Change Log

DLS Method Code: 6103.01
Procedure Name: Specific Organophosphorous Pesticides, Synthetic Pyrethroids, Selected Herbicides and DEET.

Date	Changes Made	By	Reviewed By	Date Reviewed
02/15/2009	Add Appendix I	Roberto Bravo	DBB	2/15/09

Laboratory Procedure Manual

Analyte: **Specific Organophosphorous Pesticides, Synthetic Pyrethroids, Selected Herbicides and DEET**

Matrix: **Urine**

Method **High-Performance Liquid Chromatography-Atmospheric Pressure Chemical Ionization & Heated Electrospray Ionization Mass Spectrometry**

Method No: 6103.01

Revised:

as performed by:

Organic Analytical Toxicology Branch
Division of Laboratory Sciences
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Important Information for Users

CDC periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated

Division of Laboratory Sciences
Laboratory Protocol

Analyte:	***Specific Organophosphorous Pesticides, Synthetic Pyrethroids, Selected Herbicides and DEET***
Matrix:	**Urine**
Method:	**Solid Phase Extraction and High Performance Liquid Chromatography-Tandem Mass Spectrometry (HPLC-MS/MS).**
Method Code:	**610301-A00**
Branch:	**Organic Analytical Toxicology (OAT)**

Prepared By: Roberto Bravo, Ph.D. _____ Signature _____ 6/1/2007 date

Supervisor: Dana B. Barr, Ph.D. _____ Signature _____ 6/1/07 date

Branch Chief: Larry L. Needham, Ph.D. _____ Signature _____ 8/1/07 date

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1. Clinical Relevance and Summary of Test Principle

In 1999, an estimated 415000 tons of conventional pesticides were applied in the United States. The most commonly used pesticide for home and garden was the herbicide 2, 4-D with an estimated use of 3000-4000 tons in those applications, giving an average domestic use per capita of ~ 15g/year. During the same year, the most abundantly applied pesticide in U. S. agriculture was atrazine, with application of 35000 tons. The widespread use of pesticides and the scientific interest in potential adverse health effect of pesticides exposure have increased the demand for fast and robust analytical methods for measuring markers of possible pesticides.

This method assesses human exposure to select pesticide metabolites of non-persistent pesticides. It does not directly test for any disease. This method includes **specific organophosphorous insecticide metabolites** (5-chloro-1-isopropyl-[3H]=1, 2, 4-triazol-3-one; 2-diethylamino-6-methyl pyrimidin-4-ol; 2-isopropyl-6-methyl-4-pyrimidiol; 3-chloro-4-methyl-hydroxycoumarin; 2-[(dimethoxyphosphorothioyl) sulfanyl] succinic acid; 3, 5, 6-tricholor-2-pyridinol; 4-nitrophenol, **synthetic pyrethroids** (3-[(1Z)-2-carboxyprop-1-en-1-yl]-2,2-dimethylcyclopropanecarboxylic acid; 3-phenoxybenzoic acid; 4-fluoro-3-phenoxybenzoic acid; cis-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane-1-carboxylic acid; trans-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane-1-carboxylic acid; trans-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane-1-carboxylic acid), **herbicides** (Acetochlor mercapturate; alachlor mercapturate; atrazine mercapturate; metolachlor mercapturate; 2,4-dichlorophenoxyacetic acid; 2,4,5-trichlorophenoxyacetic acid), and an **insect repellant** N, N-diethyl-m-toluamide (DEET)**.** The analytes are extracted and concentrated from the urine matrix using solid phase extraction cartridges, separated through high pressure liquid chromatography, and analyzed on a triple quadrupole mass spectrometer.

Table1.
Analytes Measured, their Parent and Class Pesticides

Analyte	Parent Pesticide	Pesticide Class
2-isopropyl-4-methyl-pyrimidinol **(IMPY)**	Diazinon	Organophophorous
Malathion dicarboxylic acid **(MDA)**	Malathion	Organophosphorous
3-phenoxybenzoic acid **(3-PBA)**	Permethrin, cypermethrin, cyfluthrin, others	Pyrethroid
Para-nitronphenol **(PNP)**	Methyl parathion, parathion	Organophosphorous
5-chloro-1,2-dihydro-1-isopropyl-[3H]-1,2,4-triazol-3-one **(CIT)**	Isazaphos, isazaphos methyl	Organophosphorous
3-chloro-4-methyl-7-hydroxycoumarin **(CMHC)**	Coumaphos	Organophosphorous
2-diethylamino-6-methyl pyrimidin-4-ol **(DEAMPY)**	Pirimiphos methyl, pirimiphos	Organophosphorous
3,5,6-tricholor-2-pyridinol **(TCPY)**	Chlorphrifos, chlorpyrifos methyl	Organophosphorous
2-[(dimethoxyphosphorothioyl) sulfanyl] succinic acid **(CDCA)**		Pyrethroid
4-fluoro-3-phenoxy-benzoic acid **(4F-3PBA)**	Cyfluthrin	Pyrethroid
Cis-dichlorovinyl-dimethylcyclopropane carboxylic acid **(cis-DCCA)**	Permethrin, cypermethrin	pyrethroid
Trans-dichlorovinyl-dimethylcyclopropane carboxylic acid **(trans-DCCA)**	Permethrin, cypermethrin	Pyrethroid
Cis-dibromovinyl-dimethylcyclopropane carboxylic acid **(DBCA)**	Deltamethrin	Pyrethroid
N,N-diethyl-m-toluamide **(DEET)**	DEET	Insect Repellant
2,4-dicholorphenoxyacetic acid **(2,4-D)**	2,4-D	Herbicide
2,4,5-tricholorphenoxyacetic acid **(2,4,5-T)**	2,4,5-T	Herbicide
Alachlor mercapturate **(ALA)**	Alachlor	Herbicide
Atrazine mercapturate **(ATZ)**	Atrazine	Herbicide
Metolachlor mercapturate **(MET)**	Metolachlor	Herbicide
Acetochlor mercapturate **(ACE)**	Acetochlor	Herbicide

2. Safety Precautions

a. Reagent Toxicity or Carcinogenicity

The reagents can be both toxic and carcinogenic. Special care should be taken to avoid inhalation or dermal exposure to the acids and solvents necessary to carry out the procedure.

b. Radioactive Hazards

None

c. Microbiological Hazards

Although urine is generally regarded as less infectious than serum, the possibility of exposure to various microbiological hazards exists. Take appropriate measures to avoid contact with the specimen (see “Protective equipment” below). A hepatitis B vaccination series is usually recommended for health care and laboratory workers who are exposed to human fluids and tissues. Observe universal precautions.

d. Mechanical Hazards

Following standard safety practice while performing this procedure minimizes the risk for mechanical hazards. Avoid any direct contact with the electronic components of the mass spectrometer unless all power to the instrument has been shut off. Only qualified technicians should perform electronic maintenance and repair.

e. Protective Equipment

Use standard personal protective equipment when performing this procedure. Wear a lab coat; safety glasses; and if appropriate, durable gloves. Use chemical fume hood for this procedure.

f. Training

Anyone performing this procedure must be trained and experienced in the use of a triple-quadrupole mass spectrometer. Formal training is not necessary; however, personnel should be trained appropriately by an experienced operator of the instrument.

g. Personal Hygiene

Be careful when handling any biological specimen. Use gloves and wash hands properly.

h. Disposal of Wastes

Always dispose of solvents and reagents in an appropriate container clearly marked for waste products, and temporarily store them in a flame-resistant cabinet (follow CDC's guidelines entitled Hazardous Chemical Waste Management) containers, glassware, etc., that come in direct contact with the specimens. Autoclave or decontaminate with 10% bleach. Wash the glassware and recycle or dispose it in an appropriate. Computerization; Data-System Management

3. Computerization: Data-System Management

a. Software and Knowledge Requirements

A database named **PSTARS** was developed on the (DLS-PC) network using Microsoft Access. This database is used to store, retrieve, and analyze data from the pesticide-residue analyses. Perform statistical data analyses using Statistical Analysis System (SAS) ® software packages (or their equivalent) are required to use and maintain the data-management structure.

b. Sample Information

Electronically transfer or manually enter into the database information pertaining to particular specimens. If you manually enter data, include the sample-identification (ID) number, the notebook number associated with the sample preparation, the sample type, the standard number, and any other information not associated with the mass-spectral analysis. Electronically transfer the analytical information

obtained from the sample to the database via a PC-based instrument interface. Then transfer the data electronically into the database.

c. Data Maintanance

See method 6112.01 for data handling, maintenance, and management procedures.

4. Specimen Collection, Storage and Handling Procedures; Criteria for Specimen Rejection

a. Sample Handling

Urine can be collected in standard urine collection cups. Samples should be refrigerated as soon as possible and transferred to specimen vials within 4 hours of collection. If possible, collect at least 10-mL of urine and pour it into sterile vials (30-mL Qorpak ® vials with screw-caps tops are suggested). Label the specimens, freeze them to ≤-20 ^{o}C, and ship them on dry ice. Carefully pack vials to avoid breaks during shipment. Store all samples at ≤-20 ^{o}C until analysis.

b. Sample Rejection

Reject specimens with volumes less than 0.1-mL because they cannot be reliably processed.

5. Procedures for Microscopic Examinations; Criteria for Rejecting Inadequately Prepared Slides

Not applicable for this procedure.

