve linearity and calculate the standards recovery at each level. Evaluate the linearity and recovery based on the criteria set in **Section 13.0.**

16.14 QC and Unknown Samples

16.14.1 Apply the linear regression calibration curve generated from the calibration standards to all QC and unknown samples to calculate the concentration in ng/mL of each analyte using the instrument quantitation software.

16.14.2 For medical marijuana samples, this value must then be converted to a sample concentration in ng/g using the following equation:

Equation 12. Where:

$$C_S\left(\frac{ng}{g}\right) = \frac{C_E\left(\frac{ng}{mL}\right) * V_F(mL) * D}{M_I(mg) * 0.001\left(\frac{g}{mg}\right)}$$

C_S = Concentration of analyte in sample (in ng/g).
C_E = Concentration of analyte in solvent (in ng/mL) (from software)
V_F = Final volume of extract (in mL)
M_I = Initial mass of sample (in mg)
0.001 mg = Conversion from g to mg
D = Dilution factor, if applicable.

16.15 To determine the precision, of the **MS/MSD** calculate the relative percent difference (RPD).

Equation 13. RPD = (|MS-MSD|/((MS+MSD)/2)) x 100

Where: RPD is in percent (%).
MS = **MS** concentration (in ng/mL).
MSD = **MSD** concentration (in ng/mL).

16.16 Reporting of Results

16.16.1 Non-detected analytes are reported as (<) the **LOQ** as specified in **Table 1.**

16.16.2 Analytes detected at a concentration at or above the **LOQ** are reported using 2 significant figures.

17.0 **Data Assessment, Acceptance Criteria and Corrective Actions for Out-of-Control Data.**

17.1 All analytical batches must meet all quality control criteria as described within this procedure and all quality control results must be documented.

17.2 The acceptance criteria for standards and quality control samples are defined in **Section 13.0**. The sections below (**Sections 17.3 – 17.13**) outline the most common corrective action procedures for nonconforming data and inconsistent chromatograms. Since reinjection of a standard or sample is a routine corrective action for most nonconformities, it is not included in each individual section below, but are used whenever applicable.

17.3 Failure to meet **QC** criteria for a Calibration Curve Correlation Factor of **≥0.995**.

17.3.1 A correlation factor of **≥0.995** must be achieved before sample analysis can begin. If samples were analyzed before an acceptable calibration curve is established, all affected samples must be reanalyzed under an acceptable curve or the results is appropriately qualified.

17.3.2 Assess the calibration curve to determine whether there is one standard that appears to be prepared incorrectly. If so, reprepare that standard and analyze. If more than eight (8) hours has elapsed since the original failing calibration standard is analyzed, then all calibration standards must be reanalyzed.

17.3.3 If more than one standard appears to be prepared incorrectly, or the calibration curve is erratic, reprepare all calibration standards and analyze. This may involve repreparing the working standard solution or opening new stock standard solutions.

17.3.4 If necessary, perform instrument maintenance.

17.4 Failure to meet required **QC** criteria for **CCR** of 80-120% recovery for major components and 70 -130 % for the minor components.

17.4.1 Check the calibration curve linearity, calibration curve response (**Section 13.3**), and internal standards response (**Section 16.0**).

17.4.2 Check **LFB** recovery value **(Section 14.5**).

17.4.3 If the **LFB** and other responses of the calibration curve appear normal, then the current **CCR** is likely compromised and a new **CCR** is prepared and reanalyzed from current stock standards. If the reprepared **CCR** fails, then the analyst must analyze a new **CCR** from freshly prepared **PS** or P**WS**.

17.4.4 If a newly prepared **CCR** still doesn't meet the required criteria, the instrument is recalibrated with new calibration standards, which may be prepared from new or existing working standard solutions or stock standard solutions. A new calibration curve must be prepared, run on the instrument and be verified with a **CCR** using the new curve.

17.4.5 All samples must have an acceptable **CCR**. Any samples that are analyzed without an acceptable **CCR** must be reanalyzed when an acceptable **CCR** is achieved or a new calibration is established.

17.5 Failure to meet required **QC** criteria for **CCV** of 80% to 120% recovery for major components and 70 -130 % recovery for the minor components.

17.5.1.1A new **CCV** is prepared and reanalyzed; it may be necessary to prepare from new working or stock solutions.

17.5.1.2If a newly prepared **CCV** still does not meet the required criteria, the instrument is recalibrated with new calibration standards, which is prepared from new or existing working standard solutions or stock standard solutions. A new calibration curve is prepared and analyzed on the instrument and is verified with a **CCR**.

