

DATA
CHEM
LABORATORIES, INC.
A SORENSON COMPANY

December 8, 1999

Tammy Chang
Parsons Engineering Science
8000 Centre Park Drive #200
Austin, TX 78754

Dear: Tammy Chang,

Attached are corrective action items due to you on December 10th, 1999. The attached documents include:

SOP: OL-SW-8330 with revisions in sections 8.2.5 and 8.2.6.

SOP: XX-DC-024 "Manual Integration"

Retention Time Studies - For SW-846 Method 8330 on the primary and confirmation columns.

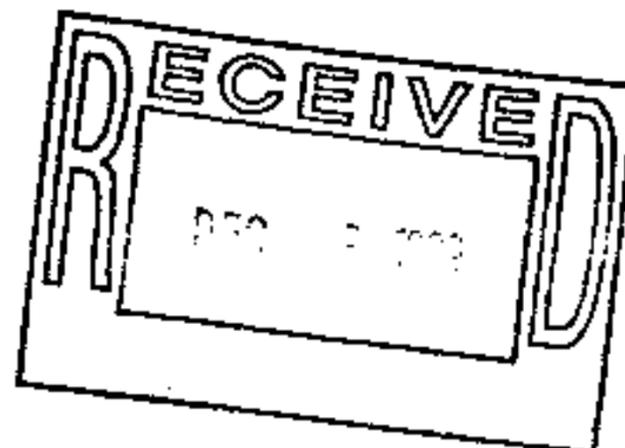
Please feel free to call me anytime regarding these responses at (801) 266-7700. Thank you for your time and the efforts you have taken to approve DataChem for this important project.

Sincerely,



Robert P. Di Rienzo
Vice President
Quality Assurance / Information Technology
DataChem Laboratories, Inc.

Enclosures: SOP # OL-SW-8330 Revision 6
SOP # XX-DC-024 Revision 0
Retention time Studies for SW-846 Method 8330



DATA CHEM LABORATORIES, INC.

STANDARD OPERATING PROCEDURE APPROVAL SHEET

SOP TITLE: Determination of Explosives by EPA Method 8330

DOCUMENT CONTROL NUMBER: OL-SW-8330 Revision 6

EFFECTIVE DATE: December 14, 1999

APPROVALS:

MANAGER

Richard H. Fide

Date 12-14-99

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Date 12/14/99

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Date 12/14/99

STANDARD OPERATING PROCEDURE

DETERMINATION OF EXPLOSIVES BY EPA METHOD 8330

1.0 SCOPE AND APPLICATION

- 1.1 This SOP is applicable to the determination of nitroaromatics and nitramines in soil and water using HPLC analysis with UV detection. This SOP provides procedures and conditions used in performance of EPA Method 8330.
- 1.2 The compounds which are determined by following this SOP are listed below:

<u>Abbreviation or Analyte Code</u>	<u>Compound</u>
HMX	Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
RDX	Hexahydro-1,3,5-trinitro-1,3,5-triazine
NB	Nitrobenzene
TETRYL	Methyl-2,4,6-trinitrophenyl nitramine
135TNB	1,3,5-Trinitrobenzene
13DNB	1,3-Dinitrobenzene
246TNT	2,4,6-Trinitrotoluene
24DNT	2,4-Dinitrotoluene
26DNT	2,6-Dinitrotoluene
2NT	2-Nitrotoluene
3NT	3-Nitrotoluene
4NT	4-Nitrotoluene
2-Am-DNT	2-Amino-4,6-Dinitrotoluene
4-Am-DNT	4-Amino-2,6-Dinitrotoluene

- 1.3 This SOP is restricted to use by or under the supervision of analysts experienced in the use of HPLC, skilled in the interpretation of chromatograms, and experienced in handling explosive materials. Furthermore, the analyst and extractionist performing this method will demonstrate the ability to generate acceptable data by providing accuracy and precision data which meet the requirements of the method.
- 1.4 The presence of additional compounds with retention times equal to the analytes of interest which absorb at 254 μm will interfere with the analysis and may result in inaccurate qualitative and quantitative results. The interfering compounds may arise from solvents, reagents, glassware, or instrumentation. For this reason, method blanks will be analyzed with each analytical batch to demonstrate the absence of interferences.

2.0 SUMMARY OF METHOD

- 2.1 Method 8330 provides high performance liquid chromatographic (HPLC) analysis and detection of ppb levels of certain explosive residues. A salting-out extraction procedure is used for low level water samples. Direct injection of diluted and/or filtered sample is used

for water samples of higher concentration.

- 2.2 For the analysis of low level water samples, a 770-mL portion of the sample is saturated with sodium chloride and then extracted with acetonitrile. Equal volumes of extract are then thoroughly mixed with a 1% calcium chloride solution containing internal standard. The resulting mixture is analyzed using an isocratic HPLC system equipped with UV detection and a column heater.
- 2.3 For the analysis of soil samples, a 2-g portion of the sample is mixed with acetonitrile and then sonicated for 18 hours. Equal portions of the sample supernatant and 1% calcium chloride containing internal standard are mixed thoroughly. The resulting mixture is analyzed using an isocratic HPLC system equipped with a column heater and UV detection.
- 2.4 Positive results in field samples are qualitatively confirmed on a secondary column. The second column analysis is not quantitative and is used only to confirm the presence of each compound. Only the target compounds which are confirmed in the second column analysis are reported, using quantitative results from the primary column analysis.
- 2.5 The surrogate, 3,4-Dinitrotoluene (3,4-DNT), is added before extraction to monitor extraction efficiency.
- 2.6 The internal standard, ortho-cresol (OC), is added with the calcium chloride solution before analysis. The internal standard is not used for quantitation but is used to determine the consistency of injections and to evaluate instrument performance.

3.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

- 3.1 Field samples collected for analysis by this method require no preservation other than refrigeration at $4^{\circ}\text{C}\pm 2^{\circ}\text{C}$.
- 3.2 Soil samples collected in the field are transferred into 5-ounce amber, wide-mouth bottles with Teflon-lined caps for transport to the laboratory. Water samples are transferred into two 1-liter amber bottles for transport to the laboratory.
- 3.3 Sample integrity is maintained at the laboratory until analysis can be performed by storing the field samples at $4^{\circ}\text{C}\pm 2^{\circ}\text{C}$.
- 3.4 Holding Times
 - 3.4.1 Field samples for method 8330 have an extraction holding time of seven days for water and 14 days for soil samples from the date of sampling. The extracts from the field samples have an analysis holding time of 40 days from the date of extraction.

4.0 SAFETY PRECAUTIONS

- 4.1 Good laboratory technique dictates the careful handling of all laboratory samples and reagents. Eye protection and a laboratory coat are minimum requirements. The following

specific additional safety measures are recommended:

- 4.1.1 For sample receipt and log-in, a hood should be used until the sample containers are found to be intact. Gloves should be worn while handling the sample containers.
- 4.1.2 For sample transfer and splitting, *etc.*, a hood and gloves should be used.
- 4.1.3 For sample sonication, a hood and gloves should be used.
- 4.1.4 **Special hazard warning:** The analytes are explosive and therefore should be handled with care at all times, especially when grinding a dry sample. Do not dry the samples in an oven; air dry only.

5.0 INSTRUMENTATION AND EQUIPMENT

5.1 All glassware must be acid washed to prevent decomposition of the explosives.

5.2 Glassware/Hardware - Soil

5.2.1 Syringes: 10-, 25-, 250-, and 1,000- μ L - Gas tight

5.2.2 Volumetric flasks: 10-, 25-, and 100-mL - Class A

5.2.3 10 mL syringe

5.2.4 Sample vials

5.2.5 Sonicator bath

5.2.6 200 mm vials

5.2.7 Analytical Balance - Metler

5.2.8 Nylon Acrodisk filters - Gelman or equivalent

5.3 Glassware/Hardware - Water

5.3.1 Syringes: Various volumes as required - Gas tight

5.3.2 Volumetric flasks: Various volumes as required - Class A

5.3.3 Disposable Pasteur pipets

5.3.4 Graduated cylinders: Various volumes as required - Class A

5.3.5 0.45- μ m nylon 66 filters, 47-mm - Gelman or equivalent

5.3.6 1 Liter Erlenmeyer flasks

- 5.3.7 15-mL blow-down tubes
- 5.3.8 Blow-down apparatus
- 5.3.9 N₂ blow-down needles, size 13
- 5.3.10 3" and 1" stir bars
- 5.3.11 Stir plate
- 5.3.12 Teflon caps
- 5.3.13 Pipets: 10-mL and 1-mL - Class A
- 5.3.14 Timer

5.4 HPLC system consisting of an Hewlett Packard 1050 pump, solvent degasser, autosampler, UV detector, and column heater, or Hewlett Packard 1090 HPLC system or equivalent.