6. Preparation of Reagents, Calibrators (Standards), Controls, and All Other Materials; Equipment and Instrumentation

a. Reagents and Sources

Table 2.
Reagents and their Suggested Manufactures

Reagents	Suggested Manufacturers
Acetonitrile	Tedia Company Inc
Acetone	Tedia Company Inc
Methanol	Tedia Company Inc
Glacial Acetic Acid	JT Baker
Deionized water	NANOpure Infinity ultrapure water system
Sodium Acetate	Anachemia Chemicals NY
Hexane	Tedia Company Inc
β-glucuronidase	Sigma-Aldrich Co.
Oxypyrimidine-methyl-4,5,6	Cambridge Isotope Labs
Malathion Dicarboxylic Acid	EPA-Pesticide Repository
3-phenoxybenzoic acid	Sigma-Aldrich

4-nitrophenol	Sigma-Aldrich
5-chloro-1,2-dihydro-1-isopropyl-[3H]-1,2,4-trizaol-3-one	Cambridge Isotope Labs
3-chloro-4-methyl-7-hydroxycoumarin	Cambridge Isotope Labs
2-diethylamino-6-methyl pyrimidin-4-ol	Cambridge Isotope Labs
3,5,6-trichloro-2-pyridinol	Chem Service
2-[(dimethoxyphosphorothioyl) sulfanyl] succinic acid	Los Almos National Labs
4-fluoro-3-phenoxy benzoic acid	Aldrich
Cis-dichlorovinyl-dimethylcyclopropane carboxylic acid	Cambridge Isotope Labs
Trans-dichloro-dimethyl-cyclopropane carboxylic acid	Cambridge Isotope Labs
Cis-dibromovinyl-dimehtylcyclopropane carboxylic acid	Cambridge Isotope Labs
N,N-diethyl-m-toluamide	Cambridge Isotope Labs
2,4 Dichlorophenoxyacetic Acid	Cambridge Isotope Labs
2,4,5-trichlorophenoxyacetic acid	Cambridge Isotope Labs
Alachlor Mercapturate	Cambridge Isotope Labs
Atrazine Mercapturate	Cambridge Isotope Labs
Metolachlor mercapturate	Cambridge Isotope Labs
Acetochlor mercapturate	Cambridge Isotope Labs
Oxypyrimidine-methyl-4,5,6-$^{13}C_4$	Cambridge Isotope Labs
D_7 Malathion Dicarboxylic Acid	Cambridge Isotope Labs
Phenyl-$^{13}C_6$-3-phenoxybenzoic acid	Cambridge Isotope Labs
$^{13}C_6$-paranitrophenol	Cambridge Isotope Labs
$^{13}C_6$-5-chloro-1,2dihydro-1-isopropyl-[3H]-1,2,4-triazol-3-one	Cambridge Isotope Labs
$^{13}C_6$-3-chloro-4-methyl-7-hydroxycoumarin	Cambridge Isotope Labs
$^{13}C_6$-2-diethylamino-6-methyl pyrimidin-4-ol	Cambridge Isotope Labs
$^{13}C_5$-^{15}N-3,5,6-tricholor-2-pyridinol	Chem Service
$^{13}C_6$ 2,4-dicholorphenoxyacetic Acid	Cambridge Isotope Labs
2-[(dimethoxyphosphorothioyl) sulfanyl] succinic acid	Los Almos National Labs
D_6-cis/trans-dichlorovinyl-dimehtylcyclopropane carboxylic acid 1:1	Ehrenstorfer
Cis-dibromovinyl-dimehtylcyclopropane carboxylic acid	Cambridge Isotope Labs
$^{13}C_6$-N,N-diethyl-m-toluamide	Cambridge Isotope Labs
2,4-Dichlorophenoxyacetic-ring-$^{13}C_6$	Cambridge Isotope Labs

$^{13}C_6$-2,4,5-trichlorophenoxyacetic acid	Cambridge Isotope Labs
Alachlor acetylcysteine Adduct-ring-$^{13}C_6$	Cambridge Isotope Labs
Atrazine Mercapturate-ring-$^{13}C_3$	Cambridge Isotope Labs
Metolachlor Mercapturate-ring-$^{13}C_6$	Cambridge Isotope Labs
Acetochlor Mercapturate-ring-$^{13}C_6$	Cambridge Isotope Labs

b. Reagent Preparation

Liquid chromatography mobile phases:

Mobile Phase A= 0.1% Acetic Acid in aqueous solution. For example, pipette 1 mL of Acetic Acid in 1000 mL in deionized water and mix. Mobile Phase B= 100% Acetonitrile.

0.2 M Buffer Acetate Enzyme Solution:

To hydrolyze possible glucoronide or sulfate conjugated metabolites in urine samples, β glucuronidase type H-1 from *Helix pomatia* (Sigma Chemical Co., St, Louis, MO). To each sample an amount of enzyme giving 1000 units of activity dissolved in 1.5 mL of 0.2 Acetate buffer. For example, to prepare 1L of the buffer acetate, combine 9,7 g of sodium acetate, 3.1 mL of glacial acid acetic, and 1 l of deionized water.

c. Standard Preparation

1) Stock Solutions of Analytes (200 ng/mL)

Individually weigh an appropriate amount of each analyte in a volumetric flask (for example 5 mg in a 25 mL flask). Add a few milliliters of acetonitrile to the flask and the swirl the flask to dissolve. Once the analytes are dissolved, dilute the contents of the flask to volume with acetonitrile and mix. Divide stock solutions into aliquots (for example, 10 mL) and place them in ampules or vials. If ampules are used, flame-seal the ampules and store them at ≤-20 ^{o}C.

2) Stock Solutions of Labeled Isotopes (200 ng/mL)

Individually weigh an appropriate amount of each analyte in a volumetric flask. Add a few milliliters of acetonitrile to the flask and the swirl the flask. Once the analytes are dissolved, dilute the contents of the flask to volume with acetonitrile and mix. Divide stock solutions into aliquots (for example, 5-mL aliquots) and place them in ampules. Flame-seal the ampules and store them at ≤-20 ^{o}C.

3) Working Labeled Isotopes Solution (ISTD)

Make the working labeled stock isotopes solution by adding an aliquot of each of the stock solutions into a 50 mL volumetric flask and dilute contents of the flash with acetonitrile. Calculate concentration so that 50 µL aliquot of working solution in 2 mL of urine gives the concentration of 12 ppb (ng/mL).

4) Working Standard Solutions

Make nine solutions of varying concentrations ranging from 0.03 ppb to 8.0 ppb from the stock analyte solutions. Add an aliquot of the stock solutions of each analyte in

acetonitrile to make the standard. Calculate concentrations so that a 50 µL aliquot of working standard in 2 mL of urine gives the desired standard concentration (ranging from 0.39 to 100 ppb in urine)

5) Calibration-Verification Materials

CLIA defines testing calibration materials as "a solution which has a known amount of analyte weighed in or has a value determined by repetitive testing using a reference or definitive test method". According to this definition, our quality control (QC) materials qualify as calibration verification materials.

6) Proficiency-Testing Standards (PT 1-3)

Proficiency testing materials are matrix-based samples (typically spiked samples) with a known or characterized concentration. These samples may be spiked or have endogenous levels of the target analytes.

d. Equipment/Supplies

1) TurboVap® LV evaporator (Zymark Corporation, Framingham, MA).
2) Sartorius Ultramicro® Microbalance (Westbury, NY).
3) 15-mL, conical tubes (Kimble Glass Inc).
4) EDP2® pipettes (Rainin Instrument Co. Woburn, MA).
5) Presterilized filter pipette tips (Rainin)
6) Vacuum Box – Supelco Visiprep 24TM DL
7) Vortex Genie® vortex mixer (Scientific Industries Inc., Springfield, MA).
8) Qorpak bottles (Lab Depot, Inc., Cumming, GA).
9) Vials with screw caps SN ST 23x85 (Durham)
10) OASIS® HLB 3cc Extraction Cartridges (Waters)
11) Disposable Flow Control – Valve Linear for the Visiprep TM – LD (Supelco)
12) Micro auto sampler vials with insert (Kimble Glass Inc)
13) Compressed nitrogen, helium, and argon, liquid nitrogen (Holox Ltd)

e. Instrumentation

The Finnigan TSQ Quantum, a high resolution triple-quadrupole mass spectrometer (ThermoFinnigan, San Jose, CA) is equipped with a syringe pump, a divert/inject valve, an atmospheric pressure ionization (API) interface, a heated electrospray ionization (H-ESI) interface, and the Xcalibur data system. Also, the TSQ quantum is connected to the Agilent 1100 Series LC System with a degasser, a quaternary pump and a heated column compartment and an autosampler. The entire system is controlled via ThermoFinnigan software Xcalibur.

1) Mass-Spectrometer TSQ Quantum

- Interfaces used: atmospheric pressure ionization (APCI) and heated electrospray ionization (H-ESI)
- Scan mode: Selective reaction monitoring (SRM), product ion scan
- The instrument needs to be calibrated and tuned every 3-6 months) for accurate results. Also after each calibration a gain check (tuning of the multiplier) is needed.
- The instrument needs to be calibrated and tuned periodically for better applications.

- Table 4 shows an example of the optimized tuning parameters for the two instrument methods.
- Table 5 shows the optimized precursor/product ion pairs as well as the collision off-set energy for the target compounds.