17.5.2 All samples must be bracketed by an acceptable **CCV**. Any samples that are analyzed without an acceptable bracket must be reanalyzed when an acceptable **CCV** is achieved or a new calibration is established. If reanalysis in not possible due to lack of remaining extract or sample, the original sample results will be appropriately qualified.

17.6 Failure to meet required **QC** criteria for **LOQ** of 70% to 130% recovery for major components and 60 -140 % for the minor components.

17.6.1 A new **LOQ** standard is prepared and reanalyzed (where necessary); it may be necessary to prepare this from new working and/or stock solutions.

17.6.2 If the newly prepared **LOQ** standard still does not meet the required criteria, the instrument is recalibrated with new calibration standards, which are prepared from new or existing working standard solutions or stock standard solutions. A new calibration curve is prepared and run on the instrument and verified with a **CCR**.

17.6.3 A **LOQ** standard within 70-130% recovery for major components and 60 -140 % for the minor components must be achieved before analysis can begin. If samples were analyzed before an acceptable **LOQ** is achieved, all affected samples must be reanalyzed after an acceptable **LOQ** is achieved.

17.7 Failure to meet required **QC** criteria for **SBLK** of <1/3 **LOQ** for target analyte(s).

17.7.1.1 Bake-out the column at an appropriate temperature based on column manufacturer's recommendations.

17.7.1.2 Inject multiple **SBLKs** and run them through the system until background contamination is removed or reduced to an acceptable level.

17.7.1.3 An acceptable **SBLK** must be achieved before sample analysis begins. If samples have already been analyzed, then any samples containing target analytes must be reanalyzed. If reanalysis of suspect samples is not possible due to lack of remaining extract or sample, the original sample results will be appropriately qualified.

Exception: If the samples do not contain target analytes at or above the **LOQ**, the original results is reported without reanalysis and qualification is not necessary.

17.8 Failure to meet required **QC** criteria for **LRB** of <1/3 **LOQ** for target analyte(s).

17.8.1 Analyze a **SBLK** to ensure that the system is free from background contamination. If background contamination is discovered in the **SBLK**, follow the corrective actions described above (**Section 17.8).**

17.8.2 Reinject the **LRB** once a contaminant-free system is achieved.

If the reinjection still fails, request that a new **LRB** be extracted and analyze to ensure that a systemic problem does not exist.

17.8.3 An acceptable **LRB** must be achieved before sample analysis begins. If samples have already been analyzed, then any samples containing target analytes must

be reanalyzed (if system contamination is suspected) or re-extracted and analyzed (if extraction contamination is suspected). If reanalysis or re-extraction of suspect samples is not possible due to lack of remaining extract or sample, the original sample results will be appropriately qualified.

Exception: If the samples do not contain target analytes at or above the **LOQ**, the original results are reported without reanalysis and qualification is not necessary.

17.8.4 If subsequent **LRBs** continue to show unacceptable levels of background contamination, the extraction of additional samples must be halted until the source of the contamination can be determined and eliminated or reduced to acceptable levels.

17.9 Failure to meet required **QC** criteria for **LFB** as described in **Section 14.0**

17.9.1 Check to determine whether there is an interference peak that is not identified.

17.9.2 Reanalyze the **LFB** sample. If it is still out of the range, check the **MS** for a similar problem.

17.9.3 If the **MS** also fails, the problem is likely related to the spiking solution. Discard the problematic solution and reprepare the spiking solution, **LFB**, and **MS** and **MSD**.

17.9.4 If the **MS** passes, indicating that the spiking solution is not the problem, the **LFB** must be reprepared.

17.10 Failure to meet required **QC** accuracy (recovery) criteria for **MS** as described in **Section 14.0**.

17.10.1 Check the **LFB** recovery to see if it is related to the spiking solution.

17.10.2 If the **LFB** also fails, discard problematic spiking solution and reprepare the spiking solution, **LFB**, **MS**, and **MSD**.

17.10.3 If the subsequent **MS** is prepared with a newly prepared spiking solution and meets acceptance criteria, no further action is required.

17.10.4 If the **MS** fails to meet the acceptance criteria, but the **LFB** is acceptable, then it is recommended that the **MS** and/or **MSD** be reprepared and analyzed if sufficient sample remains; this requires preparation from a new stock standard.

17.10.5 If reanalysis is performed, and the new **MS** and/or **MSD** meets the acceptance criteria, only report those results.