6.0 CHEMICALS, REAGENTS, AND STANDARD SOLUTIONS

6.1 The reagents used in this method are:

- 6.1.1 Methanol, HPLC grade or equivalent - Burdick & Jackson.
- 6.1.2 Acetonitrile, UV grade or equivalent - Burdick & Jackson.
- 6.1.3 Ortho-Cresol Solution: 10 mg of ortho-cresol diluted in 5.0 mL of UV grade acetonitrile (2000 µg/ml).
- 6.1.4 1% Calcium Chloride/Internal Standard Solution: 1 gram of calcium chloride (reagent grade) and 1.0 mL ortho-cresol solution diluted in 100 mL of ASTM Type II water.
- 6.1.5 ASTM Type II water.

7.0 SAMPLE PREPARATION

7.1 Soil

- 7.1.1 Air dry the samples at room temperature or colder. Do not expose the samples to heat or direct sunlight.
- 7.1.2 Grind and homogenize the air-dried samples in an acetonitrile- rinsed mortar and pestle to pass through a 30-mesh sieve.
- 7.1.3 Place 2 grams of homogenized sample into a 200 mm test tube, record the weight, add 1000 µL of 40 µg/mL solution of 3,4-DNT in acetonitrile. This provides a surrogate concentration of 20 µg/g for each sample.

- 7.1.4 Add 1.0 mL of matrix spiking solution to the LCS, MS and MSD samples. The concentration of the matrix spiking solution is specified in Table 5.
- 7.1.5 Add the appropriate volume of acetonitrile to each sample to bring the final volume up to 10.0 mL. Cap each tube with a Teflon-lined screw cap.
- 7.1.6 Vortex each sample for one minute, then sonicate in a cooled ultrasonic water bath for 18 hours. The temperature of the water bath should be kept at room temperature or below to avoid breakdown of tetryl and possibly other explosives. Check often.
- Note:** To ensure efficient sonication, fill the bath to a level greater than or equal to the level of the sample and visually check the samples for cloudiness periodically. If samples are not cloudy, investigate the performance of the bath. Poor recoveries are obtained when the ultrasonic water bath is not operating properly.
- 7.1.7 Allow the samples to settle for 30 minutes. Filter 5 mL of the extract through a 0.45- μ m Teflon filter, discarding the first 2 mL and collecting the remainder for analysis.
- 7.1.8 Store the extracts in amber glass, acid washed vials with Teflon-lined screw caps. Store at 4°C \pm 2°C and protect from light.

7.2 Water

7.2.1 High-Level Method

7.2.1.1 Direct injection following the high-level method is recommended for aqueous samples with concentrations greater than 200 μ g/L. It is also recommended that process waste samples or other potentially high level samples be screened with the high level method to determine if the low-level method is required. Concentrations less than about 50 μ g/L will require the low level method.

7.2.1.2 **Sample Filtration:** Place a 5-mL aliquot of each water sample in a scintillation vial, add 5 mL of acetonitrile and mix. Filter through an acetonitrile rinsed 10-mL syringe equipped with a 0.45 μ m PTFE filter. Discard the first 3 mL of filtrate and retain the remainder in a Teflon-capped vial for HPLC analysis. HMX quantitation can be improved with the use of methanol rather than acetonitrile for dilution before filtration.

7.2.2 Low-Level Method (salting out extraction)

7.2.2.1 **NOTE:** Rinse all glassware with acetonitrile.

7.2.2.2 Add a large stir bar then add 325 g of sodium chloride to a 1 L Erlenmeyer flask. Measure out 770 mL of a water sample (using a 1 L graduated cylinder) and transfer it to the flask containing the salt. Spike

samples with 1000 μL of a 10.0- $\mu\text{g}/\text{mL}$ solution of 3,4-DNT in acetonitrile as surrogate. Add 1000 μL of matrix spiking solution for water 8330/MS-W (see Table 7) to the LCS, MS and MSD samples. Mix contents at maximum obtainable speed on a magnetic stirrer for 15 minutes. **DO NOT STOP THE STIRRING OF THE SALT.**

- 7.2.2.3** Add 164 mL of acetonitrile (measured with a 250-mL graduated cylinder) while the solution is being stirred, tightly cap with a Teflon cap. Stir for an additional 30 minutes, this time is critical and must be followed exactly. Turn off the stirrer and allow the phases to separate for 15 minutes.
- 7.2.2.4** Add a small stir bar to a 110-mL volumetric flask and add 84 mL of saturated salt water (425 g NaCl must be added per liter of reagent water to be sure the salt water is saturated).
- 7.2.2.5** Remove the acetonitrile (upper) layer (about 15 mL) with a Pasteur pipet, and transfer it to a 110-mL volumetric flask containing 84 mL of saturated salt water. If the solution is greater than the 6 to 8 mL mark, then another 110-mL flask must be used with 84 mL of saturated salt solution to accommodate all of the acetonitrile from the 1-liter flask. Cap the 110-mL flask with a Teflon cap.
- 7.2.2.6** Pipet 10 mL of fresh acetonitrile to the water sample in the 1-liter flask. Cap and again stir the contents at maximum obtainable speed for exactly 30 minutes followed by 15 minutes for phase to separate.
- 7.2.2.7** Combine the second acetonitrile portion with the initial extract in the first 110-mL flask if it is below the 6 mL mark; or, in the second 110-mL flask if the first one has reached the 6 to 8 mL mark. If the solution is below the 6 mL mark on the second 110-mL volumetric flask, transfer solution from the 1-L flask until such volume is obtained. As much as possible, pipet upper layer of the 1-L solution. Cap and stir the contents on a magnetic stirrer at maximum obtainable speed for 30 minutes followed by 15 minutes for phase separation.
- 7.2.2.8** **CAREFULLY** transfer the acetonitrile phase to a 40-mL blow-down test tube using a Pasteur pipet. Also, transfer the acetonitrile phase from the second 110-mL volume flask if one was used. There must **NOT** be any salt water solution transferred with the acetonitrile. The salt water contains a high concentration of an interference which produces a large peak at the beginning of the chromatogram and interferes with the HMX determination.
- 7.2.2.9** Pipet an additional 1.0 mL of acetonitrile to the 110-mL volumetric flask. If the solution is below the 2.0 mL mark on the 110-mL volumetric flask, transfer upper portion of the solution from the 1-L flask until such volume is obtained. Cap and stir the contents of the flask for 30 minutes

followed by 15 minutes for phase separation. Combine the second acetonitrile portion with the initial extract in the 15-mL tube. Nitrogen blow-down to 5 mL. Then transfer to a 5- mL volumetric flask. Dilute to volume with acetonitrile.

- 7.2.2.10 If the diluted extract is cloudy, filter it through a 0.45- μ m PTFE filter using an acetonitrile rinsed 10-mL syringe. Discard the first 0.5 mL of filtrate and retain the remainder in a Teflon-capped vial for HPLC analysis.

8.0 CALIBRATION

- 8.1 The calibration ranges for each analyte in this method are listed below. The low values represent the lowest standard analyzed during initial calibration and are the PQLs (practical quantitation limits). The PQLs will be used as the lower reporting limit unless project specific reporting limits are provided.

<u>Analyte Code</u>	<u>Soil</u>	<u>Water</u>
HMX	0.20 μ g/g to 100 μ g/g	0.26 μ g/L to 130 μ g/L
RDX	0.20 μ g/g to 100 μ g/g	0.26 μ g/L to 130 μ g/L
NB	0.20 μ g/g to 100 μ g/g	0.26 μ g/L to 130 μ g/L
TETRYL	0.20 μ g/g to 100 μ g/g	0.26 μ g/L to 130 μ g/L
135TNB	0.10 μ g/g to 50 μ g/g	0.13 μ g/L to 65.0 μ g/L
13DNB	0.10 μ g/g to 50 μ g/g	0.13 μ g/L to 65.0 μ g/L
246TNT	0.20 μ g/g to 100 μ g/g	0.26 μ g/L to 130 μ g/L
24DNT	0.10 μ g/g to 50 μ g/g	0.13 μ g/L to 65.0 μ g/L
26DNT	0.20 μ g/g to 200 μ g/g	0.52 μ g/L to 260 μ g/L
2NT	1.00 μ g/g to 200 μ g/g	0.52 μ g/L to 260 μ g/L
3NT	1.00 μ g/g to 200 μ g/g	0.52 μ g/L to 260 μ g/L
4NT	1.00 μ g/g to 200 μ g/g	0.52 μ g/L to 260 μ g/L
2-Am-DNT	0.20 μ g/g to 100 μ g/g	0.26 μ g/L to 130 μ g/L
4-Am-DNT	0.20 μ g/g to 100 μ g/g	0.26 μ g/L to 130 μ g/L

- 8.2 Preparation of Calibration Standards: Dry each standard using a vacuum desiccator. This step can be omitted if the standard is stored appropriately to prevent water addition and if the ICV criteria are met against a second source purchased as a solution from an external vendor.