Table 4.
Tuning Parameters

MS Parameter	Setting	
Ionization type	APCI	H-ESI
Ion polarity mode	Positive mode	Negative mode
Heated capillary	270º C	250º C
Vaporizer temperature	450º C	150º C
Sheath gas pressure	25	37
Discharge current	4.0	4.5
Aux Gas Pressure	5	10
Collision gas	Argon at 1.5 mT	Argon at 1.5 mT

Table 5a
Precursor/Product ion pairs and Collision off-set Energies for Positive Mode

Analyte	Precursor (m/z)	Product (m/z)	Collision Energy (V)
IMPY-Q	153	84	26
IMPY-C	153	70	27
IMPY-L	157	88	26
DEAMPY-Q	182	154	25
DEAMPY-C	182	84	32
DEAMPY-L	188	158	25
CIT-Q	162	120	20
CIT-C	164	122	20
CIT-L	169	121	19
ATZ-Q	343	214	27
ATZ-C	343	172	37
ATZ-L	346	217	27
ALA-Q	365	162	27
ALA-C	365	130	10
ALA-L	371	168	27
ACE-Q	351	130	10
ACE-C	351	148	28
ACE-L	357	130	10
DEET-Q	192	119	26
DEET-C	192	91	38
DEET-L	198	91	38

("Q" = Quantification ion; "C" = Confirmation ion)

Table 5b
Precursor/Product ion pairs and Collision off-set Energies for Negative Mode

Analyte	Precursor (m/z)	Product (m/z)	Collision Energy (V)
MDA-Q	273	141	16
MDA-C	273	157	27
MDA-L	280	147	16
CDCA-Q	197	69	25
CDCA-C	197	97	16
CDCA-L	204	99	22
PNP-Q	138	108	32
PNP-C	138	92	32
PNP-L	144	114	23
MET-Q	409	280	22
MET-C	409	150	35
MET-L	415	286	22
CMHC-Q	209	145	27
CMHC-C	209	173	21
CMHC-L	213	148	27
2,4-D-Q	219	161	19
2,4-D-C	221	163	19
2,4-D-L	225	167	19
TCPY-Q	196	196	7
TCPY-C	198	198	7
TCPY-L	202	202	7
2,4,5-T-Q	253	195	21
2,4,5-T-C	255	197	21
2,4,5-T-L	261	203	21
*Cis &Trans*_DCCA-Q	207	207	7
*Cis &Trans*_DCCA-C	209	209	7
*Cis &Trans*_DCCA-L	210	210	7
4F-3-PBA-Q [(1)]	231	93	42
4F-3-PBA-C [(1)]	231	187	19
3-PBA-Q	213	93	42
3-PBA-C	213	169	17
3-PBA-L	219	99	42
DBCA-Q	294	79	31
DBCA-C	296	79	31
DBCA-L	303	79	31

("Q" = Quantification ion; "C" = Confirmation ion)
(1) 3-PBA-L was used as Internal Standard

2) Agilent 1100 Series LC System

Chromatographic separation is performed using an Agilent 1100 Series Capillary LC System composed of a Capillary Pump, Micro Vacuum Degasser, Thermostatted Micro Autosampler, and Thermostatted Column Compartment.

7. Calibration and Calibration-Verification Procedures

a. Calibration Plot

1) Construct a-point calibration plot by performing a linear regression analysis of relative response factor (i.e., area native/area label) versus standard concentration.

Perform a minimum of five repeat determinations are performed for each point on the standard curve.

2) The lowest point on the calibration curve is at or below the measurable detection limits and highest point is above the expected range of results.

3) Determine the slope and intercept of this curve is by linear least squares fit using SAS® software.

4) R-squared values for the curve must be greater than 0.99. Linearity of standard curves should extend over the entire standard range. Intercepts, calculated from the least squares fit of the data, should not be significantly different from 0; if they are, identify the source of this bias.

5) Periodically recalculate the standard curve to incorporate the newest data points. Whenever you prepare a new, combined, labeled-isotope solution, re-establish the standard curve.

b. Verification of Calibration

Calibration verification for the overall test system is performed by analyzing three concentrations of calibration verification materials (which are included in each run as QC materials) in the same manner as unknown samples. The concentrations span the useable range of the method but are weighted toward the expected unknown concentrations. The overall system is considered calibrated if the calculated concentrations are within ±3 standard deviations of the characterized mean values for each of the analytes measured in the method. Calibration verification of the overall test system is performed with every analytical run. No data are reported unless the overall test system passes calibration verification. Calibration results and any significant corrective actions are documented either in the PSTARS database or other ancillary documents.

In addition, eight calibrators which span the linear range of the method are run with each analytical run which satisfies the requirements for calibration verification according to the Clinical Laboratory Improvements Amendments (CLIA) brochure #3 which states, “If the test system’s calibration procedure includes three or more levels of calibration materials, and includes a low, mid, and high value, and is performed at least once every six months, then the requirement for calibration verification is also met.”

c. Proficiency Testing

Proficiency testing should be performed a minimum of once every 6 months. Because no formal PT testing program exists for the target analytes of this method, an in-house program is used. This in-house program currently includes pools prepared in-house or individually spiked but may also include independently prepared materials whose preparation was contracted out to an external laboratory. Where applicable, NIST matrix-based certified reference materials may be included as PT materials. Five randomly selected PT materials will be analyzed in the same manner as unknown samples. These PT

materials will be selected from among three different concentration ranges spanning the linear range of the method. The concentration range for each sample will be blinded to all analysts. The analytical results are evaluated by an auditor (e.g., branch statistician) who is independent of the laboratory performing the analyses. A passing score is obtained if at least four of the five samples fall within the prescribed limits established by the auditor. The auditor will notify our laboratory of its PT status (i.e. pass/fail). If a PT challenge is failed, a second attempt to demonstrate proficiency by analyzing a second set of PT samples is undertaken. If the second attempt fails, laboratory operations will cease until an appropriate corrective action is taken. After corrective action is taken, laboratory operations can resume.

8. Operating Procedures; Calculations; Interpretation of Results

a. Analytical Runs

Allow the following to thaw and reach room temperature: unknown urine samples (usually 20), blank urine, three QC samples, and two blank urines used for standards.

b. Sample Preparation

Two mL of urine are pippetted into round bottom tubes with screw caps and spiked with 50 µl of the labeled internal standard stock solution. The urine samples are vortexed and to that mix 1.5 mL of acetate/β-glucaroindase buffer solution is added. The samples are then placed in an incubator at 37°C for at least 6 hours. Samples are removed from incubator and allowed to come to room temperature. Samples are run thru Oasis® 3cc cartridges that are conditioned with Acetone and 1% Acetic Acid in water solutions. Cartridges are washed with 1.5 mL of a solution of 5% 1:5:94 Acetic Acid/MeOH/dH_2O. Cartridges are dried for 5 minutes. Cartridges are then eluted with 3.0mL of Acetone and 3.0mL of Hexane. Samples are blown to dryness in TurboVap® LV evaporator (40°C, 10-12 psi). Sample tubes are vortexed with 2.0mL Acetonitrile and blown to dryness and again with 0.5mL Acetonitrile. Samples are finally reconstituted with 100 µl 1:1 ACN:water mixture. Samples are stored in labeled HPLC autosampler vials and in a freezer if not analyzed immediately.

c. Liquid Chromatography Conditions

For analytes analyzed in negative H-ESI mode the chromatographic separation is done on a Betasil C-18, narrow-bore 4.6 x 100-mm, 3.0 µm (ThermoElectron Corporation) column using a gradient eluent program. The solvent used are; HPLC grade water with 0.1% acetic acid in an aqueous solution (Solvent A) and acetonitrile (Solvent B). The gradient program follows as: t=0 min – 65% solvent A and 35% solvent B, t=3 min – 60% solvent A and 40% solvent B, t=4 min – 55% solvent A and 45% solvent B, t=7 min – 50% solvent A and 50% solvent B, t=9min – 35% solvent A and 65% solvent B, t=10 min – 25% solvent A and 75% solvent B, t=11min – 15% solvent A and 85% solvent B, t=12-13 min – 0% solvent A and 100% solvent B, and 3 min for equilibration at 65% solvent A and 35% solvent B before the next injection. The column used is a Betasil C-18, narrow-bore 4.6 x 100-mm, 5.0 µm (ThermoElectron Corporation). The analytes elute between 2 and 12 minutes. The run time for each analyte is 16.5 minutes. The flow rate is 700 µl/min and the injection volume is 10 µl. The divert valve

is programmed to go to waste for the first two minutes and the last three minutes of the run.

For analytes analyzed in positive APCI mode the chromatographic separation is done on an Aquasil C-18, narrow-bore 4.6 x 100-mm, 5.0 µm (ThermoElectron Corporation) column using a gradient eluent program. The solvent used are; HPLC grade water with 0.1% acetic acid in an aqueous solution (Solvent A) and acetonitrile (Solvent B). The gradient program follows as: t=0 min – 75% solvent A and 25% solvent B, t=2 min – 75% solvent A and 25% solvent B, t=2.5 min – 70% solvent A and 30% solvent B, t=3 min – 65% solvent A and 35% solvent B, t=4min – 58% solvent A and 42% solvent B, t=5 min – 50% solvent A and 50% solvent B, t=6min – 42% solvent A and 58% solvent B, t=7 min – 32% solvent A and 68% solvent B, t=9 min – 32% solvent A and 68% solvent B, t=9.5-10.5min – 0% solvent A and 100% solvent B and 3 min for equilibration at 75% solvent A and 25% solvent B before the next injection. The column used is an Aquasil C-18, narrow-bore 4.6 x 100-mm, 5.0 µm (ThermoElectron Corporation). The analytes elute between 2 and 10 minutes. The run time for each analyte is 12.0 minutes. The flow rate is 750 µl/min and the injection volume is 10 µl. The divert valve is programmed to go to waste for the first two minutes and the last three minutes of the run.

d. Mass Spectrometry Conditions

1) Sequence Setup

- Open Xcalibur - Xcalibur allows to import data that has been created by Microsoft Excel.
- To import a sequence: Choose **File > Import Sequence** to open the Import Sequence dialog box.
- Use the Browse button to select the file for import.
- In the select Columns to Import group box, select the sequence columns to be included in the sequence file.
- Click on **OK** to import the sequence: Xcalibur displays the imported file in Sequence Setup.
- Manually define the following parameters:
- Path → Enter the directory path where the sample's raw file will be stored;
- Inst. Meth → Enter the path and filename of the instrument method file; and
- Proc Method → Enter the path and filename of the processing method file.
- For Save File: Choose **File > Save AS**.

2) Autosampler

On the Agilent 1100 Autosampler, place the vials into the sample tray, according the position on the sequence file. The tray will hold 100 vials arranged in 10 rows of 10 samples.

