DETERMINATION of ACEQUINOCYL, ACEQUINOCYL-OH, and AKM-18 in SOIL

This summary level document modifies the original validated method by incorporating minor method changes employed during the independent laboratory validation.

The original method: "Determination of Acequinocyl and Acequinocyl-OH and AKM-18 in Soil" (Morse Method 136, Appendix 1 of MRID 46182602) was validated a by Morse Laboratories, LLC 1525 Fulton Avenue, Sacramento, California 95825. (Appendix I of MRID 46182602)

The Independent laboratory Validation "Independent Laboratory Validation for the Determination of Acequinocyl, Acequinocyl-OH, and AKM-18 in Water and Soil" (MRID 46182602) was performed by Pyxant Labs Inc, 4720 Forge Road, Suite 108, Colorado Springs, CO 80907

Reasons for Revision:

1) All glassware was silylated prior to any standard or sample preparation and was resilylated again before each use.
2) All the lights in the laboratory were turned off during the preparation of standards, fortification, extraction, partition, and throughout the rotary evaporation process because of the sensitivity of acequinocyl and acequinocyl-OH to light
3) Plastic powder funnels were substituted for glass powder funnels
4) No 500 mL glass Erlenmeyer flasks were used
5) The only graduated mixing cylinders needed were 250 and 25 mL sizes
6) Opaque brown centrifuge bottles were used instead of standard HDPE bottles
7) HPLC sample filters were not used in ILV; use of filters is left to the analyst's discretion.
8) Polypropylene pipettes were used in place of the glass Pasteur pipets
9) Serological pipettes were not used
10) No solid phase extraction apparatus was used
11) Sample filtration was not done in ILV, so Luer-lock syringes were not used there; sample filtration is left to the analyst's discretion.
12) Keeper Solution was prepared as 1% v/v not w/v (1 mL decanol per 100 mL acetone).
13) Specific volumes and concentrations used for standard preparation were changed in order to improve accuracy and convenience.
14) At step 7.8, extracts were concentrated to 11-16 mL and the quantitative transfer was made with more than one 2 mL acetonitrile rinse
15) Separatory funnels were not centrifuged in the ILV,
16) At step 8.7, the concentrated extracts were not blown down to dryness because a small amount of water and keeper remained in the flask, and could not be completely evaporated within a reasonable amount of time
17) Silica column cleanup as described in section 9.0 of the method was modified. Section 3.8 of this report describes the column cleanup
18) The combined eluates in section 9.10 of the method were concentrated using 15 in. Hg for the first 20 minutes, then adjusted to 18-21 in. Hg for the final 40-45 minutes
19) The transfer of concentrated extracts in section 9.11 occurred with one 2 mL acetone rinse and two 1 mL rinses instead of one 1 mL rinse and two 0.5 mL rinses
20) The *LC/MS/MS* instrument and instrument parameters, HPLC column, and mobile phase were changed from what is stated in the method. Section 3.5, Analytical Instrumentation and Equipment outlines the parameters and equipment that were used.

These minor modifications are not considered significant and will have no effect on the quality of the data.

1. PRINCIPLE

The method described herein is capable of determining acequinocyl, acequinocyl-OH, and AK-18 in soil.

All three targeted analytes are extracted from soil with acetonitrile:water (90:10, v/v) using a multiple extraction technique. The extracts are combined, then concentrated by rotary evaporation. To the concentrate is added a saturated sodium chloride solution along with solid sodium chloride and hexane. Following vigorous shaking, the layers are separated by centrifugation, with the upper hexane/acetonitrile layers containing all three targeted analytes. The lower aqueous layer is extracted one additional time with a fresh aliquot of hexane to insure adequate recovery of the analytes from the aqueous phase. The organic extracts are combined, concentrated, and then purified by means of silica liquid chromatographic column cleanup. Two different eluants are employed to recover all analytes, the eluates being combined upon collection. The resulting solution is evaporated to dryness, reconstituted in acetone:acetonitrile: 0.4% aqueous formic acid (2:2:1, v/v), then submitted to HPLC analysis.

During routine analysis, determination and quantitation of the analytes are conducted using high pressure liquid chromatography (HPLC) employing mass spectrometric (MS/MS) detection. The limit of quantitation (LOQ) in soil for all three analytes is ~0.01 ppm. (Detector sensitivity to each analyte is generally not the same resulting in different actual LOQs for each analyte. 0.01 ppm is the targeted LOQ for the least sensitive of the three analytes.)