8.2.1 Separate primary stock standards are prepared at a concentration of 1.00 mg/mL by dissolving and/or diluting known amounts of each compound in an appropriate amount of acetonitrile (see Table 1). Preparation is recorded in the concentrated stock standard preparation logbook. Primary stock standards are stored at 4°C \pm 2°C in glass test tubes with Teflon-lined screw caps.

8.2.2 A combined standard solution of intermediate concentration is prepared by following the procedures outlined in Table 2. The intermediate solution is stored at

4°C ± 2°C in a glass container with a Teflon-lined screw cap. Replace after six months, or sooner if comparison with check standards indicates a problem. Intermediate standards are replaced after 30 days.

- 8.2.3 Working standards are prepared by transferring, via syringe, portions of the intermediate solution (from Table 2) to 1 mL volumetric flasks then diluting to final volume with acetonitrile. Refer to Table 3 for the preparation of the working standards. Working standards are prepared fresh on the day of calibration.
- 8.2.4 Alternatively, commercially prepared solutions are used if they are certified by the manufacturer or an independent source. They are diluted in an appropriate amount of acetonitrile to yield the concentrations listed in Table 2.
- 8.2.5 An initial calibration verification (ICV), prepared by a third party (other than QC or the analyst) or purchased from an outside source, is prepared in the same manner and at the same concentration as the calibration standard SW8330/#4 (see Table 3). The ICV is stored at 4°C ± 2°C in a glass container with a Teflon-lined screw cap. Working standards are prepared fresh on the day of analysis.
- 8.2.6 A continuing calibration standard (CCS) is prepared in the same manner and at the same level as the calibration standard SW8330/#4 (see Table 3). The CCS is stored at 4°C ± 2°C in a glass container with a Teflon-lined screw cap. Replace after two months, or sooner if comparison with check standards indicates a problem.

8.3 Initial Calibration

- 8.3.1 The instrument is allowed to warm up for approximately 30 minutes or until a stable baseline has been established. This allows the column and detector to equilibrate.
- 8.3.2 Each of the initial calibration standards listed in Table 3 is analyzed in triplicate. For each analyte, instrument response is plotted versus standard concentration using an average response of the triplicate injections for each concentration level. A linear fit of the calibration data is used to construct the initial calibration curves. The acceptance criteria for the initial calibration curve is a correlation coefficient of 0.995 or higher. Once the acceptance criteria are met, sample results are then quantitated against the initial calibration curves.
- 8.3.3 The ICV solution is analyzed after the initial calibration standards but before any samples are analyzed. The ICV is used to verify the accuracy of the standard solutions used for calibration. The ICV solution quantitated against the initial calibration curve must be within 25% of the actual concentration of the ICV. Project specific ICV criteria will be used when available.

8.4 Continuing Calibration

- 8.4.1 A Continuing Calibration Standard (CCS) is analyzed in triplicate at the beginning of sample analysis and injected once after every ten samples and at the end of

analysis.

8.4.2 The initial calibration curve for each analyte is used to quantitate each CCS analyzed and all field samples.

8.4.3 The results for each analyte in each CCS must be within $\pm 15\%$ of the target concentration for that analyte.

8.4.3.1 Sample analyses must be bracketed between continuing calibration standards that meet the $\pm 15\%$ criteria.

8.4.4 Following appropriate corrective action, a new initial calibration for an analyte must be prepared if the CCS is not within $\pm 15\%$ of the target concentration for the analyte.

8.5 Retention Time Windows

8.5.1 Retention time windows are calculated by averaging the absolute retention times of the analytes in the continuing calibration standards collected over 72 hours, determining the standard deviation, and setting the window equal to \pm three times the standard deviation.

8.5.2 Adjustments to the retention times, if necessary, will be made based on the absolute retention time of the CCSs. Windows may be expanded based on analyst's judgement, but must be documented.

9.0 ANALYSIS

9.1 The instrument is adjusted to the parameters listed below:

9.1.1 Primary column suggested instrument conditions:

9.1.1.1 Column: 250 x 4.6 mm Phenomenex Ultracarb C18, 5 μ m ODS(20)

9.1.1.2 Eluent flow rate: 0.8 mL/minute

9.1.1.3 Column oven temperature: 28.5°C

9.1.1.4 Injection volume: 25 μ L

9.1.1.5 UV absorbance wavelength: 254 nm

9.1.1.6 Eluent profile: 46% water, 54% methanol; isocratic

9.1.2 Second column suggested instrument conditions:

9.1.2.1 Column: Waters Nova-Pak C8 and CN-HP in series, with 5- μ m particle size packing, 3.9mmX100mm each.

- 9.1.2.2 Eluent flow rate: 1.0 mL/minute
- 9.1.2.3 Column oven temperature: 29°C
- 9.1.2.4 Injection volume: 50 µL
- 9.1.2.5 UV absorbance wavelength: 254 nm
- 9.1.2.6 Eluent profile: 82% water, 18% Isopropanol; isocratic
- 9.1.3 Adjust the run time for primary or secondary column analysis so that all the analytes of interest elute off the column.
- 9.2 The instrument is operated until a stable baseline is achieved (approximately 30 minutes).
- 9.3 Mix the soil or water sample extracts and calibration standards with an equal volume of 1% calcium chloride/ortho-cresol solution. If the sample appears "cloudy," filter again through a 0.45-micron PTFE filter. The filtrate is transferred to a vial and inserted into the autosampler.
- 9.4 Perform initial calibration and continuing calibration according to Section 8.0.
- 9.5 Once calibration requirements have been met, inject the samples into the HPLC for analysis.
- 9.6 Positive results in field samples from the primary column analysis are qualitatively confirmed on the secondary column.
 - 9.6.1 A standard at the low end of the calibration range is analyzed before and after the field samples to update retention times and verify adequate sensitivity.
 - 9.6.2 A method blank is analyzed to verify the absence of interferences on the secondary column.
 - 9.6.3 Confirmation is based on the presence of a peak within an appropriate retention-time window.
 - 9.6.4 Confirmed analytes are reported using quantitative results from the primary column analysis.

10.0 QUALITY CONTROL

- 10.1 Acceptance criteria for evaluation of quality control data may be statistically determined performance based limits or fixed upper and lower acceptance limits specified by a client, contract or analytical method. The type of limits to be used and the use of project specific acceptance limits will be specified in the Project Protocol Worksheet. Refer to DCL SOP QC-DC-001 "Establishing and Updating Control Limits" and DCL SOP XX-DC-018 "Evaluation of Quality Control Data."

10.2 Method Blank

- 10.2.1 One method blank will be extracted with each analytical batch, with a maximum of 20 field samples per method blank.
- 10.2.2 The method blank must be evaluated for contamination. If any target compound is present in the method blank then follow the procedure in Figure 1.

10.3 Laboratory Control Sample (LCS)

- 10.3.1 One LCS will be extracted with each analytical batch which will not exceed 20 field samples.
- 10.3.2 The LCS verifies that the method is in control. If the LCS acceptance criteria are not met, the entire analytical batch is reextracted and reanalyzed. For evaluation of LCS data and corrective action to be taken, follow the procedures in Figure 2.

10.4 Matrix Spike Samples

- 10.4.1 A matrix spike and matrix spike duplicate will be extracted and analyzed with each analytical batch which will not exceed 20 field samples.
- 10.4.2 For evaluation of MS and MSD data and corrective action to be taken, follow the procedures in Figure 3. Specific projects may require reextraction of MS and MSD when outside the project specific acceptance criteria.