The first vial position is 1 and the last position is 100.

Ensure that the solvent reservoir contains sufficient running solvent for all the samples and that the solvent rinse reservoir contains enough of the rinse solvent (100% Acetonitrile) for a complete rinse.

3) Gases

Ensure that there is enough N2 gas (utilized as the sheath and auxiliary gases) and argon (for the collision induced dissociation gas – CID).

4) Run the sequence

- In Tune Master, click on the On/Standby button on the Control/Scan Mode toolbar to turn on the mass spectrometer.
- On the Control/Scan Mode toolbar, click on the AS/LC Direct Control Button to display the Inlet Direct Control view.
- Set up the Agilent 1100 pump to deliver the initial condition of the instrument method you are about to run.
- Let the instrument and pump system to be on conditioning for about 30 minutes before start running samples. Always run a standard check before running real samples.
- Highlight the samples you want to run. Click on the left-most column of the first sample and drag to the last sample on the sequence.
- Choose Actions > Run Sequence or click on the Run Sequence toolbar button. The Run Sequence dialog box is then displayed.
- On the Sequence dialog box on the After Sequence Set System select (STANDBY) to put the instrument in standby mode and turn off the pump after the last sample has been analyzed.
- Click on OK to start to analyzed the run.
- Always run a probe wash and column wash program after the last sample in the sequence.

e. Processing data

- To process a batch of samples:
- Select the rows to be processed from the current sequence → Click on the left-most column of the first sample and drag to the last sample on the sequence.
- Choose Actions>Bach Reprocess or click on the Bach Reprocess toolbar Button to display the Batch Reprocess Setup Dialog box.
- Select the Quan check box and select the Peak Detection & Integration and Quantification boxes.
- Click on OK. Xcalibur initiates batch reprocessing of the select samples.

f. Quantification

- After processing the sample batch, manually evaluate for correct peak detection and baseline selection in the Quan® browser.
- Export data files to EXCEL® using the long report format.
- Quan Browser allows: to view quantitative results, to evaluate standard curve, QCs and unknowns samples, to integrate chromatogram peaks manually, and to analyze detailed quantification information.
- To start Quan browser:
- Click on the Quan Browser icon on the Home Page.
- Quan Browser displays an Open dialog to select an exiting file (.SLD).
- Xcalibur displays the View Sample Types dialog box that offers the following viewing options to choose:

- Show standard and QC samples types and Show All Samples types.
- Click on OK to start the session.
- Save the settings in a Quan Browser file (*.XQN). Choose File>Save As.
- Export data files to EXCEL → File > Export Excel > Long Report.

g. Rearrangement of Data Files

Data are automatically rearranged into a single worksheet (Excel format)® that is compatible with our existing database using an Excel® macro. This macro also allows for analyst evaluation of quantification and confirmation ions.

h. Transfer of Data

Transfer the file to a mobile drive disk.

i. Importation of Data into Database

Select “Import new data” option in database. A password is required to import the data.

j. Statistical analysis and interpretation of data

Export data from the database to a fixed ASCII file and import it into SAS®. SAS® programs for calibration plot generation, QC analysis, blank analysis, limit-of-detection (LOD) determination, unknown calculations, data distribution, etc., have been created and may be executed in SAS® when this information is needed.

k. Routine and Periodic Maintenance of Key Components

1) Routine Maintenance

a) Mass Spectrometer

Flush the APCI/H-ESI probe at the end of each working day by flowing a wash (100% Acetonitrile), a probe-wash (50:50 methanol: water solution), and a condition (initial conditions-probe specific) from the LC through the APCI/H-ESI source.

b) Agilent 1100 Autosampler

The autosampler requires only a few simple maintenance procedures to keep it in optimum working condition.

- Checking the solvent tubing and connections for leaks
- Clearing a plugged line or needle
- Replacing column filter
- Check needle position into vials.

Ensure that the solvent reservoir contains sufficient running solvent for all samples and that the solvent rinse reservoir contains enough of the rinse solvent (100% Acetonitrile) for a complete rinse.

c) Column, pre-filters and tubing

- Clean the column after each working day by flushing pure acetonitrile through it for at least 30 min at the flow rate were you normally operate.
- Replacing pre column filter as needed

2) Periodic Maintenance

In general, these maintenance procedures are performed if you detect a decrease in the system performance (sensitivity or S/N ratio) without any other apparent technical reasons.

I. Calibration

- Place the LC/MS System in Standby
- Install the ESI Probe

Use the following procedure to set up the syringe pump for introducing tuning and calibration solution into ESI source:

- Install a sample transfer line between the syringe holder unit on the instrument and the sample inlet on the ESI probe.
- Load a clean, 500 µL Hamilton® syringe with 420 µL of the polytyrosine-1, 3, 6 tuning and calibration solution.
- Insert the tip of the syringe needle into the end of the Teflon® tube on the syringe adapter assembly. Make sure to push the handle of the syringe holder down until it comes in contacts the syringe plunger.

Set up the mass spectrometer for tuning and calibration as follows:

- In Quantum Tune Master, click on the On/Standby button on the Control/Scan Mode toolbar to turn on the mass spectrometer. (The Tune Master is automatically placed in the ESI source mode.)
- Open the tune file called “posESI calibration parameters” (Capillary temperature 2700C, Sheath gas pressure 5 psi, Auxiliary gas pressure 5 psi, spray voltage 4.0 kV). Also make sure the mass spectrometer is put in positive ion mode.
- Choose Setup>Syringe Pump & Sample Loop to set up the Syringe pump; specify flow rate (5-10 µL/min), syringe type (Unimetrics or Hamilton) and syringe size (10-500 µL).
- Start off by monitoring the three dominant peeks that the polytyrosine-1, 3, 6 tuning and calibration solution will produce in full scan mode by clicking on the Instrument Method Development Workspace button and select: Scan Type→ Full Scan; Scan Mode→ Q1MS; Scan Range/Entry Mode → FM/LM; enter First mass → 150 and Last mass → 1050; PeakWidth/Q1 box → enter 0.70; Scan time enter 0.70; Tack the Data Processing box → Average. Confirm AutoSIM box is not selected and that Micro Scans is set to 1 and Accurate Mass Mode is OFF. Click Apply.
- Three dominant peeks m/z 182.082 (polytyrosine monomer), 508.2080 (polytyrosine trimer) and 997.3980 (polytyrosine hexamer) should now be visible. Make sure that these three really are the dominant peaks in their m/z range. Do not continue unless they are. Use a new calibration solution if needed.
- Click on the Creep toolbar button to normalize the spectrum. The Y-axle will now show relative intensities. Also make sure that the intensities are in their 106- 107.

Establish a stable ion beam:

- Zoom in to look at mass m/z 508.2080. Click on the Instrument Method Development Workspace button Select: Scan Type→ Full Scan; Scan Mode→ Q1MS; Scan Range/Entry Mode → Center Mass; enter Center mass → 508.208 and Scan With → 10.000; PeakWidth/Q1 box → enter 0.70; Scan time enter 0.20; Tack the Data Processing box → Average. Confirm AutoSIM box is not selected and Micro Scans is set to 1 and Accurate Mass Mode is OFF. Click Apply.
- Click on the Creep toolbar button to normalize the spectrum. Look at the m/z 508 peek and make sure it does not change more than 30% in intensity from scan to scan. Also make sure that the peek is nicely gauss shaped not split in the top. These are indications of whether the ion beam produce are stable or not.

Dealing with an unstable ion beam:

- Adjust the sheath and/or auxiliary gas pressure.
- Move the ESI needle tip closer or further away from the capillary transfer tube opening. (There are knobs on the ion max source housing to move the needle tip in x- and y-axis.)

When establishing a stable ion beam:

- Click on System Tune and Calibration Workspace button and select Auto Tune-Calibration (to specify a full tune and calibration) and Both (to tune and calibrate in both Q1 and Q3).
- Click on Start to start the automatic calibration.

When tune and calibration has completed successfully

- Click on Accept (if calibration went through), and then Save Calib. As (to save the created tune and the calibration files). Finally click on Print (to print the data from the calibration).
- Click on Gain to perform a tuning of the multiplier. When gain check has been completed successfully, add these new settings into the calibration file just created. In Quantum Tune Master save under File>Save Calibration As>.
- Finnish calibration by flushing tubing and probe with a (50:50) solution of HPLC-grade methanol and water.

a) Creating Tuning Files in ESI, H-ESI, and APCI mode.

- Place the LC/MS System in Standby
- Install the appropriate probe
- Install a T-connection so mobile phase from the LC-pump and liquid from the syringe can be mixed up before entering the probe.
- Open the Quantum Tune Master.
- Turn on the Instrument on the On/Standby button and the Control/Scan Mode toolbar
- Turn on the LC pump and let it run at the operating flow rate. (Acetonitrile:water 50:50).

- Fill the syringe with the compound (10-20 ng/µL desolved in acetonitrile) of interest (the compound the tuning file is going to be based up on) and put the flow to 10µL/min.
- Click on the Define Scan Button in Tune Master. Zoom in on the parent mass.

Click on the Optimize Compound Dependent Devises button on the Control/Scan Mode toolbar to display the Compound dependent parameters

- Spray Voltage (ESI/H-ESI)
- Discharge Current (APCI)
- Sheath Gas Pressure
- Aux Valve Flow
- Vaporizer Temperature (APCI/H-ESI)
- Capillary Temperature
- Tube Lens Offset
- Source CID
- Collision Pressure
- Collision Energy
- Quad MS/MS Bias

Check one or several at a time (or all of them at the same time) and hit the Start button and the software will to find the optimum settings for the compound of interest. When the tuning has completed successfully: click on Accept and then Save As to save the tuning file.

b) Maintaining the APCI probe

The APCI probe requires a minimum of maintenance.