2. EQUIVALENCE STATEMENT

During the conduct of this analysis, comparable apparatus, solvents, glassware, and techniques (such as sample extract evaporation) may be substituted for those described in this method, except where specifically noted otherwise. In the event a substituted piece of equipment or technique is used, its use will be documented in the study records.

3. APPARATUS AND EQUIPMENT

Note:
It is recommended that all glassware used for the processing of sample extracts be silylated to facilitate transfer of residues at various analytical steps. Resilylate at least every 2-3 weeks during active use. Silylation before each use may also be employed if recoveries diminish with each successive use. The silylation method is described in Appendix II.

Assorted laboratory Glassware	As required

Balances:	Analytical balance capable of weighing to ± 0.1 mg.
	Top-loading balance capable of weighing to ± 0.1 mg.
Beakers	Glass; 50 mL
Centrifuge:	IEC Model HN-SII (Damon IEC Division, Needham Hts., MA)
Centrifuge bottles:	Opaque brown 250 mL VWR® HDPE (high-density polyethylene) bottles with screw cap closures
Chromatographic columns:	Glass; 30 cm x 11mm i.d., with solvent reservoir
Evaporation flasks:	Round or flat-bottom, glass, 125 250, and 500 mL
Evaporators:	Rotary evaporator equipped with a Dewar condenser (Labconco Corp., Kansas City, Mo.)
	N-Evap™ Laboratory Sample Evaporator Model 115 attached to a nitrogen source (Organomation Associates, South Berlin, MA)
Funnels:	Powder, plastic, 100 mm diameter
Graduated cylinders:	Glass; 1000, 100, and 25 mL
Graduated mixing cylinders:	Glass; 250 and 25 mL
HPLC System:	PE Sciex API 4000 LC/MS/MS system with a Perkin Elmer series 200 autosampler, an integrated Shimadzu chromatograph consisting of (2) LC-I0ADvp Liquid Chromatograph units and a DGU-14A Degasser. The system is controlled and data processed by PE Sciex Analyst Software.
HPLC column:	15 cm x 2.0 mm i.d. Luna Phenyl-Hexyl, 3 µ particle size, part no. 00F-4256-BO (Phenomenex, Torrence, CA)
HPLC sample filter:	Nylon 66 filters, 13 mm, 0.45 mm (Varian Sample Preparation Products, Harbor City, CA)
Micro-liter syringes:	Various sizes, (Hamilton Co., Reno, NV)
Pasteur pipets:	Polypropylene, disposable
Platform shaker:	Eberbach model 6000, variable speed, reciprocating (Eberbach Corp., Ann Arbor, MI)
Separatory funnels:	Glass; 1000 and 250 mL

Syringes:	Glass, 2.5 mL, Hamilton Teflon® Luer-Lok (Hamilton Co., Reno, NY)
Test (culture) tubes:	Glass; 13 X 100 mm,
Ultrasonic bath:	Branson Model 2210 ultrasonic bath (VWR Scientific, Bridgeport, NJ)
Volumetric flasks: Glass;	500, 250, 100, 50, and 25 mL
Vortex mixer:	VWR Vortexer 2 (Scientific Industries, Inc., Bohemia, NY)

4. REAGENTS AND MATERIALS

Acequinocyl	Analytical grade
Acequinocyl-OH	Analytical grade
AKM-18	Analytical grade
Acetonitrile	OmniSolv® (EM Science, Gibbstown, NJ)
Acetone	Ultra Resi-analyzed® (J.T. Baker Chemical Co., Phillipsburg, NJ)
Dimethyldichlorosilane:	Catalog #3-3009 (Supelco, Inc., Bellefonte, PA) also referred to as "DMDCS"
1-Decanol	"Baker" (J.T. Baker Co., Phillipsburg, NJ)
Ethyl acetate:	OmniSolv® (EM Science, Gibbstown, NJ)
Formic acid:	Guaranteed Reagent: ACS (EM Science, Gibbstown, NJ)
Glass wool:	Silylated, see Appendix II
Hexane:	(95% n-hexane),Ultra Resi-analyzed® (J.T. Baker Chemical Co., Phillipsburg, PA)
Methanol:	HPLC Grade, (Burdick and Jackson, Muskegon, MI)
Silica gel:	Fisher brand, grade 923 (chromatographic), 100-200 mesh (fisher scientific, Fair Lawn, NJ)
Sodium chloride	Guaranteed Reagent: ACS (EM Science, Gibbstown, NJ)

Sodium sulfate: Analytical Reagent, anhydrous granular, #8024 (Mallinckrodt, St. Louis, MO)

Water: Deionized (DI) water (Polymetrics System, Morse Laboratories, Inc.)