10.5 Surrogate Standards

- 10.5.1 All blanks, LCS, MS, MSD as well as all other field samples will contain 3,4-Dinitrotoluene as a surrogate standard.
- 10.5.2 For evaluation of surrogate data and corrective action to be taken, follow the procedures in Figure 4.

11.0 CALCULATIONS

- 11.1 The concentration of each analyte in the sample extract is calculated by comparing the instrument responses for sample to initial calibration curves constructed from calibration standards. The final result is reached by applying correction factors for concentration steps and dilution steps.
- 11.2 The concentration of each analyte in the sample extract is calculated using a linear regression program. The curve-fit equation is:

$$\text{Conc. in sample extract } (\mu\text{g/mL}) = \frac{\text{Instrument Response} - \text{Y Intercept}}{\text{Slope}}$$

The final result is calculated by the following equation:

Final Results ($\mu\text{g/L}$) = A x W (water concentration factor)

Final Results ($\mu\text{g/g}$) = A x S (soil concentration factor)

Where: A = Concentration in sample extract ($\mu\text{g/mL}$)

$$W = \frac{\text{Total Volume of Extract (mL)}}{\text{Volume of Sample taken for Analysis (L)}} = \frac{5\text{ mL}}{0.770\text{ L}} = 6.49\text{ mL/L}$$

$$S = \frac{\text{Total Volume of Extract (mL)}}{\text{Volume of Sample taken for Analysis (L)}} = \frac{10\text{ mL}}{2\text{ g}} = 5\text{ mL/g}$$

11.3 CCS, surrogate and check standard recovery are calculated as follows:

$$\% \text{ Recovery} = \frac{A \times W \times 100}{T} \text{ or } \frac{A \times S \times 100}{T}$$

Where: T = Target concentration in $\mu\text{g/L}$ or $\mu\text{g/g}$

11.4 Matrix spike recovery is calculated as follows:

$$\% \text{ Recovery} = \frac{(C - D) \times 100}{T}$$

Where: C = $\mu\text{g/L}$ or $\mu\text{g/g}$ result of the spiked sample

D = $\mu\text{g/L}$ or $\mu\text{g/g}$ result of the unspike sample

11.5 Relative percent difference:

$$\% \text{ RPD} = \left(\frac{C_o - C_D}{\frac{C_o + C_D}{2}} \right) \times 100$$

Where: C_o = concentration of original sample

C_D = concentration of duplicate sample

12.0 REPORTING RESULTS

12.1 Results are reported in units of $\mu\text{g/g}$ or $\mu\text{g/L}$ unless specific contracts or QA plans specify alternate reporting units.

12.2 Reporting formats and data deliverables should be in accordance with the specific contract or QA plan governing the sample analysis. See table 9 in the appendix for Explosive Compounds (Soil & Water).

13.0 REFERENCES

- 13.1 EPA Method 8330, SW846, Revision 0, September 1994. United States Environmental Protection Agency, Office of Soil Waste and Emergency Response.
- 13.2 "Spiking Solution Control," Tom Jenkins, 1987.
- 13.3 "Comparison of RP-HPLC Determination for Explosives in Water," Cold Regions Research and Engineering Laboratory, April 1988.
- 13.4 DCL SOP XX-DC-018 "Evaluation of Quality Control Data."
- 13.5 DCL SOP XX-QC-001 "Establishing and Updating Control Limits."

APPENDIX

Selected physical properties of the analytes are:

<u>Analyte Code</u>	<u>m.p.</u>	<u>Density</u>
HMX	NA	NA
RDX	203 C	1.82 g/mL
NB	5.7 C	1.20 g/mL
TETRYL	NA	NA
135TNB	122.5 C	1.76 g/mL
13DNB	89 C	1.58 g/mL
246TNT	80.1 C	1.65 g/mL
24DNT	70 C	1.32 g/mL
26DNT	66 C	1.28 g/mL
2NT	-2.9°C	1.16 g/mL
3NT	15 C	1.16 g/mL
4NT	52°C	1.30 g/mL
2-Am-DNT	NA	NA
4-Am-DNT	NA	NA
3,4-DNT	61°C	1.26 g/mL

Table 1. PRIMARY STOCK STANDARDS

<u>Analyte Code</u>	<u>Analyte Weight</u>	<u>Dilution Volume</u>	<u>Name of Concentrated Stock</u>	<u>Concentrated Stock Standard Concentrations</u>
HMX	5.00 mg	5.00 mL	SW8330-HMX/A	1,000 mg/mL
RDX	5.00 mg	5.00 mL	SW8330-RDX/A	1,000 mg/mL
135TNB	5.00 mg	5.00 mL	SW8330-135TNB/A	1,000 mg/mL
13DNB	5.00 mg	5.00 mL	SW8330-13DNB/A	1,000 mg/mL
TETRYL	5.00 mg	5.00 mL	SW8330-TETRYL/A	1,000 mg/mL
246TNT	5.00 mg	5.00 mL	SW8330-246TNT/A	1,000 mg/mL
NB	5.00 mg	5.00 mL	SW8330-NB/A	1,000 mg/mL
24DNT	5.00 mg	5.00 mL	SW8330-24DNT/A	1,000 mg/mL
26DNT	5.00 mg	5.00 mL	SW8330-26DNT/A	1,000 mg/mL
2NT	5.00 mg	5.00 mL	SW8330-2NT/A	1,000 mg/mL
3NT	5.00 mg	5.00 mL	SW8330-3NT/A	1,000 mg/mL
4NT	5.00 mg	5.00 mL	SW8330-4NT/A	1,000 mg/mL
34DNT	5.00 mg	5.00 mL	SW8330-34DNT/A	1,000 mg/mL
2A46DT	5.00 mg	5.00 mL	SW8330-2A46DT	1,000 mg/mL
4A26DT	5.00 mg	5.00 mL	SW8330-4A26DT	1,000 mg/mL

Note: Solvent of dilution is acetonitrile

Table 2. STANDARD SOLUTIONS OF INTERMEDIATE CONCENTRATION

<u>Name of Source Solution</u>	<u>Volume of Source Solution</u>	<u>Flask Volume</u>	<u>Name of Intermediate Solution</u>	<u>Analyte Code</u>	<u>Concentration of Intermediate Solution</u>
SW8330-HMX/A	200 µL	10mL	SW8330/I	HMX	20.0 µg/mL
SW8330-RDX/A	200 µL			RDX	20.0 µg/mL
SW8330-135TNB/A	100 µL			135TNB	10.0 µg/mL
SW8330-13DNB/A	100 µL			13DNB	10.0 µg/mL
SW8330-TETRYL/A	200 µL			TETRYL	20.0 µg/mL
SW8330-246TNT/A	200 µL			246TNT	20.0 µg/mL
SW8330-NB/A	200 µL			NB	20.0 µg/mL
SW8330-24DNT/A	100 µL			24DNT	10.0 µg/mL
SW8330-26DNT/A	200 µL			26DNT	20.0 µg/mL
SW8330-2NT/A	400 µL			2NT	40.0 µg/mL
SW8330-3NT/A	400 µL			3NT	40.0 µg/mL
SW8330-4NT/A	400 µL			4NT	40.0 µg/mL
SW8330-34DNT/A	200 µL			34DNT	20.0 µg/mL
SW8330-2A46DT	200 µL			2A46DT	20.0 µg/mL
SW8330-4A26DT	200 µL			4A26DT	20.0 µg/mL
	200 µL				20.0 µg/mL

Table 3. INITIAL CALIBRATION WORKING STANDARDS

<u>Name of Source Solution</u>	<u>Volume of Source Solution</u>	<u>Final Volume</u>	<u>Name of Working Standard</u>	<u>Analyte Code</u>	<u>Initial Calibration Standards Concentration</u>		
					<u>µg/mL</u>	<u>µg/g (soil)</u>	<u>µg/L (water)</u>
SW8330/I	2.0 µL	1.00 mL	SW8330/S2	A	0.020	0.10	0.13
				B	0.040	0.20	0.26
				C	0.080	0.40	0.52
SW8330/I	10 µL	1.00 mL	SW8330/S10	A	0.100	0.50	0.65
				B	0.200	1.00	1.30
				C	0.400	2.00	2.60
SW8330/I	50.0 µL	1.00 mL	SW8330/S50	A	0.500	2.50	3.25
				B	1.00	5.00	6.5
				C	2.00	10.	13.0
SW8330/I	200 µL	1.00 mL	SW8330/S200	A	2.00	10.0	13.0
				B	4.00	20.0	26.0
				C	8.00	40.0	52.0
SW8330/I	1000 µL	1.00 mL	SW8330/S1000	A	10.0	50.0	65.0
				B	20.0	100.0	130.0
				C	40.0	200.0	260.0

Note: ¹ A is 135TNB, 13DNB, and 24DNT.