Remove the APCI Nozzle:

- Place the instrument in Standby mode
- Hold onto the APCI probe body with one hand and grasp the head of the APCI nozzle assembly.
- Rotate the head of the nozzle assembly until the Flat sides of the head are facing towards the retention flanges. The Nozzle assembly is now free of the probe.
- Carefully pull the nozzle assembly straight out of the APCI probe.
- Place the assembly on a clean, lint free tissue.
- Cleaning the APCI probe components:
- Check the condition O-rings on the APCI nozzle.
- Clean the interior APCI components (excluding the ceramic heater) with a 50:50 solution of HPLC-grade methanol and distilled/deionized water and a lint-free swab.

Removing the APCI Sample Tube:

- With a 3/8-in. open-end wrench, remove the sample tube inlet fitting (P/N 70005-20250), 0.239-in. ID O-ring (P/N 00107-04000), and sample tube from the APCI manifold.
- Remove the exit-end nut (P/N 70005-20220), 0.016-in. ID, PEEK

Ferrule (P/N 00101-18120), and sample tube from the sample tube inlet fitting.

- Discard the old sample tube.

c) Housing Maintenance

The Ion Max ion source housing is designed to be serviced by trained Service Engineers only. User maintenance is limited to cleaning the ion source housing as necessary. To clean the housing, remove the housing from the instrument and in an appropriate fume hood, rinse the interior of the housing with methanol and allow to dry before installing on the instrument.

9. Reportable Range of Results

The linear range of the standard calibration curves determines the highest and lowest analytical values of an analyte that are reportable. The calibration verification of the method encompasses this reportable range. However, you can dilute and re-analyze urine samples whose analytical data values exceeds the highest reportable limit so that the result will be in the reportable range.

a. Linear Limits

Analytical standard were linear for all analytes through the range of concentrations evaluated. The linear range for all analytes was LOD to 100 ppb. Resample urine samples whose concentrations exceed these ranges, and re-analyze them using a smaller aliquot.

b. Analytical Sensitivity

The detection limits (LODs) for all analytes were calculated as $3S_0$, where S_0 is the standard deviation (SD) at zero concentration and is determined by linear regression analysis of the absolute standard deviation (SD) versus concentration. The detection limits vary based upon the current operating precision and the cleanliness of the analytical system. The range of detection limits determined to date for the analytes are presented in Table 8.

Table 8.
Analyte Detection Limits

Analyte	$3S_0$ ng/ml
IMPY_Q	0.06
CIT_Q	0.18
DEAMPY_Q	0.06
ATZ_Q	0.51
ACE_Q	0.18
ALA_Q	0.36
DEET_Q	0.06
MDA-Q	0.05
CDCA-Q	0.21

PNP-Q	0.10
MET-Q	0.10
CMHC-Q	0.18
2,4-D-Q	0.10
TCPY-Q	0.18
2,4,5-T-Q	0.10
DCCA-Q	0.30
4F-3-PBA-Q	0.12
3-PBA-Q	0.20
DBCA-Q	0.30

c. Accuracy

The accuracy of this method was determined by enriching urine samples with know concentrations of the pesticides residues and comparing the calculated and expected concentrations. The accuracy was consistent across the entire linear range. The accuracy can be expressed as the slope of a linear regression analysis of the expected value versus the calculated value. A slope of 1.0 indicates that the results are identical. Another way of expressing a method's accuracy is as a percentage of the expected value.

Table 9.
Accuracy of the Method

Analyte	Slope (Calculated vs. Expected)
IMPY_Q	0.9998
CIT_Q	0.9997
DEAMPY_Q	0.9996
ATZ_Q	0.9996
ACE_Q	1.0294
ALA_Q	1.0330
DEET_Q	1.0000
MDA-Q	0.999
CDCA-Q	0.999
PNP-Q	0.999
MET-Q	0.999
CMHC-Q	0.998
2,4-D-Q	0.998
TCPY-Q	0.999
2,4,5-T-Q	0.999
DCCA-Q	0.997
4F-3-PBA-Q	0.999
3-PBA-Q	0.999
DBCA-Q	0.961

d. Precision

The precision of this method is reflected in the variance of (QC) samples over time. The major contributor to the overall calibration verification is the variation between runs. Variation and the total coefficient of variation were determined from multiple analyses of quality control materials throughout the study. The total calibration verifications of the method vary from study to study and are dependent upon instrument, operators, and sample preparation analyst. The calibration verifications shown in Table 10 are representative of the most recent study.

Table 10.
Precision of the Method

Analyte (ppb)	QCL			QCM			QCH		
	Con ng/ mL	Stdv	CV (%)	Con ng/ mL	Stdv	CV (%)	Con ng/ mL	Stdv	CV (%)
IMPY_Q	1.2	0.1	14	8.6	0.9	11	16.5	1.8	11
CIT_Q	1.1	0.2	16	8.4	1.1	13	16.5	2.8	17
DEAMPY_Q	1.1	0.1	11	8.6	1.1	12	16.7	2.3	14
ATZ_Q	1.3	0.2	18	10.2	1.3	13	18.9	2.6	14
ACE_Q	1.4	0.2	14	10.6	1.1	10	19.4	2.3	12
ALA_Q	1.4	0.2	15	10.6	1.2	11	19.2	2.1	11
DEET_Q	1.3	0.2	12	8.8	0.9	10	16.7	2.0	12
MDA-Q	1.7	0.2	15	13.6	1.1	8	25.2	2.0	8
CDCA-Q	2.2	0.4	18	10.4	0.8	7	18.3	1.4	8
PNP-Q	1.1	0.1	11	8.3	0.7	8	15.4	1.3	8
MET-Q	1.2	0.1	9	9.9	0.8	8	18.8	1.7	9
CMHC-Q	1.2	0.1	17	7.8	1.2	15	16.0	1.1	7
2,4-D-Q	1.2	0.1	11	10.2	0.9	9	18.8	1.5	8
TCPY-Q	1.2	0.2	14	9.0	0.9	11	16.6	1.5	9
2,4,5-T-Q	1.2	0.1	8	9.4	0.8	8	18.1	1.4	8
Trans-DCCA-Q	1.2	0.2	18	8.6	1.0	12	16.8	2.2	13
Cis-DCCA-Q	1.4	0.2	14	8.7	0.7	8	16.0	2.0	12
4F-3-PBA-Q	1.1	0.1	10	8.9	0.7	8	16.2	1.2	8
3-PBA-Q	1.1	0.2	15	8.7	0.8	9	16.5	1.4	9
DBCA-Q	1.1	0.2	17	8.9	1.0	11	16.5	1.3	8

Con = Average Concentration
Stdv= Standard deviation of the QC Values
CV= coefficient of variation of the QC Values

e. Analytical Specificity

This is a highly selective method that requires the following of each analyte detected: 1) that it be at a specific retention time, 2) that it has two precursor ions at specific masses, 3) that it has two specific product ions formed from each of the two precursor ions at

specific masses, and 4) that the ion ratios of the two product ions be within a predetermined range.

10. Quality Control (QC) Procedures

a. QC Materials

Urine pools enriched with known amounts of pesticide residues as the control materials used for each unknown run.

b. Collection of Urine for QC Pools

Prepare and use three QC pools in each run of unknown samples. Collect the urine and screen the urine to ensure that the endogenous levels of pesticide residues are low or non-detectable. Combine and homogenize the urines samples to form a base pool.

c. Urine Enrichment

Split the base pool equally into four smaller urine pools. Reserve one pool for blank and standard analyses (see "Sample Preparation" section). Enrich one of the pools with an appropriate amount of the stock solution of each pesticide residue to yield an approximate concentration of 0.5 µg/L (QCL). Enrich the other pool to yield an approximate concentration of 5 µg/L (QCM) Enrich the final pool with an appropriate amount of each pesticide stock solution to yield an approximate concentration of 15 µg/L (QCH).

d. Filtration and Dispensing

Clean filter each pool to 0.2 µ. Dispense the urine in 12-mL aliquots into 25-mL, sterile, screw-capped vials. Label the vials appropriately, and then freeze the QC materials at -20°C until needed.

e. Characterization of QC Materials

Characterize the QC pools (including the unspiked pool) by 20 consecutive runs of each QC material. Use the data from these runs to establish the mean and upper-and lower-99th and 95th confidence intervals. Determine the confidence intervals and adjust them according to the number of each QC material analyzed in each run.

f. Use of QC Samples

During each analytical run, analyze one blank urine and three QC materials: QCL, QCM, QCH.

g. Final Evaluation of Quality Control Results

Evaluate QC material are evaluated using standard Westgard multirule criteria (www.westgard.com). Repeat out-of-control runs if residual sample is available. No data from runs considered out-of-control will be reported.

11. Remedial Action If Calibration or QC Systems Fail to Meet Acceptable Criteria

If the calibration or QC systems fail to meet acceptable criteria, suspend all operations until you identify the source or cause of failure is identified and corrected. If the source of failure, for example, failure of the mass spectrometer or a pipetting error, correct the

problem immediately. Otherwise, prepare fresh reagents and clean the mass spectrometer system. Before beginning another analytical run, re-analyze several QC materials (in the case of QC failure) or calibration-verification samples (in the case of calibration failure). After re-establishing calibration or QC, resume analytical runs.

12. Limitations of Method; Interfering Substances and Conditions

This method is an isotope-dilution mass spectrometry method, which is widely regarded as the definitive method for the measurement of organic toxicants in human body fluids. By using high-resolution tandem mass spectrometry, you can eliminate most analytical interferences. Because of the matrix used in this procedure, occasional interfering, unknown substances have been encountered. Interferences with the internal standards result in rejection of that analysis. If repeat analysis still results in an interference with the internal standard do not report the results for that analyte.