HPLC Grade water (Fisher Scientific, Fairlawn, NJ)

4.1. Reagents and Materials to be Prepared (including typical preparation instructions)

4.1.1. 1% (v/v) keeper solution: Place 1.0 mL 1-decanol in a 100 mL volumetric flask. Fill to mark with acetone. Mix well.

4.1.2. Silica column wash and elution mixtures:

Ethyl acetate:hexane (l:99. v/v): To a 1000 mL graduated mixing cylinder, add 10 mL of ethyl acetate. Bring to a final volume of 1000 mL with hexane. Mix well. Sufficient for approximately 20 samples.

Ethyl acetate:hexane (5:95. *v/v):* To a 1000 mL graduated mixing cylinder, add 50 mL of ethyl acetate. Bring to a final volume of 1000 mL with hexane. Mix well Sufficient for approximately 13 samples.

Water:acetonitrile (2:98, *vlv):* To a 1000 mL graduated mixing cylinder, add 20 mL of DI water. Bring to a final volume of 1000 ml with acetonitrile. Mix well. Sufficient for approximately 16 samples.

4.1.3. Activated silica gel: Activate the silica gel in an oven set at approximately 130°C for a minimum of 24 hours. Transfer the hot silica into a glass container with ground glass stopper. Let cool to ambient temperature before use (mixing with hexane).

4.1.4. 5% (v/v) dimethyldichlorosilane in hexane: To a glass-stoppered glass container (such as a 100 mL mixing cylinder), add 95 mL of hexane. Slowly add 5 mL of DMDCS. Stopper and invert to mix. Larger or smaller volume can be prepared using the proportions discussed above.

4.1.5. Acetone:acetonitrile:0.4% aqueous formic acid (2:2:1; v/v): All samples and standards injected into the HPLC must be dissolved in this specific solvent combination. Typically 200 mL are prepared which are sufficient for the preparation of the four calibration standards and any dilutions that may be necessary for the analysis of the samples. Prepare 200 mL of an *acetone: acetonitrile:0.4% aqueous formic acid (2:2:1; v/v)* solution as follows: To a 250 mL mixing cylinder, add 80 mL of acetone, 80 mL of acetonitrile, and 40 mL of 0.4% aqueous formic acid. M ix thoroughly. Smaller volumes may be prepared by decreasing the individual volumes and keeping the ratio constant. Store in an air-tight container. Prepare weekly.

4.1.6. 0.4% formic acid in water: To a 500 mL volumetric flask, add 2.0 mL of formic acid. Bring to volume with HPLC grade water. Mix well. Prepare weekly.

4.1.7. Acetonitrile:water (90:10, v/v): Prepare volumes as needed, i.e., prepare 100 mL of solution by mixing 10 mL of DI water with 90 mL of acetonitrile in beaker of suitable size.

4.1.8. Saturated sodium chloride solution: Place 88 g of sodium chloride into a 250 mL mixing cylinder. Fill to 250 mL mark with deionized water. Shake to dissolve all the salt that will dissolve (~30 seconds). If necessary, add additional water to make a 250 mL final volume. Shake for an additional ~30 seconds. Some crystals will remain undissolved. This is to be expected and indicates the solution is indeed saturated. Sufficient for approximately 25 samples.

4.1.9. HPLC mobile phases:

0.1% formic acid in methanol: To a 1 liter graduated cylinder, add methanol to the 1000 mL mark. Add 1.0 mL of formic acid using a 2.0 mL graduated pipet. Transfer the entire solution to the HPLC solvent reservoir and once transferred, mix thoroughly.

0.1% formic acid in water: To a 1 liter graduated cylinder, add HPLC grade water to the 1000 mL mark. Add 1.0 mL of formic acid using a 2.0 mL graduated pipet. Transfer the entire solution to the HPLC solvent reservoir and once transferred, mix thoroughly.

5. STANDARD PREPARATION

Note:
Specific volumes and concentrations used in standard preparation may be changed in at the analyst's discretion.