² B is RDX, NB, 246TNT, TETRYL, HMX, 26DNT, 34DNT, 2A46DT, and 4A26DT.

³ C is 2NT, 3NT, and 4NT.

⁴ Solvent of dilution is acetonitrile.

Table 4. CONCENTRATED STOCK STANDARDS FOR LCS AND MATRIX SPIKE SAMPLES

<u>Analyte Code</u>	<u>Analyte Weight</u>	<u>Dilution Volume</u>	<u>Name of Primary Stock</u>	<u>Concentrated Stock Concentration</u>
RDX	60.0 mg	3.00 mL	SW8330-RDX/C	20.0 mg/mL
135TNB	50.0 mg	5.00 mL	SW8330-135TNB/C	10.0 mg/mL
246TNT	60.0 mg	3.00 mL	SW8330-246TNT/C	20.0 mg/mL
NB	60.0 mg	3.00 mL	SW8330-NB/C	20.0 mg/mL
24DNT	50.0 mg	5.00 mL	SW8330-24DNT/C	10.0 mg/mL
2NT	120 mg	3.00 mL	SW8330-2NT/C	40.0 mg/mL
2A46DT	60.0 mg	3.00 mL	SW8330-2A46DT	20.0 mg/mL

Note: Solvent of dilution is acetonitrile.

Table 5. LCS AND MATRIX SPIKING SOLUTION – SOIL

<u>Name of Source Solution</u>	<u>Volume of Source Solution</u>	<u>Flask Volume</u>	<u>Name of Matrix Spiking Solution</u>	<u>Analyte Code</u>	<u>Matrix Spiking Solution Concentrations</u>
SW8330-RDX/C	250 µL	100 mL	SW8330/MS-S	RDX	50.0 µg/mL
SW8330-135TNB/C	250 µL			135TNB	25.0 µg/mL
SW8330-246TNT/C	250 µL			246TNT	50.0 µg/mL
SW8330-NB/C	250 µL			NB	50.0 µg/mL
SW8330-24DNT/C	250 µL			24DNT	25.0 µg/mL
SW8330-2NT/C	250 µL			2NT	100.0 µg/mL
SW8330-4A26DT/C	250 µL			2A46DT	50.0 µg/mL

Note: Solvent of dilution is acetonitrile.

Table 6. LCS AND MATRIX SPIKING SAMPLES - SOIL

<u>QC Sample</u>	<u>Name of Source Solution</u>	<u>Volume of Source Solution</u>	<u>Sample Weight</u>	<u>Analyte Code</u>	<u>QC Sample Concentrations</u>
LCS	SW8330/MS-S	1.0 mL	2 g	RDX	25.0 µg/g
				135TNB	12.5 µg/g
				246TNT	25.0 µg/g
				NB	25.0 µg/g
				24DNT	12.5 µg/g
				2NT	50.0 µg/g
				2A46DT	25.0 µg/g
MS/MSD	SW8330/MS	1.0 mL	2 g	RDX	25.0 µg/g
				135TNB	12.5 µg/g
				246TNT	25.0 µg/g
				NB	25.0 µg/g
				24DNT	12.5 µg/g
				2NT	50.0 µg/g
				2A46DT	25.0 µg/g

Table 7. LCS AND MATRIX SPIKING SOLUTION - WATER

<u>Name of Source Solution</u>	<u>Volume of Source Solution</u>	<u>Flask Volume</u>	<u>Name of Matrix Spiking Solution</u>	<u>Analyte Code</u>	<u>Matrix Spiking Solution Concentrations</u>
SW8330-RDX/C	96.3 μ L	200 mL	SW8330/MS-W	RDX	9.63 μ g/mL
SW8330-135TNB/C	96.3 μ L			135TNB	4.81 μ g/mL
SW8330-246TNT/C	96.3 μ L			246TNT	9.63 μ g/mL
SW8330-NB/C	96.3 μ L			NB	9.63 μ g/mL
SW8330-24DNT/C	96.3 μ L			24DNT	4.81 μ g/mL
SW8330-2NT/C	96.3 μ L			2NT	19.3 μ g/mL
SW8330-4A26DT/C	96.3 μ L			2A46DT	9.63 μ g/mL

Note: Solvent of dilution is acetonitrile.

Table 8. LCS AND MATRIX SPIKING SAMPLES – SOIL

<u>QC Sample</u>	<u>Name of Source Solution</u>	<u>Volume of Source Solution</u>	<u>Sample Weight</u>	<u>Analyte Code</u>	<u>QC Sample Concentrations</u>
LCS	SW8330/MS-W	1.0 mL	770 mL	RDX	25.0 µg/L
				135TNB	12.5 µg/L
				246TNT	25.0 µg/L
				NB	25.0 µg/L
				24DNT	12.5 µg/L
				2NT	50.0 µg/L
				2A46DT	25.0 µg/L
MS/MSD	SW8330/MS-W	1.0 mL	700 mL	RDX	25.0 µg/L
				135TNB	12.5 µg/L
				246TNT	25.0 µg/L
				NB	25.0 µg/L
				24DNT	12.5 µg/L
				2NT	50.0 µg/L
				2A46DT	25.0 µg/L

Table 9: Explosive Compounds (Soil & Water)

Compound	Soil ug/g		LCS	MS/MSD	RPD
	MDL	PQL	Control Limit	Control Limit	Control Limit
1,3,5-Trinitrobenzene	0.0970	0.10	65-125	65-125	35
1,3-Dinitrobenzene	0.0626	0.10	65-125	65-125	35
2,4,6-Trinitrotoluene	0.133	0.20	65-125	65-125	35
2,4-Dinitrotoluene	0.0721	0.10	65-125	65-125	35
2,6-Dinitrotoluene	0.130	0.20	65-125	65-125	35
2-Amino-4,6-dinitrotoluene	0.132	0.20	65-125	65-125	35
2-Nitrotoluene	0.209	0.40	65-125	65-125	35
3-Nitrotoluene	0.253	0.40	65-125	65-125	35
4-Amino-2,6-dinitrotoluene	0.119	0.20			
4-Nitrotoluene	0.191	0.40	65-125	65-125	35
HMX	0.0830	0.20	65-125	65-125	35
Nitrobenzene	0.0584	0.20	65-125	65-125	35
RDX	0.133	0.20	65-125	65-125	35
Tetryl	0.145	0.20	65-125	65-125	35
3,4-Dinitrotoluene	0.182	0.2	65-125		

Compound	Water ug/L		LCS	MS/MSD	RPD
	MDL	PQL	Control Limit	Control Limit	Control Limit
1,3,5-Trinitrobenzene	0.0557	0.65	82.2 - 117.6	74.5 - 123.1	15
1,3-Dinitrobenzene	0.0507	0.65	90.7 - 121.3	78.0 - 134.0	15
2,4,6-Trinitrotoluene	0.0797	0.26	87.7 - 112.3	75.2 - 124.4	17
2,4-Dinitrotoluene	0.0521	0.65	78.3 - 116.7	68.7 - 125.7	15
2,6-Dinitrotoluene	0.0957	0.26	82.5 - 121.5	76.2 - 127.8	14
2-Amino-4,6-dinitrotoluene	0.116	0.26	89.0 - 121.0	77.5 - 130.5	15
2-Nitrotoluene	0.349	0.52	89.8 - 112.2	78.7 - 119.9	14
3-Nitrotoluene	0.446	0.52	85.5 - 113.1	77.7 - 119.1	16
4-Amino-2,6-dinitrotoluene	0.153	0.26			
4-Nitrotoluene	0.296	0.52	87.5 - 116.5	79.6 - 122.4	16
HMX	0.0991	0.26	75.4 - 115.6	71.8 - 116.8	14
Nitrobenzene	0.0670	0.26	89.7 - 112.3	78.4 - 120.6	13
RDX	0.0599	0.26	78.6 - 116.2	65.5 - 122.2	14
Tetryl	0.0993	0.26	87.8 - 120.2	64.1 - 143.9	17
3,4-Dinitrotoluene	0.159	0.26	50-150		

The values given in this table change due to specific project requirements and/or updates by the laboratory.