13. Critical-Call Results ("Panic Values")

It is unlikely that any result would be a "critical call," which would only occur with poisonings. Report test results in this laboratory in support of epidemiological studies, rather than clinical assessments. Data will help determine critical exposures.

14. Specimen Storage and Handling during Testing

Refrigerate urine samples overnight to expedite thawing prior to aliquoting the sample. Store the urine extracts in autosampler vials in a -20°C freezer after analysis. Stability studies suggest that the extracts remain stable at room temperature for up to 5 days.

15. Alternate Methods for Performing Test and Storing Specimens If Test System Fails

The method is designed to run on a LC/MS/MS instrument and is not generally transferable to other instrumentation. If the system has failed, store sample vials may in a refrigerator. You can store the extract samples for as long as 3 weeks. If you anticipate long-term interruption, store samples between -80°C and -20°C.

16. Test-Result Reporting System; Protocol for Reporting Critical Calls (If Applicable)

Once the validity of the data has been established by the QC/Quality Assurance system outlined above and has been verified by a DLS QAO, generate one hard copy and one electronic copy of the data. Route these data, a cover letter, and a table of method specifications and reference range values through the appropriate channels for approval (i.e., supervisor, branch chief, division director). Once division personnel have approved, the information, send it to the contact person who requested the analyses.

Report data in support of epidemiological or health survey studies. At this time there is not protocol for reporting critical calls.

17. Transfer or Referral of Specimens; Procedures for Specimen Accountability and Tracking

Use standard record-keeping systems (i.e., notebooks, sample logs, data files, creatinine logs, demographic logs) to keep track of all specimens. Transfer of refer to CLIA-specimens only certified laboratories. Any transfer of study samples is handled through the DLS special studies coordinator.

18. References

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19. Notes

1. Special care should be taken to avoid loss of sample when thawing because cracks may occur in the sample containers. It is recommended to put samples in refrigerator over night so that samples thaw slowly.

2. QC limits and means may vary over time as additional studies are completed and their QC data are added in the characterization.

3. Method specifications, including LOD and CV, are calculated for individual study, so may vary slightly.

4. The analytical detection limits and QC limits may vary from study to study as more data are available for statistical calculations. QC limits do not vary within studies, but remain constant.

5. The expiration time for the standard working solutions is determined by monitoring the peak intensity for each standard over time in the analytical runs.

6. The expiration time for the Quality Control material is determined by monitoring the concentration of each QC over time in the analytical runs.

7. The frequency of cleaning the components of the mass spectrometer depends on the types and amounts of samples and solvents that are introduced into the instrument.

20. Appendix I "Robustness Test"

Introduction

The robustness of an analytical method is an estimation of its capability to remain unaffected by small but deliberate changes in method variables. It provides a qualitative estimation of reliability when analyses are performed in standard conditions. The robustness test performed on this method includes:

a. Sample Stability after Preparation.
b. Solid Phase Extraction without Condition and Equilibration Steps.
c. Solid Phase Extraction without Wash Step.
d. Re-inject Repeatability.
e. TurboVap Evaporator Temperature.

(a) Sample Stability after Preparation.

Test Description

The stability of Universal Pesticides stored in urine extract for extended periods was evaluated. The sample stability preparation time was determined at two concentrations, around 10 ppb and 20 ppb. Five runs consistent of five QC medium samples, five QC high samples, and a standard series were prepared the same day. Runs were combined and thoroughly mixed to ensure homogeneity. The combined runs were subsequently aliquoted into four runs. One run was analyzed the same day and the other three runs were stored at -10 ^{o}C and analyzed during different days (see Table 1). Average measured concentration values from each day were compared with the averaged measured concentration values from the first day.

Results/Discussion

In the "delayed injection" test, all mean values measured on subsequent days after the first day were within 10% of the mean values on the first day (see Table1). The highest percent of deviation for sample stability after preparation was found for TCPY_Q (8%) between days 8 and 1.

(b) Solid Phase Extraction (SPE) without Conditioning and Equilibration Steps.

Test Description

A major cause of poor recovery in SPE stems from the need to pre-wet traditional reversed-phase sorbents with a water-miscible organic solvent and keeping them wetted

before applying the aqueous sample matrix. If the sorbent dries out before loading the sample, retention of the analytes falls sharply. Then the capacity is severely compromised, and sample breakthrough can be significant.Polymeric solid phase extraction cartridges such as Oasis HLB ® are designed to simplify and improve the sample preparation by combining the right sorbent chemistry, device format and methodology. They incorporate a hydrophilic-lipophilic-balanced water-wettable copolymer optimal for any sample cleanup. Oasis HLB® cartridges are unique in their purity, reproducibility, stability, and retention characteristics. The water wettable capability of these OASIS cartridges was evaluated by comparing two sets of five QC mediums and five QC high with and without conditioning and equilibration steps.

Results/Discussion

In the "Solid Phase Extraction without conditioning and equilibration steps" test, all mean values were measured and compared with the mean values for the regular clean up procedure. All the results from the procedure without the conditioning and equilibration steps were within 10% of the mean values of the procedure that included the conditioning and equilibration steps (see Table2). The highest percent of deviation for the solid phase extraction with and without conditioning and equilibration steps was found for ALA_Q (12.5%). By using these polymeric solid phase cartridges, we can expect robust SPE methods without having to endure irreproducible results and low recoveries caused by undesirable silanol activity, sorbent drying, pH limitations, and breakthrough of polar compounds/metabolites.

(c) Solid Phase Extraction without Wash Step.

Test Description

The possible wash effects in the solid phase extraction procedure were evaluated by preparing two sets of five QC mediums, five QC highs, and a calibration curve. One of the sets was prepared excluding the wash step during the sample clean up and the other set was prepared following the regular clean up procedure. Both sets were prepared and injected the same day.

Results/Discussion

In the "Solid Phase Extraction without wash step" test, all mean values from extraction without wash step were measured and compared with the mean values for regular clean up procedure. All the results from the procedure without wash step were within 10% of the mean values of the procedure that included the wash step (see Table3). The highest percent of deviation for solid phase extraction with and without wash step was found for *Cis*_DDCA_Q (8%).

(d) Re-inject Repeatability.

Test Description

To investigate effects on precision and accuracy caused by repetitively injecting and analytical run, a set of 6 QC mediums, 6 QC highs, and a completed calibration curve was prepared using the regular sample clean up. Samples were analyzed the same day that they were prepared. Then, samples were recapped and stored a -10 ^{o}C overnight. The next day, samples were removing from the refrigerator and allowing them to reach room temperature before been re-injected into the HPLC-MS/MS system. The injection vials were recapped and stored at – 10 ^{o}C for the next four (4) days. Finally, samples were removing from the refrigerator and allowing them to reach room temperature before been re-injected for the third time into the HPLC-MS/MS system. Mean values from the second and fifth injections were compared to mean values from the first injection. The accuracy of the second and fifth day injections were expressed as percentage deviations from the mean values obtained the first day.

Results/Discussion

In the "Re-inject repeatability" test, all mean values measured on subsequent days after the first day were within 10% of the mean values on the first day (see Table 4). The highest percent of deviation for re-inject repeatability was found for CIT_Q and DEAMPY_Q (9.7%) between days 5 and 1.

(e) Turbo_Vap Evaporator Temperature.

Test Description

Thermal stability of the analytes during the sample clean up was evaluated by preparing three different sets of six QC mediums, six QC highs and a calibration curve under different turbo-vap temperatures (30 ^{o}C; 40 ^{o}C, and 50 ^{o}C). Average measured concentration values from each temperature (40 ^{o}C, and 50 ^{o}C) were compared with the averaged measured concentration values from the regular temperature (30 ^{o}C). All three sets were prepared and injected the same day.

Results/Discussion

In the "Turbo_vap" test, all mean values measured at 40 ^{o}C, and 50 ^{o}C were within 15% of the mean values on the regular procedure at 30 ^{o}C (see Table 5). The highest percent of deviation turbo-vap temperature was found for MDA_Q (10.2%) between samples clean up at 50 ^{o}C and samples clean up at 30 ^{o}C

Table 1. Data from robustness test, "Sample Stability after Preparation". Mean concentrations of QCs (mediums and high levels) and a comparison of the percent deviations of these values. The absolute percent deviations are calculated by comparing each day's mean values to the mean values of the first day such as % Dev (1) comparing day 2 versus day 1; % Dev (2) comparing day 4 versus day 1, and % Dev (3) comparing day 8 versus day 1. (n=5)