Acequinocyl and its derivatives are extremely sensitive to photo-degradation. Care should be taken to avoid direct illumination of the standard or sample solutions.

5.1 Stock standard solution:
Typically, 10.0 mg (corrected for purity) of each analytical standard is accurately weighed and quantitatively transferred to a separate 50 mL volumetric flask. Acequinocyl and AKM-18 are brought to volume with acetonitrile and acequinocyl-OH is brought to volume with acetone. The resulting concentration of each solution is 200 µg/mL. These solutions are to be stored in the dark at 1 to 8° C when not in use.

5.2 Fortification solutions:
Typically, the following concentrations of acequinocyl and acequinocyl-OH are prepared. Suitable mixtures may be prepared accordingly. All solutions are stored at 1 to 8°C when not in use.

100 µg/mL: Transfer 12.5 mL of 200 µg/mL standard solution to a 25 mL volumetric flask. Bring to volume in acetonitrile. Mix well.

10 µg/mL: Transfer 2.5 mL of 100 µg/mL standard solution to a 25 mL volumetric flask. Bring to volume in acetonitrile. Mix well.

1 µg/mL: Transfer 250 µL of 100 µg/mL standard solution to a 25 mL volumetric flask. Bring to volume in acetonitrile. Mix well.

5.3 HPLC (Calibration) Standard Solutions

All standard solutions prepared in this section are to be stored at 1 to 8°C when not in use. Typically the following concentrations of HPLC standard solution *mixtures* are prepared:

0.05 µg/mL Transfer 125 µL of 10 µg/mL standard solution to a 25 mL volumetric flask. Bring to volume in acetone/acetonitrile: 0.4% aqueous formic acid (2:2:1; v/v). Mix well.

0.1 µg/mL Transfer 250 µL of 10 µg/mL standard solution to a 25 mL volumetric flask. Bring to volume in acetone/acetonitrile: 0.4% aqueous formic acid (2:2:1; v/v). Mix well.

0.2 µg/mL Transfer 500 µL of 10 µg/mL standard solution to a 25 mL volumetric flask. Bring to volume in acetone/acetonitrile: 0.4% aqueous formic acid (2:2:1; v/v). Mix well.

.5 µg/mL Transfer 1250 µL of 10 µg/mL standard solution to a 25 mL volumetric flask. Bring to volume in acetone/acetonitrile: 0.4% aqueous formic acid (2:2:1; v/v). Mix well

6. SAMPLE FORTIFICATION

Note:
All samples should be kept in at least a semi-frozen state until the addition of the first extraction solvent and the extraction process begins.

6.1 Weigh 20.0g of semi-frozen sample into a 250-mL polyethylene centrifuge bottle.

6.2 Fortify the sample with the appropriate amount of standard solution(s) containing either single or mixed components. Use a volume of fortification solution ≤ 2.0 mL.

6.3 Proceed with Sample Extraction (Section 7).

7. SAMPLE EXTRACTION

Notes:
All samples should be kept in at least a semi-frozen state until the addition of the first extraction solvent and the extraction process begins.

Acequinocyl and acequinocyl-OH are very sensitive to light. Conduct all analytical steps away from direct light. Alternatively, protect extracts in glassware during analysis with aluminum foil or use amber/dark glassware. Specific glassware includes any items in which there may be lengthy extract storage (> 30 minutes) such as: evaporation flasks, mixing cylinders, separatory funnels, test tubes. Cover rotary evaporator water bath, to exclude light, when concentrating samples.

7.1. Weigh 20.0 g of semi-frozen sample into a 250 mL polyethylene centrifuge bottle. As applicable, fortify appropriate samples at this point.

7.2 Add 100 mL of acetonitrile:water (90:10, v/v) and shake on a reciprocating shaker for 30 minutes at ~160 excursions/minute.

7.3 Centrifuge at ~2200-2500 rpm for 15 minutes.

7.4 Decant the supernatant through a powder funnel, containing a plug of glass wool, into a 250 mL graduated mixing cylinder.

7.5 Repeat Steps 7.2 through 7.4 with the solids remaining in the extraction bottle from Step 4 one additional time using 100 mL of acetonitrile:water (90:10, v/v). Decant the supernatant into the same mixing cylinder through the same funnel as in Step 4, thus combining both extracts.