FIGURE 1
ORGANIC METHOD BLANK

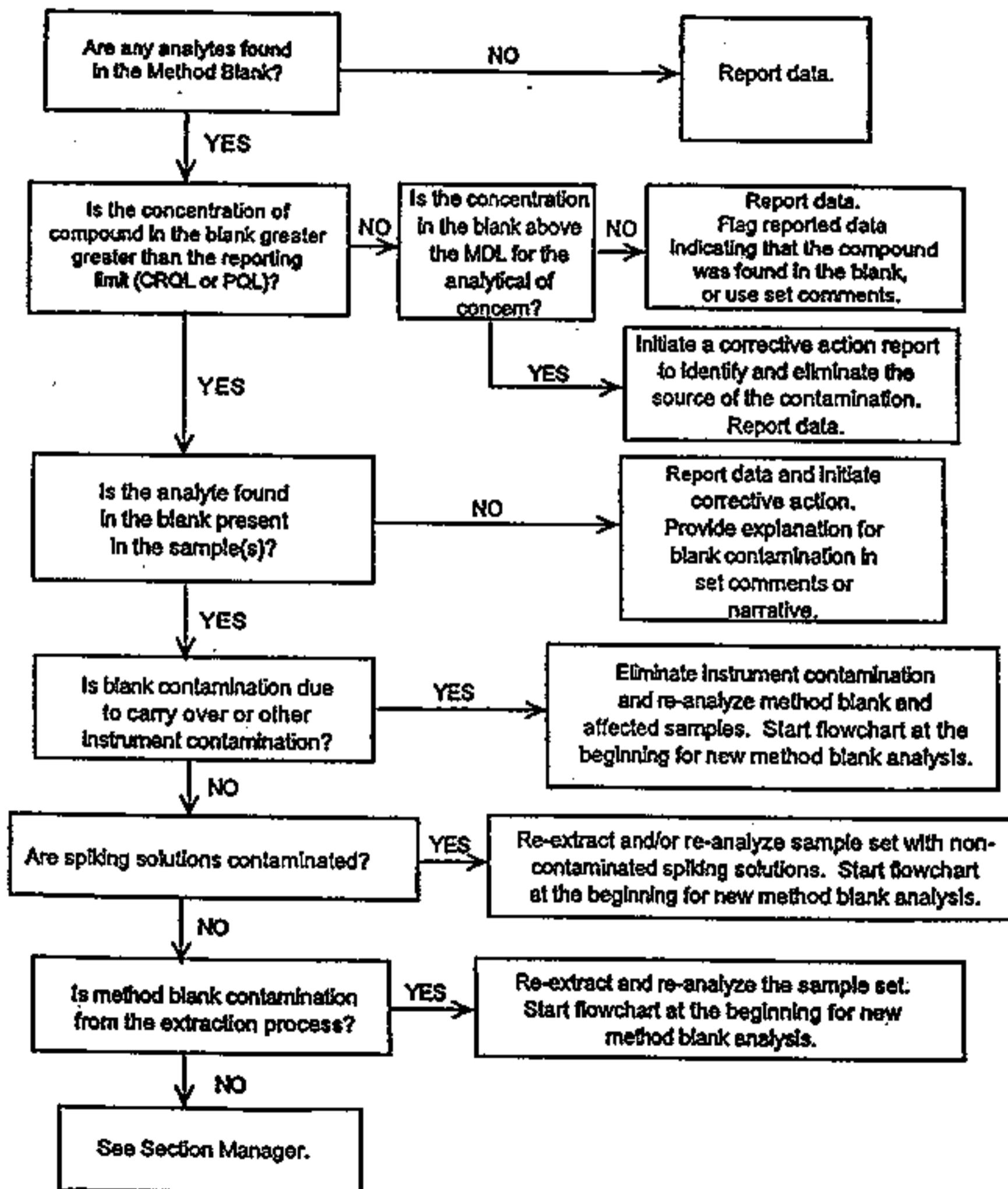


FIGURE 2
 ORGANIC LCS

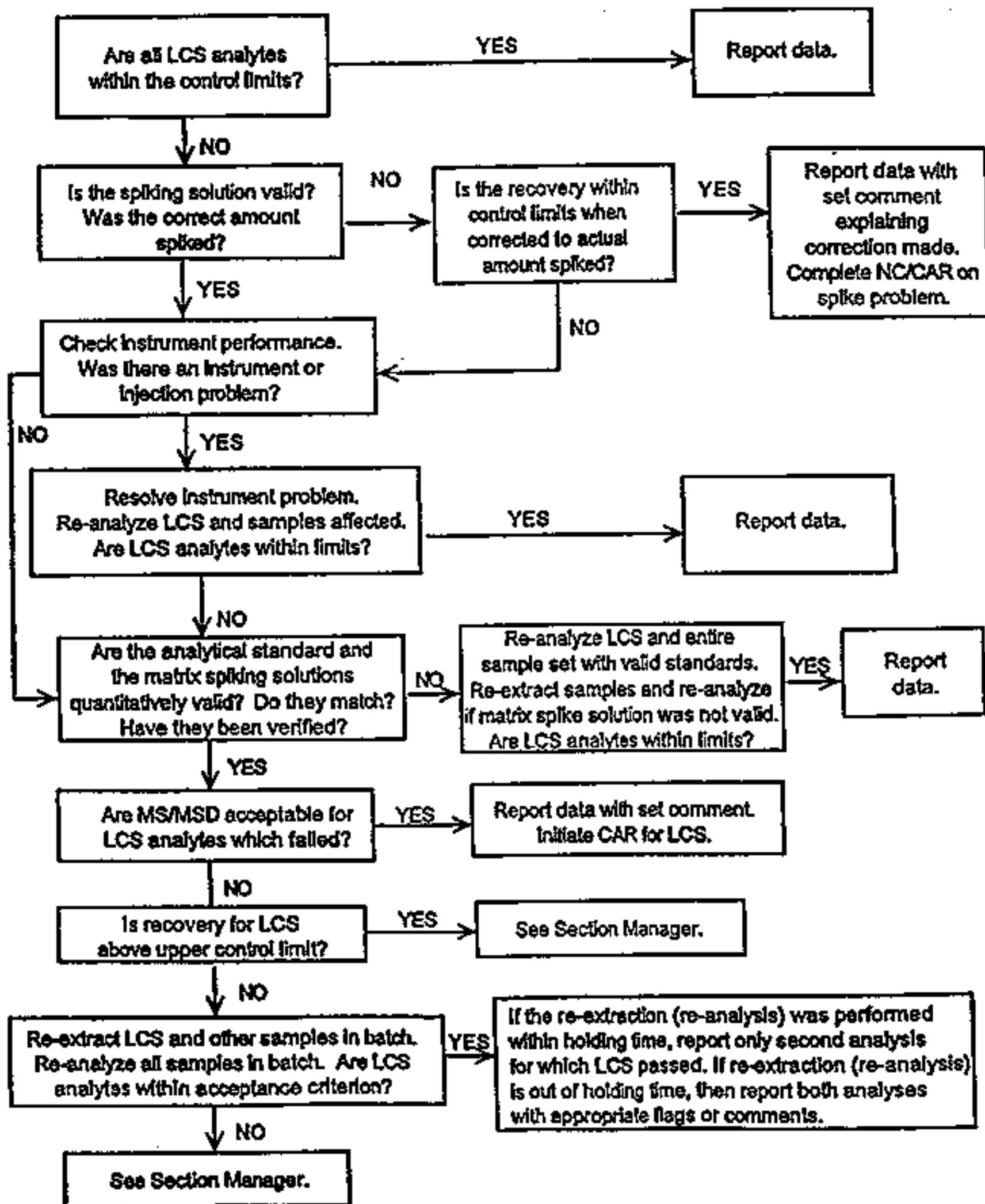


FIGURE 3