Analyte	Sample_Name	1 Day		2 Day		4 Day		8 Day		%Dev (1)	%Dev (2)	%Dev (3)
		Aver	Std	Aver	Std	Aver	Std	Aver	Std			
MDA_Q	QCM	12.4	0.2	12.2	0.5	12.8	0.8	12.2	0.1	1.6	3.2	1.9
	QCH	22.6	1.4	22.5	1.6	23.6	0.8	22.1	0.5	0.6	4.4	2.2
CDCA_Q	QCM	9.9	0.2	9.1	0.7	9.4	0.2	9.9	0.1	7.7	5.0	0.6
	QCH	18.1	0.5	18.2	1.7	17.3	0.6	17.2	1.1	0.5	4.8	5.2
PNP_Q	QCM	8.1	0.1	8.0	0.9	8.4	0.9	8.1	0.5	1.2	3.4	0.8
	QCH	15.6	0.4	15.5	1.0	15.3	0.7	15.0	1.0	0.8	2.4	3.9
CMHC_Q	QCM	8.1	0.1	8.0	1.2	7.7	0.8	8.2	0.5	1.4	4.7	1.2
	QCH	15.8	1.0	15.6	0.3	15.7	0.7	15.6	1.1	1.6	0.7	1.8
MET_Q	QCM	9.7	0.3	10.0	0.8	10.0	0.2	9.6	0.4	3.3	3.9	0.7
	QCH	19.1	0.7	19.9	1.2	20.1	0.6	20.4	1.0	4.2	4.9	6.4
2,4 D_Q	QCM	10.6	0.7	10.6	1.8	10.4	0.2	9.9	0.7	0.1	1.9	6.0
	QCH	18.8	1.8	19.3	0.4	19.8	0.2	18.8	1.3	2.8	5.4	0.0
TCPY_Q	QCM	8.9	0.2	9.0	0.4	8.8	0.9	8.7	0.2	1.3	0.7	2.4
	QCH	16.7	0.6	15.9	0.2	15.8	0.4	15.4	1.0	5.1	5.5	8.0
2, 4, 5 T _Q	QCM	9.9	0.3	9.9	0.5	9.3	0.7	9.4	0.5	0.2	6.1	5.3
	QCH	19.4	0.5	19.0	1.2	19.1	0.4	19.0	0.7	1.8	1.2	1.8
3 PBA_Q	QCM	8.0	0.1	8.2	1.1	8.2	0.6	8.1	0.6	2.9	2.6	0.7
	QCH	16.1	0.8	15.8	0.5	16.3	0.9	15.8	1.2	2.3	1.0	1.8
4F-3 PBA_Q	QCM	9.0	0.2	9.2	1.6	9.0	0.6	9.1	0.7	2.3	0.8	1.3
	QCH	16.4	0.6	16.4	0.5	16.4	0.8	16.1	0.8	0.4	0.3	2.1
Trans DDCA_Q	QCM	8.9	0.5	8.7	0.1	8.4	0.4	8.7	0.7	2.1	5.0	2.0
	QCH	16.3	1.6	15.1	0.9	15.6	1.1	15.7	0.8	7.0	4.4	3.3
Cis DDCA_Q	QCM	8.4	0.1	8.2	0.9	8.0	0.5	8.1	0.4	2.3	4.8	4.0
	QCH	15.4	0.8	15.0	0.6	15.2	0.5	15.3	0.9	2.7	1.6	0.5
DBCA_Q	QCM	7.8	0.2	8.3	1.0	7.7	0.1	8.0	0.5	5.8	2.2	2.6
	QCH	15.6	0.9	15.4	0.7	15.3	0.2	15.2	0.9	1.4	2.1	2.7
IMPY_Q	QCM	9.2	0.2	8.5	0.2	8.8	0.6	8.6	0.7	8.0	4.8	6.9
	QCH	17.1	0.5	16.2	0.1	16.1	0.5	15.9	0.8	5.2	5.7	6.8
CIT_Q	QCM	8.8	0.1	8.5	0.2	8.6	0.3	8.4	0.4	3.5	2.4	4.7
	QCH	17.2	0.2	16.1	0.3	16.3	0.7	16.1	0.7	6.7	5.4	6.6
DEAMPY_Q	QCM	9.0	0.1	9.1	0.6	8.9	0.3	8.7	0.3	1.1	0.4	2.6
	QCH	17.8	0.3	16.8	0.9	16.7	1.0	16.5	1.0	5.8	6.4	7.5
ATZ_Q	QCM	10.5	0.3	10.4	0.3	10.1	0.6	9.9	0.6	0.3	3.5	5.4
	QCH	19.6	0.4	19.0	0.5	19.1	0.7	18.9	0.7	3.2	2.7	3.8
ACE_Q	QCM	10.9	0.1	10.6	0.3	10.6	0.2	10.4	0.3	2.3	3.0	4.8
	QCH	20.6	0.3	19.9	0.8	19.3	0.5	19.1	0.8	3.5	6.1	7.1
ALA_Q	QCM	10.8	0.2	10.8	0.2	10.7	0.2	10.5	0.2	0.1	0.8	2.7
	QCH	19.4	0.6	19.0	0.5	19.4	0.5	19.2	0.5	2.1	0.1	1.0
DEET_Q	QCM	8.9	0.4	8.6	0.2	8.4	0.4	8.2	0.2	3.1	5.3	7.6
	QCH	17.0	0.3	16.6	0.8	17.1	1.0	16.9	0.8	2.5	0.3	0.9

Table 2. Data from robustness test, "Solid Phase Extraction without Condition and Equilibration Steps". Mean concentrations of QCs (mediums and high levels) and a comparison of the percent deviations of these values. The absolute percent deviations are calculated by comparing set mean values from procedure without condition and equilibration steps to the set mean values from procedure with condition and equilibration steps. (n=5).

Analyte	Sample_Name	C&E for SFE_C		No C&E for SFE_C		%Dev (1)
		Aver	Std	Aver	Std	
MDA_Q	QCM	14.2	0.3	14.8	0.3	4.0
	QCH	27.5	0.9	26.9	0.8	2.3
CDCA_Q	QCM	10.0	0.5	10.2	0.2	2.8
	QCH	18.0	0.9	18.7	0.2	3.8
PNP_Q	QCM	9.0	0.3	8.7	0.2	3.6
	QCH	16.5	1.3	16.6	0.3	0.3
CMHC_Q	QCM	7.9	0.2	8.6	0.2	8.9
	QCH	16.8	0.9	17.0	0.6	1.0
MET_Q	QCM	9.9	0.3	9.9	0.4	0.5
	QCH	20.6	1.7	19.6	0.9	4.9
2,4 D_Q	QCM	10.4	0.4	10.1	0.4	3.5
	QCH	20.2	0.8	19.5	0.4	3.3
TCPY_Q	QCM	9.9	0.4	9.4	0.1	5.0
	QCH	18.6	0.9	17.6	0.3	5.3
2, 4, 5 T _Q	QCM	9.5	0.5	9.6	0.5	0.5
	QCH	19.0	0.7	18.7	0.6	1.4
3 PBA_Q	QCM	9.3	0.1	9.3	0.2	0.3
	QCH	17.9	0.5	17.7	0.2	1.1
4F-3 PBA_Q	QCM	8.4	0.3	9.1	0.5	8.3
	QCH	17.0	0.7	17.2	0.5	1.0
Trans DDCA_Q	QCM	9.6	0.9	8.9	0.3	6.6
	QCH	16.4	0.6	16.9	0.4	3.0
Cis DDCA_Q	QCM	9.2	0.3	9.1	0.4	0.7
	QCH	15.9	1.0	15.7	0.7	1.7
DBCA_Q	QCM	9.3	0.5	9.0	0.5	3.1
	QCH	16.8	0.3	17.5	0.4	4.2
IMPY_Q	QCM	8.2	0.5	8.9	0.5	8.1
	QCH	16.1	0.9	17.2	0.9	6.9
CIT_Q	QCM	8.0	0.4	7.8	0.4	2.0
	QCH	15.5	0.6	15.6	0.6	0.9
DEAMPY_Q	QCM	7.4	0.5	8.0	0.5	7.4
	QCH	17.1	1.1	17.6	1.1	2.9
ATZ_Q	QCM	10.6	0.5	10.4	0.5	1.5
	QCH	19.0	1.3	20.3	1.3	6.9
ACE_Q	QCM	10.5	0.8	10.0	0.8	4.6
	QCH	19.3	0.9	19.5	0.9	0.9
ALA_Q	QCM	9.6	0.3	10.8	0.3	12.6
	QCH	18.8	1.1	19.7	1.1	5.2
DEET_Q	QCM	8.1	0.6	8.5	0.6	5.0
	QCH	17.7	1.2	16.9	1.2	4.7

Table 3. Data from robustness test, "Solid Phase Extraction without Wash Step". Mean concentrations of QCs (mediums and high levels) and a comparison of the percent deviations of these values. The absolute percent deviations are calculated by comparing set mean values from procedure without wash step to the set mean values from procedure with wash step. (n=5).

Analyte	Sample_Name	With Wash Step		Withouth Wash Step		%Dev (1)
		Aver	Std	Aver	Std	
MDA_Q	QCM	12.3	1.3	12.3	2.1	0.0
	QCH	23.0	2.7	22.4	3.9	2.6
CDCA_Q	QCM	10.1	0.3	10.1	1.2	0.0
	QCH	22.2	1.9	22.0	1.6	0.7
PNP_Q	QCM	8.8	0.8	8.5	0.6	2.6
	QCH	18.5	1.8	18.0	1.6	2.9
CMHC_Q	QCM	9.6	0.9	10.0	1.6	4.0
	QCH	19.5	1.2	18.7	1.2	4.1
MET_Q	QCM	10.5	1.2	10.0	1.4	5.3
	QCH	19.5	1.6	19.1	1.5	1.9
2,4 D_Q	QCM	10.3	0.8	10.1	0.8	2.6
	QCH	20.1	2.0	20.6	0.9	2.1
TCPY_Q	QCM	9.7	1.1	9.8	1.1	1.6
	QCH	20.1	2.7	20.2	1.9	0.2
2, 4, 5 T _Q	QCM	9.7	0.5	9.3	0.9	4.3
	QCH	19.5	1.9	19.4	1.6	0.2
3 PBA_Q	QCM	9.3	1.0	9.1	0.9	2.5
	QCH	19.3	0.9	18.9	1.0	2.4
4F-3 PBA_Q	QCM	7.5	0.4	7.7	0.3	2.1
	QCH	19.2	0.4	19.1	1.1	0.3
Trans DDCA_Q	QCM	9.1	0.7	9.4	0.9	3.9
	QCH	19.5	1.3	20.0	2.7	2.6
Cis DDCA_Q	QCM	9.5	0.8	8.9	0.7	6.5
	QCH	17.7	1.8	17.8	2.1	0.7
DBCA_Q	QCM	9.7	0.8	9.0	0.7	8.0
	QCH	17.8	1.8	17.9	2.1	0.4
IMPY_Q	QCM	9.1	0.5	8.9	0.3	2.9
	QCH	19.8	3.3	19.4	2.2	1.7
CIT_Q	QCM	9.7	1.2	10.3	0.8	5.7
	QCH	20.6	2.3	20.3	3.0	1.2
DEAMPY_Q	QCM	9.3	0.7	9.6	1.2	2.7
	QCH	21.2	1.3	19.8	1.7	6.7
ATZ_Q	QCM	10.2	0.7	10.8	1.1	5.4
	QCH	20.1	1.0	20.8	0.7	3.5
ACE_Q	QCM	10.1	0.6	10.5	0.3	3.6
	QCH	20.7	2.5	20.5	0.6	1.0
ALA_Q	QCM	10.4	0.7	10.7	0.6	3.5
	QCH	19.8	0.7	20.8	1.3	4.7
DEET_Q	QCM	10.0	0.7	10.4	0.6	4.7
	QCH	20.1	1.0	20.7	1.2	2.6

Table 4. Data from robustness test, "Re-inject Repeatability". Mean concentrations of QCs (mediums and high levels) and a comparison of the percent deviations of these values. The absolute percent deviations are calculated by comparing each days mean values to the mean values of the first day such as % Dev (1) comparing day 2 versus day 1; % Dev (2) comparing day 5 versus day 1. (n=6).