7.6 Bring to a volume of 200 mL with acetonitrile:water (90:10, v/v) and mix well.

7.7 Transfer 100 mL of the extract (equivalent to 10.0 grams sample) to a 250 mL evaporation flask

7.8 Add 0.4 mL of 1% keeper solution (see Subsection 4.1.1) and concentrate the extract to ~11-16 mL on a rotary evaporator at ~30°C.. Quantitatively transfer the concentrate to a 25 or 50 mL graduated mixing cylinder with the aid of one or more ~2 mL acetonitrile rinse. Bring to a volume of 20 mL with acetonitrile, mix, then transfer to a 125 mL polypropylene separatory funnel.

Note:
Small variations in the volume of the extract concentrate and volume of acetone used to facilitate the transfer may be made at the discretion of the analyst.

8. SOLVENT PARTITIONS

8.1 To the acetonitrile/water extract concentrate from Step 7.0.8, add 20 mL of saturated sodium chloride solution, 3 g of solid NaCl and 60 mL of hexane. Manually shake for 1 minute.

8.2 Centrifuge inverted separatory funnels at ~2200-2500 rpm for 10 minutes. Three layers generally form. The bottom is aqueous and the top two layers are organic (hexane and acetonitrile).

8.3 Drain the lower aqueous layer into another 125 mL polypropylene separatory funnel and the upper organic layers into a 250 mL evaporation flask.

8.4 Reextract the aqueous layer with a fresh 60 mL portion of hexane, as in Step 1.

8.5 Centrifuge at ~2200-2500 rpm for 10 minutes.

8.6 Drain the lower aqueous layer to waste and the upper hexane layer into the same evaporation flask used in Step 3, combining the hexane extracts (which may contain some acetonitrile as a separate layer).

8.7 To the combined organic extracts, add 0.4 mL of 1% keeper solution and concentrate to ~0.5 mL on a rotary evaporator at ~30°C. Continue evaporation to dryness carefully with manual nitrogen blow down if dryness can be achieved in a reasonable amount of time. Small residual amounts of water and keeper solution are tolerable.

8.8 Add 5.0 mL of hexane. Sonicate for ~1 minute. Proceed with silica column chromatographic cleanup (Section 9.0.

Note:
Centrifugation of seperatory funnels may be omitted if clean breaks between solvent layers are obtained otherwise

9. SILICA COLUMN CHROMATOGRAPHIC CLEANUP

Notes:
Assess new lots of silica gel prior to use in order to ensure optimum method performance. Analyte recovery of >90% is desired to ensure silica gel is suitable for use. The elution volume and/or polarity of the suggested eluting mixture may need to be adjusted in order to obtain quantitative elutions. The analysis-is conducted on a reagent spike basis. See Appendix II for detailed instructions on evaluation of silica gel.

All elutions are conducted at ~2-3 mL/min.

Procedure:

9.1 Prepare a chromatographic column (30 cm x 11 mm i.d., with solvent reservoir) placing a glass wool plug at the bottom. Close stopcock fully.

9.2 Pour ~20 mL of hexane into the column. Add 5 g of anhydrous sodium sulfate.

Note:
Alternate techniques of preparing the silica gel column may be employed. The ILV lab slurried the silica gel in the column reservoir before allowing it to settle into the column body. Hexanes were used to wash down and silica that adhered to the column sides

9.3 Add 5 grams of activated silica gel to the column and allow it to settle. Tap the column to level the top of the silica gel and to dislodge any sorbent from the walls of the tube. Top off the mixture with 5 g anhydrous sodium sulfate.

9.4 Drain the hexane to about 0.5 cm from the top of the sodium sulfate. Discard eluate.

9.5 Further condition the column by passing another 50 mL of hexane through it. Stop elution when conditioning, solvent has drained to within 0.5 cm of the top of the sodium sulfate. Discard eluate.

9.6 Pass the hexane extract (~5 mL) from Step 8.8 through the column. Stop elution when solvent has drained to within 0.5 cm of the top of the sodium sulfate. Discard eluate.

9.7 Add 20 mL of hexane to the evaporation flask remaining from Step 6. Swirl to rinse the walls of the flask and pass this rinse through the sample-laden column. Stop elution when solvent has drained to within 0.5 cm of the top of the sodium sulfate. Discard eluate...

9.8 Wash the column with 50 mL of ethyl acetate:hexane (1 :99, v/v). Stop elution when solvent has drained to within 0.5 cm of the top of the sodium sulfate. Discard eluate.