ORGANIC MS AND MSD

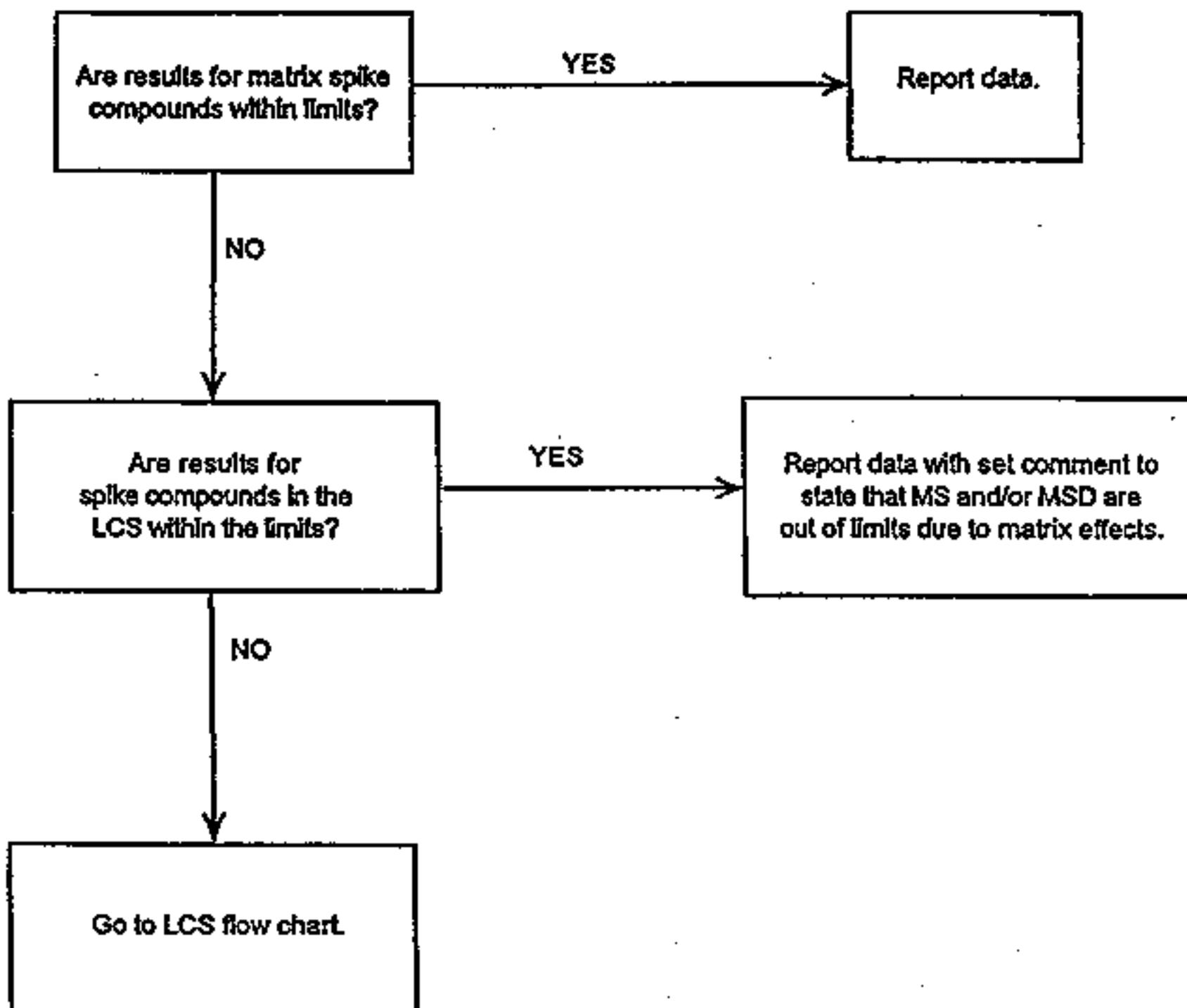
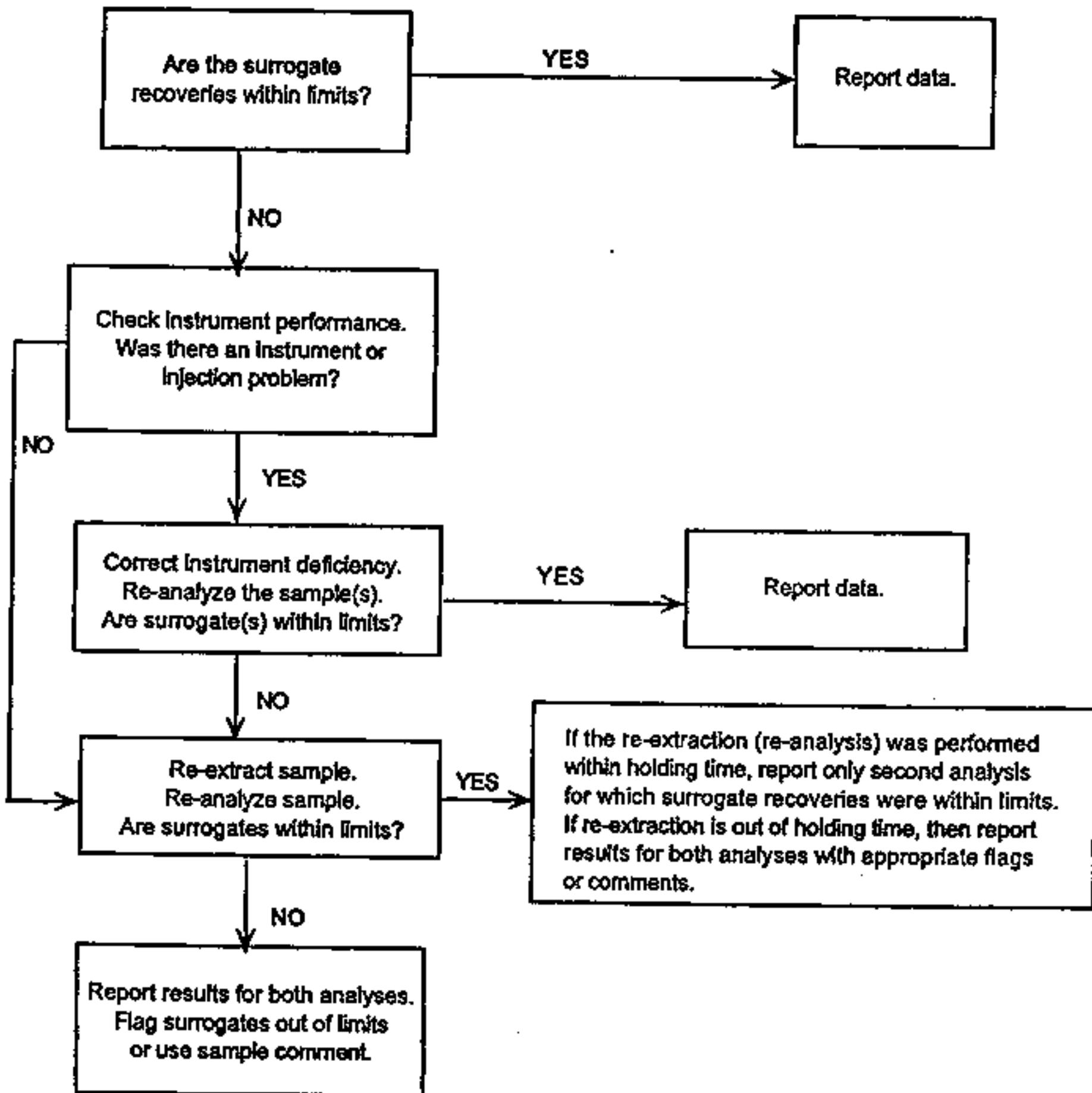
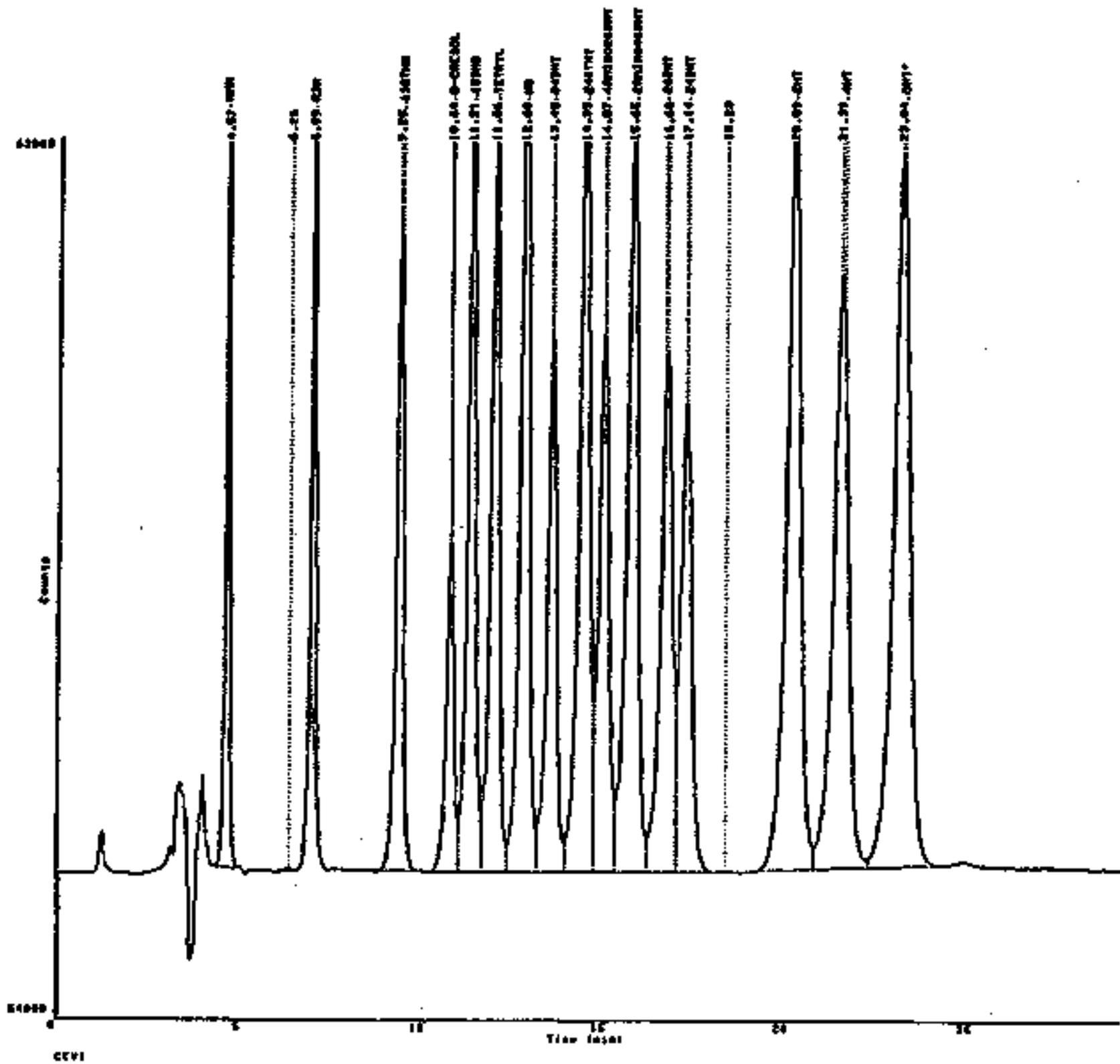