17.10.6 If the **MS** and/or **MSD** cannot be reanalyzed, or if the reanalyzed **MS** and/or **MSD** still fails, the original **MS** and/or **MSD** and all corresponding sample results will be appropriately qualified on the report.

17.11 Failure to meet required **QC** precision (**RPD**) criteria for **MSD** as described in **Section 14.0**

17.11.1 Compare the **MS** and **MSD** for **IS** Peak Area Deviation using only the **IS** peak area from these two samples.

17.11.2 If the IS deviation check fails, it indicates that the **IS** has not been accurately spiked and both the **MS** and **MSD** must be reprepared.

17.11.3 If the deviation check passes, repeat the calculation using the peak areas of both samples in place of **IS** peak area.

17.11.4 If this deviation check fails, it indicates that the **IS** has not be accurately spiked and both the **MS** and **MSD** must be reprepared.

17.11.5 If samples do not deviate from peak area or **IS** peak area, yet still fail precision criteria, both the **MS** and **MSD** must be reprepared.

17.11.6 If the reprepared **MS** and/or **MSD** still fail **RPD**, the original **MS** and/or **MSD** and all corresponding sample results will be appropriately qualified on the report.

17.12 Failure to meet required **QC** criteria for **IS** peak area variation described in **Section 14.0** and **16.0**.

17.12.1 Check to determine if there is a sample preparation error.

17.12.2 Check if there is an interference peak co-eluting with **IS**.

17.12.3 If the **IS** variation is higher than **20%,** an investigation must be performed until the reason for this variation is determined and the issue is resolved. The samples are then reanalyzed and reported. If reanalysis or re-extraction of suspect samples is not possible due to lack of remaining extract or sample, the original sample results will be appropriately qualified.

17.13 Inconsistent baseline

17.13.1 Perform appropriate instrument maintenance, if applicable.

17.13.2 Repeat the sequence using the same standards/samples. If repeat analysis is acceptable, report only those results.

17.13.3 If instrument maintenance and repeat analysis fails to produce acceptable data, the sample results will be appropriately qualified.

17.13.4 All other nonconforming data that has not been addressed within this procedure requires the completion of a nonconformance/corrective action report.

18.0 Method Performance

18.1 Detection limit studies and **DOC** results are maintained by the laboratory.

19.0 Waste Management/Pollution Prevention

19.1. It is the responsibility of the laboratory to comply with all federal, state and local regulations governing waste management, particularly regarding hazardous waste identification rules and land disposal restrictions.

19.2. Minimize solvent, chemical, reagent, and standard use whenever possible to reduce the amount of hazardous waste generated.

19.3. Dispose of solvent waste in an appropriate solvent in a properly labeled waste container.

19.4. Acetonitrile and methanol must be disposed of separately from all other solvents in a container no larger than 4 L (1-Gallon).

19.5. All other solvents are separated into two categories: chlorinated and non-chlorinated. These are disposed of in red, 5-Gallon solvent cans.

19.6. Dispose of non-hazardous aqueous waste in the laboratory sink followed by flushing with tap water.

19.7. Dispose of glassware in appropriately labeled boxes. Be sure that, whenever possible, the glass has been thoroughly rinsed and is contaminant-free before disposal.

20.0 References

20.1 Public Health Law, section 502 of the Public Health Law ("PHL"), Title 10 (Health) of The Official Compilation of Codes, Rules and Regulations of the State of New York (NYCRR) subpart 55-2 (Approval of Laboratories Performing Environmental Analysis). http://w3.health.state.ny.us/dbspace/NYCRR10.nsf/56cf2e25d626f9f785256538006c3ed7/c9252587bc832b3485256c390055920a?OpenDocument&Highlight=0,section,55).

20.2 Definition and Procedure for the Determination of the Method Detection Limit--Revision 1.11 Environmental Protection Agency, 40 CFR (7-1-95 Edition) Part 136, Appendix B.

20.3 Agilent- System User Guide https://www.agilent.com/cs/library/usermanuals/Public/G7000-90038.pdf

20.4 Harynuk, James; Marriott, Philip J. Fast. *GC_GC with Short Primary Columns*. Anal. Chem. 2006, 78, 2028-2034

20.5 Rauf, M. A; and Hanan, A. *Quality Assurance Considerations in Chemical Analysis*. Quality Qual Assur. 2009, 12, 16-21.