9.9 Place a 250 mL evaporation flask under the column. Elute acequinocyl with 75 mL of ethyl acetate:hexane (5:95, v/v). Follow this elution immediately with the elution of acequinocyl-OH and AKM-18 using 60 mL of water:acetonitrile (2:98, v/v), thus combining both eluates.

9.10 Add 0.4 mL of 1% keeper solution to the combined eluates and concentrate to ~0.5 mL on a rotary evaporator at ~30°C.

Note:
Start concentration at ~21-23 in. Hg vacuum and continue at this setting for ~20 minutes. Increase vacuum gradually to ~25 in. Hg and maintain at this setting until concentration is complete (~45-50 minutes).

Alternate vacuum settings may be used at the analyst's discretion.

9.11 Transfer the concentrated extract quantitatively to a 13 x 100mm test tube. Use one 1.0 mL acetone rinse, and two 0.5 mL acetone rinses of the flask to assist in the transfer. Sonicate each rinse prior to transfer to the test tube.

Larger rinse volumes may be used to facilitate transfer.

9.12 Add 0.4 mL of 1% keeper solution and concentrate the solution to ~0.2 mL using an N-Evap evaporator set at ~30°C, then on to dryness, with care, using manual nitrogen blow down.

9.13 Redissolve the residue in 400 µL acetone. Sonicate to insure that all residue is either dissolved or in suspension. Add 400 µL of acetonitrile and sonicate. Finally add 200 µL of 0.4% aqueous formic acid and sonicate. The combination and proportion of solvents used results in a mixture of acetone:acetonitrile:0.4% aqueous formic acid (2:2:1, v/v). Mix well and submit to HPLC analysis. Final concentration of HPLC-ready extract is 1.0 mL = 10.0 g.

Note:
Samples may require a 0.45 µm filtration just prior to HPLC analysis. Instrumentation personnel will filter the solution through a 0.45 µm Nylon 66 filter using a Hamilton Teflon® Luer-lock syringe just prior to HPLC analysis. The independent validation laboratory omitted this step. The use of filters is left to the discretion of the analyst.

10. HIGH PRESSURE LIQUID CHROMATOGRAPHIC ANALYSIS

Note:
The column and conditions stated in the method have been satisfactory for the matrices being analyzed. The specific column packing, mobile phase, column temperature and flow rate listed are typical conditions for this analysis. Alternate columns may be used depending on the need to resolve analyte and/or interfering responses. Specific conditions used will be noted on each chromatographic run and will not otherwise be documented.

10.1 Operating Conditions

Instrument: PE Sciex API 2000 LC/MS/MS system with a Perkin Elmer series 200 autosampler, an integrated Shimadzu chromatograph consisting of (2) LC-10AD*vp* Liquid Chromatograph units and a DGU-14A Degasser. The system is controlled and data processed by PE Sciex Analyst® Software.

HPLC Column: 15 cm X 2.0 mm i.d. Luna Phenyl-Hexyl, 3µ particle size

Mobile Phase: (Fisher Water; Burdick and Jackson Methanol; EM Scientific Formic Acid)

Gradient:

Time (min)	0.1% formic acid in water	0.1% formic acid in methanol
0.0-0.4	45	55
8.0	10	90
12.0-15.5	5	95
16.0-18.0	45	55

Divert Valve: Programmed to divert LC flow from column to waste (by- passing detector) from 0 to 11 minutes and again from 15 to 23.5 minutes. LC flow is directed to detector during the 11 to 15 minute window. Diversion time settings can be adjusted as necessary depending on the retention times of the analytes (i.e., beef liver and fat).

Flow Rate: 300 µL/min.

Interface: APCI (atmospheric pressure chemical ionization)

Ionization Mode: Positive (+)

Acquisition Mode: MRM

Source Temperature: 400 °C

Curtain Gas: Nitrogen @ 35 psi

Collision Gas: Nitrogen @ setting of "4"

Transitions Monitored:

	Ion, M/Z	
Acequinocyl	385 to	189
Acequinocyl-OH	343 to	189
AKM-18	347 to	149

Retention Times:

AKM-18	~11.0 minutes
Acequinocyl-OH	~12.6 minutes
Acequinocyl	~13.8 minutes

Loop Size : 50 µL

Column Temperature: 35 °C

10.2 Sample Analysis

Prepare a four-point standard curve by injecting constant volumes of mixed standard solutions. Use constant volume injections for sample extracts as well. Sample responses not bracketed by the standard curve require dilution and reinjection. Inject a curve check standard every 4-5 sample injections.