Analyte	Sample_Name	1 Day		2 Day		5 Day		%Dev (1)	%Dev (2)
		Aver	Std	Aver	Std	Aver	Std		
MDA_Q	QCM	14.2	0.3	14.8	0.3	13.9	0.3	4.7	1.8
	QCH	27.1	0.7	26.2	0.4	26.2	0.4	3.4	3.5
CDCA_Q	QCM	10.3	0.2	10.3	0.3	10.7	0.1	0.5	4.3
	QCH	18.7	0.2	18.0	1.0	18.4	1.2	3.9	1.7
PNP_Q	QCM	8.7	0.1	8.9	0.3	8.3	0.2	1.6	5.0
	QCH	16.7	0.3	15.7	0.4	15.7	0.4	5.7	5.8
CMHC_Q	QCM	8.7	0.1	9.4	0.2	8.9	0.2	7.6	2.1
	QCH	17.2	0.3	17.0	0.9	16.7	1.0	1.0	2.7
MET_Q	QCM	9.8	0.1	10.3	0.2	9.7	0.1	5.2	1.0
	QCH	19.1	0.2	18.3	0.7	18.0	0.8	3.9	5.7
2,4 D_Q	QCM	10.2	0.1	11.0	0.2	9.6	0.2	7.1	6.4
	QCH	19.5	0.4	19.2	0.7	18.0	0.9	1.5	7.8
TCPY_Q	QCM	9.5	0.1	9.2	0.2	8.9	0.1	3.1	6.1
	QCH	17.6	0.3	17.6	0.7	16.1	0.8	0.3	8.6
2, 4, 5 T _Q	QCM	9.7	0.1	10.5	0.2	9.5	0.1	7.8	2.6
	QCH	18.9	0.2	19.2	0.7	18.0	0.9	1.5	4.9
3 PBA_Q	QCM	9.2	0.1	9.6	0.3	8.9	0.2	4.6	3.1
	QCH	17.7	0.3	17.4	0.7	16.0	0.8	1.8	9.6
4F-3 PBA_Q	QCM	9.4	0.1	9.8	0.2	8.7	0.1	4.4	7.1
	QCH	16.4	0.4	16.9	0.6	15.9	0.7	3.3	2.8
Trans DDCA_Q	QCM	8.9	0.4	8.8	0.4	8.4	0.4	0.9	6.1
	QCH	16.9	0.4	16.5	0.4	16.4	0.3	2.1	2.5
Cis DDCA_Q	QCM	9.1	0.4	9.2	0.1	9.2	0.1	1.9	1.2
	QCH	17.6	0.6	16.3	0.6	16.3	0.6	7.2	7.4
DBCA_Q	QCM	9.1	0.4	9.5	0.3	8.8	0.3	3.9	3.6
	QCH	17.6	0.3	16.8	0.3	16.3	0.3	4.2	7.5
IMPY_Q	QCM	9.2	0.2	8.6	0.2	8.5	0.1	6.8	8.0
	QCH	17.1	0.5	16.2	0.6	16.2	0.7	4.9	5.2
CIT_Q	QCM	9.0	0.1	9.0	0.1	8.5	0.1	0.1	4.9
	QCH	17.8	0.3	16.7	0.6	16.1	0.7	6.0	9.7
DEAMPY_Q	QCM	9.0	0.1	9.0	0.1	8.5	0.1	0.1	4.9
	QCH	17.8	0.3	16.7	0.6	16.1	0.7	6.0	9.7
ATZ_Q	QCM	10.5	0.3	10.1	0.3	9.6	0.2	3.4	8.0
	QCH	19.6	0.4	20.0	0.3	19.0	0.4	1.7	3.3
ACE_Q	QCM	10.5	0.1	10.9	0.2	10.0	0.3	3.6	4.6
	QCH	20.2	0.3	20.0	0.4	19.4	0.4	0.8	3.7
ALA_Q	QCM	10.3	0.2	11.0	0.2	10.2	0.2	6.1	1.7
	QCH	19.0	0.6	19.9	0.7	17.8	0.7	4.7	6.2
DEET_Q	QCM	8.8	0.0	9.4	0.1	8.4	0.1	6.7	4.4
	QCH	16.9	0.3	16.9	0.7	15.9	0.7	0.1	6.4

Table 5. Data from robustness test, "Turbo_Vap Temperatures". Mean concentrations of QCs (mediums and high levels) and a comparison of the percent deviations of these values. The absolute percent deviations are calculated by comparing each temperature condition mean values to the mean values of the regular clean up at 30 °C. (n=6).

Analyte	Sample_Name	30 °C		40 °C		50 °C		%Dev (1)	%Dev (2)
		Aver	Std	Aver	Std	Aver	Std		
MDA_Q	QCM	12.5	0.7	11.3	1.1	11.9	1.7	10.2	5.0
	QCH	23.5	1.0	22.7	1.6	21.8	1.2	3.7	7.5
CDCA_Q	QCM	8.3	0.4	8.0	0.5	7.7	0.9	2.6	7.2
	QCH	15.4	0.9	15.1	0.5	14.9	1.6	2.3	3.3
PNP_Q	QCM	8.0	0.4	7.8	0.9	7.7	1.1	2.1	3.2
	QCH	14.5	1.2	14.3	1.2	15.4	2.3	1.3	6.2
CMHC_Q	QCM	7.9	0.8	8.0	0.9	8.0	0.6	1.6	2.0
	QCH	15.4	0.8	16.0	0.8	15.5	1.7	3.8	0.4
MET_Q	QCM	8.6	0.4	8.4	0.5	7.8	0.8	2.2	9.3
	QCH	17.9	0.6	17.6	0.9	18.0	2.2	2.0	0.3
2,4 D_Q	QCM	8.6	0.7	8.0	0.3	7.9	0.8	6.0	8.3
	QCH	18.3	1.2	18.0	0.8	18.3	1.0	1.4	0.1
TCPY_Q	QCM	8.1	0.4	7.7	0.7	8.1	1.1	4.1	0.4
	QCH	17.4	1.1	17.3	0.9	17.3	2.1	0.7	0.8
2, 4, 5 T _Q	QCM	8.7	0.8	8.5	0.9	8.9	0.6	2.7	2.2
	QCH	17.6	0.9	16.8	0.9	17.7	0.9	4.5	0.5
3 PBA_Q	QCM	7.3	0.3	7.4	0.7	7.8	0.9	2.0	7.4
	QCH	15.5	1.3	15.1	1.6	15.7	0.9	2.6	1.6
4F-3 PBA_Q	QCM	8.4	0.9	8.1	0.9	8.2	0.6	3.6	2.2
	QCH	17.2	1.5	17.3	1.3	17.0	1.1	0.1	1.6
Trans DDCA_Q	QCM	7.2	0.8	7.4	0.6	7.2	0.8	2.8	0.7
	QCH	15.0	1.3	15.0	0.9	15.2	1.0	0.1	1.2
Cis DDCA_Q	QCM	7.7	0.8	7.7	0.8	7.9	0.3	1.0	2.0
	QCH	17.5	1.0	16.4	0.8	17.4	0.7	6.2	0.7
DBCA_Q	QCM	6.7	0.7	7.0	1.5	6.9	0.8	4.1	2.6
	QCH	16.0	1.1	15.8	0.7	15.9	1.2	1.3	0.8
IMPY_Q	QCM	8.2	0.4	7.6	0.8	8.2	0.7	7.4	0.9
	QCH	16.9	1.3	16.9	1.4	16.9	1.5	0.4	0.3
CIT_Q	QCM	7.8	0.5	8.0	0.3	8.0	0.7	2.2	2.4
	QCH	19.1	1.1	19.4	0.9	19.7	0.7	1.4	3.1
DEAMPY_Q	QCM	8.4	1.2	7.7	0.9	8.7	1.2	7.7	3.3
	QCH	18.6	0.7	18.7	0.6	18.8	1.0	0.7	1.0
ATZ_Q	QCM	10.0	1.0	9.6	1.0	9.0	0.6	4.2	9.8
	QCH	18.4	0.7	18.8	0.4	18.7	0.6	2.2	1.7
ACE_Q	QCM	10.5	1.3	9.8	0.9	9.6	1.3	6.0	8.3
	QCH	18.3	0.7	18.7	0.5	18.7	1.1	1.9	2.0
ALA_Q	QCM	9.6	0.7	9.5	0.8	9.3	0.5	1.3	3.9
	QCH	18.9	0.5	19.1	0.4	19.1	0.9	0.7	1.0
DEET_Q	QCM	9.1	0.6	8.7	0.5	9.1	0.5	5.2	0.0
	QCH	18.0	0.7	17.4	1.1	17.9	0.8	2.9	0.3