21.0 Supporting Documents

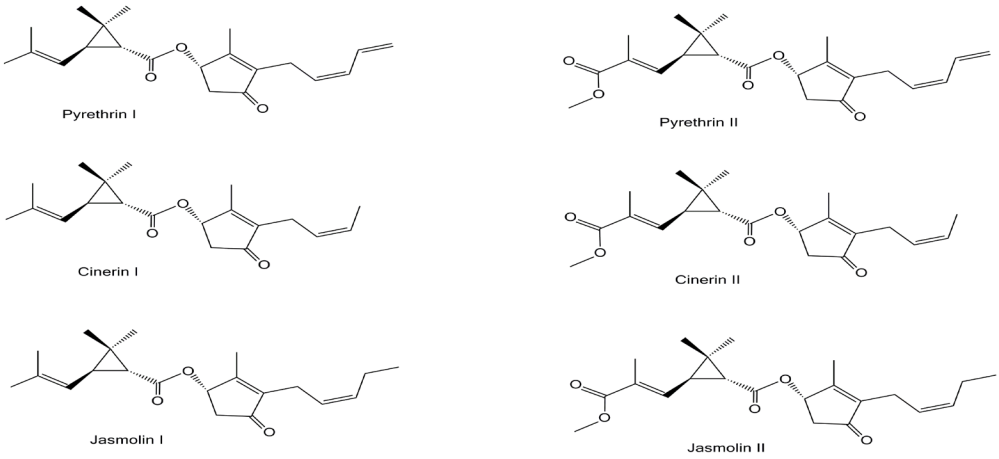
21.1 Structures and chromatograms of analyte list (MML-308 Appendix A)
21.2 Natural Pyrethrin and Internal Standard with corresponding retention times (Appendix B)

22.0 Appendices

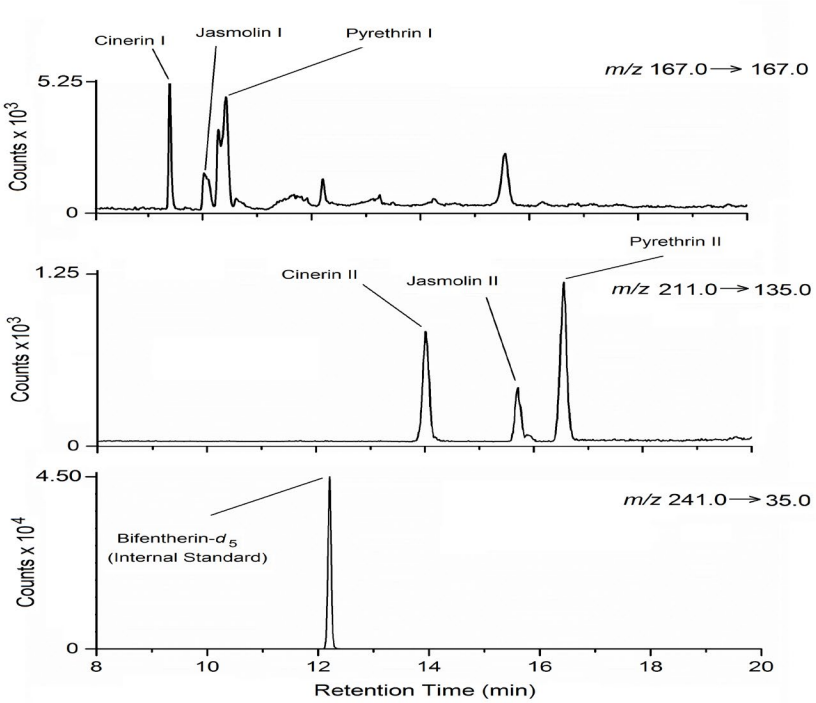
Appendix A

Structures and chromatograms of analyte list

Natural Pyrethrin Structures



Natural Pyrethrin Chromatograms



Appendix B

Natural Pyrethrin and Internal Standard with corresponding retention times.

Analyte**	**Retention Time***
Pyrethrin Group I:	
Cinerin I	9.40
Jasmolin I	10.04
Pyrethrin I	10.42
Pyrethrin Group II:	
Cinerin II	14.04
Jasmolin II	15.72
Pyrethrin II	16.55
Internal Standard (IS):	
Bifenthrin-(biphenyl-2′,3′,4′,5′,6′-*d*5)	12.20

*Retention Time Acceptable within 2%

Retention times are approximate based on current column setup and may vary slightly over different column installations and the lifetime of the columns

Columns and analytical conditions are described in **Section 10.0**