11. CALCULATIONS

Calculations for instrumental analysis are conducted using a validated software application to create a standard curve based on linear regression. The regression functions are used to calculate a best fit line (from a set of standard concentrations in µg/mL versus peak response) and to determine concentrations of the analyte found during sample analysis from the calculated best fit line.

The equation used for the least squares fit is:

$$y = mx + b$$

Where:

y	=	peak response
x	=	µg/mL found for peak of interest
m	=	slope
b	=	y-intercept

11.1 The calculations for ppm found and percent recovery (for fortified samples) are:

The amount of analyte, either acequinocyl or acequinocyl-OH (in ppm), found in the sample is calculated according to the following equation:

$$ppm = \frac{\mu g}{mL} \times \frac{HPLC\ final\ vol.(mL)}{sample\ wt.(g)} \times \frac{mL\ ext.solv.}{mL\ aliq.} \times X\ GPC\ dil.factor\ \times HPLC\ dil.factor$$

Where:

µg/mL found	= µg/mL of analyte found from standard curve
Sample weight	= gram weight of sample extracted
mL ext. solv.	= volume of extraction solvent
mL aliq.	= volume of sample extract taken through procedure (typically 300 mL)
HPLC final	

Volume	= volume of final extract submitted to HPLC
HPLC dil. Factor	= dilution of sample extract required to produce an analyte response bracketed by standards

11.2 The percent recovery for fortified control samples is calculated as follows:

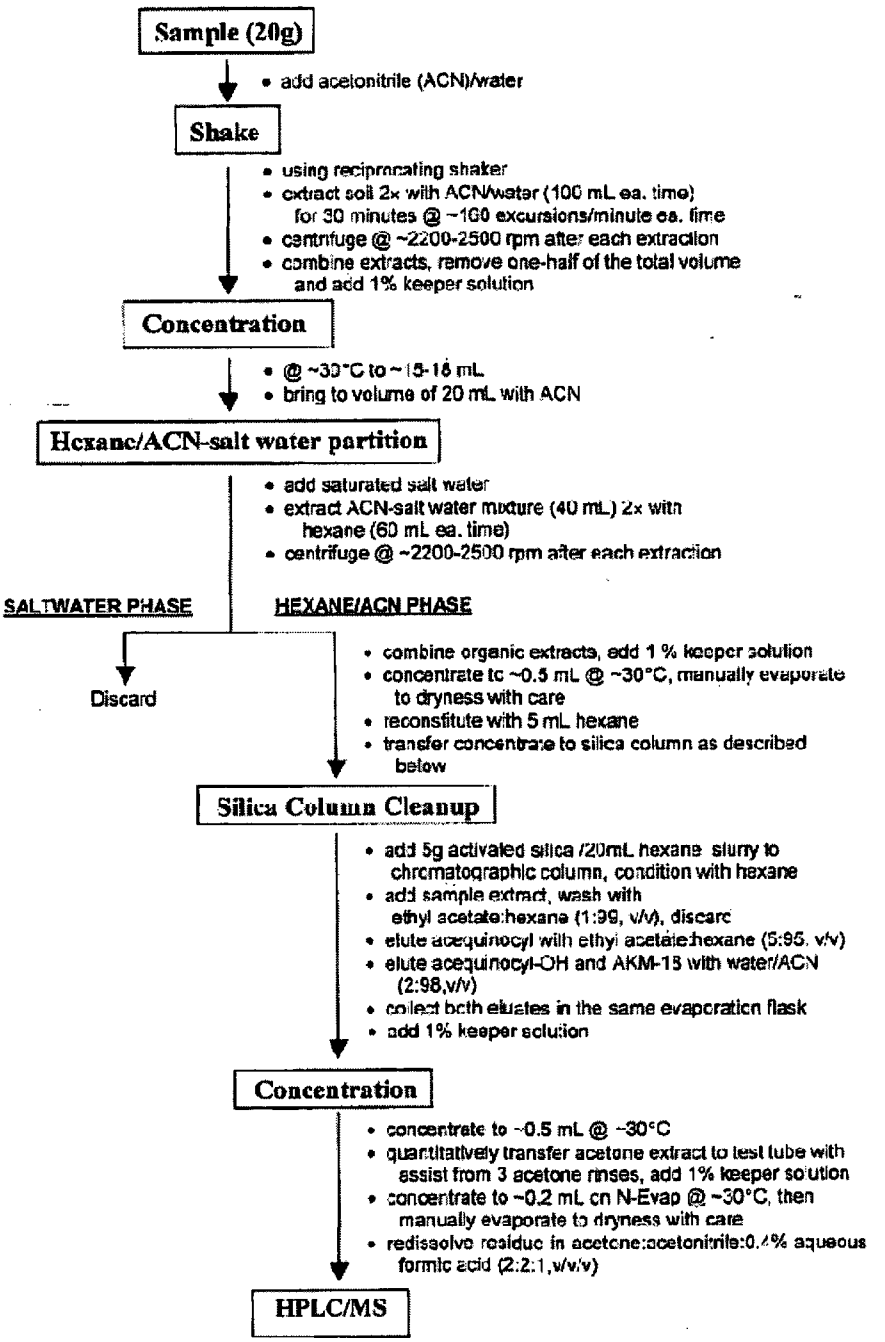
$$\%\ Rec. = \frac{ppm\ found\ in\ fortified\ control\ (spike) -\ ppm\ found\ in\ control}{fortification\ level\ (ppm)} \times 100$$

12. REFERENCES

Morse Laboratories, Inc. Analytical Method # Meth-133, Revision #2, November 22, 2000, *Determination of Acequinocyl And Acequinocyl-OH in Fruit Crops.*

APPENDIX I

FLOWCHART FOR THE DETERMINATION OF ACEQUINOCYL, ACEQUINOCYL-OH AND AKM-18 IN SOIL



APPENDIX II

Silylation of Glassware

Silylation is a process used to chemically treat glassware or other products in order to prevent or minimize binding of analyte residues to the glass surface.

Caution: **Do not allow dimethyldichlorosilane (DMDCS) to come in contact with water. Chlorine gas or hydrogen chloride gas will be produced.**

This procedure must be conducted inside an efficient fume hood. Heavy latex gloves must be worn.

1. Pour a small amount of the 5% DMDCS solution into the glassware to be treated. Stopper bulk container. Rotate the glassware to thoroughly coat the inside surfaces. Pour excess solution into the next piece of glassware to be treated.

   Note: Moisture in the air tends to inactivate this reagent. To insure maximum activity of the silylating agent during the coating process, limit the exposure (to the atmosphere) of the silylating agent to approximately 5 minutes.

2. Allow the treated glassware to dry (approximately 20 minutes). Rinse thoroughly with hexane, then reagent acetone. Again allow to dry.

3. Glassware is now ready for use.

   Notes: Any glassware that is cleaned with a brush after it has been silylated, must be resilylated.

   Store pure DMDCS at room temperature.

   5% solutions of DMDCS in hexane are stable for 5 days when stored well stoppered at room temperature. Choose a storage container with minimum air space above the surface of the solution.

Silylation of Glass Wool:
In a fume hood, while wearing heavy latex gloves, place a quantity of glass wool into a large wide mouth glass jar with Teflon®-lined lid (e.g., ~400 mL capacity). Saturate the glass wool with the silylating solution (using approximately 200 mL). **To assure that thorough saturation has occurred, stir to thoroughly coat the glass wool in the glass jar with a sturdy glass rod.** Cap tightly and allow to stand for approximately 20 minutes.

Decant the silylating solution from the glass jar and while wearing latex gloves, squeeze the excess silylating solution from the glass wool. Pull the wet glass wool apart to aid in drying. Place on clean paper towels in a hood to thoroughly dry. This drying may require as long as 4-8 hours.

When completely dry, return the silylated glass wool into a glass jar and rinse thoroughly three times with hexane followed by three rinses with acetone.

Allow the glass wool to dry on clean paper towels. Store the dry, silylated glass wool in a covered glass container. **The washed and dried silylated glass wool may be used for up to one month.**

APPENDIX III

Quality Control for Silica Gel

Add 50 µL of an appropriate standard or standard mixture @ 10 ,ug/mL (in acetonitrile) to a 50 mL evaporation flask. Add 0.4 mL 1% keeper solution and evaporate to dryness using manual nitrogen blow down. Add 5 mL hexane. Vortex mix. Follow steps 9.1 through 9.13 of the procedure. Final concentration is 0.5 ,ug/mL.