FIGURE 4
ORGANIC SURROGATE



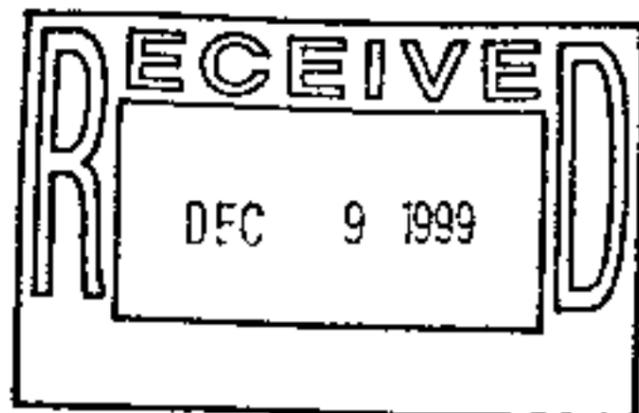


8330 72 Hour RT Windows

Sequence: LC8-1101

Analyte	RT Low	RT High	CCV RT	0 Hr RT	Mid Hr RT	72 Hr RT	3xStDv
HMX	4.547	4.571	4.559	4.567	4.563	4.559	0.012
RDX	7.032	7.102	7.067	7.089	7.071	7.067	0.035
1,3,5-Trinitrobenzene	9.629	9.713	9.671	9.699	9.682	9.671	0.042
1,3-Dinitrobenzene	11.734	11.870	11.802	11.847	11.821	11.802	0.068
Tetryl	12.566	12.732	12.649	12.704	12.675	12.649	0.083
Nitrobenzene	13.387	13.541	13.464	13.515	13.492	13.464	0.077
3,4-Dinitrotoluene	14.417	14.619	14.518	14.585	14.558	14.518	0.101
2,4,6-Trinitrotoluene	15.255	15.431	15.343	15.401	15.381	15.343	0.088
4-Amino-2,6-dinitrotoluene	15.806	16.094	15.950	16.046	16.002	15.950	0.144
2-Amino-4,6-dinitrotoluene	16.603	16.915	16.759	16.863	16.816	16.759	0.156
2,6-Dinitrotoluene	17.767	18.013	17.885	17.968	17.943	17.885	0.128
2,4-Dinitrotoluene	18.324	18.598	18.461	18.550	18.522	18.461	0.137
2-Nitrotoluene	21.684	21.990	21.837	21.933	21.914	21.837	0.153
4-Nitrotoluene	23.147	23.479	23.313	23.418	23.396	23.313	0.166
3-Nitrotoluene	24.969	25.315	25.142	25.250	25.231	25.142	0.173

Default Minimum Window=+/- 0.030 minutes



RT Windows For Analytical Sequence From 72 Hr RT Study

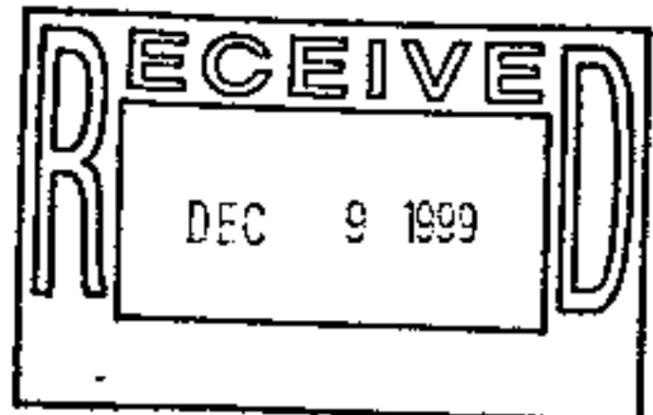
Sequence: LC3-1201,LX3-1202, LC3-1203

	RT Low	RT High	CCV RT	0 Hr RT	Mid Hr RT	72 Hr RT	3xStDv
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Datafile:

Analyte

HMX	4.285	4.307	4.296	4.296	4.289	4.293	0.011
RDX	5.081	5.099	5.090	5.090	5.084	5.086	0.009
1,3,5-Trinitrobenzene	4.007	4.017	4.012	4.012	4.009	4.012	0.005
1,3-Dinitrobenzene	6.382	6.410	6.396	6.396	6.392	6.401	0.014
Tetryl	9.567	9.605	9.586	9.586	9.576	9.588	0.019
Nitrobenzene	8.958	9.030	8.994	8.994	8.991	9.013	0.036
3,4-Dinitrotoluene	16.085	16.291	16.188	15.848	15.804	15.872	0.103
2,4,6-Trinitrotoluene	7.311	7.339	7.325	7.325	7.321	7.330	0.014
4-Amino-2,6-dinitrotoluene	17.827	18.237	18.032	18.032	17.992	18.125	0.205
2-Amino-4,6-dinitrotoluene	16.047	16.343	16.195	16.195	16.161	16.258	0.148
2,6-Dinitrotoluene	14.580	14.796	14.688	14.688	14.671	14.740	0.108
2,4-Dinitrotoluene	12.346	12.464	12.405	12.405	12.394	12.432	0.059
2-Nitrotoluene	19.990	20.400	20.195	20.195	20.159	20.291	0.205
4-Nitrotoluene	19.475	20.015	19.745	19.745	19.874	19.918	0.270
3-Nitrotoluene	22.060	22.490	22.275	22.275	22.235	22.374	0.215

Default Minimum Window= \pm 0.030 minutes

December 22, 1999

Via Facsimile

Bob DiRienzo
DataChem Laboratories, Inc.
960 West LeVoy Drive
Salt Lake City, UT 84123

Subject: SOP for Manual Integration on GC, HPLC, IC, and GC/MS Data Systems

Dear Mr. DiRienzo:

Parsons Engineering Science, Inc. (Parsons ES) received DataChem's Standard Operation Procedure (SOP) for Manual Integration on GC, HPLC, IC, and GC/MS Data Systems (Document Control Number XX-DCX-024 Revision 0) on December 9, 1999. After reviewing the document, Parsons ES has concluded that the submitted SOP does not meet either AFCEE's or Parsons' project requirements.

Specifically, Section 1.2 of the above-referenced SOP states "This SOP process supersedes any client, method or other SOP QC requirements." Parsons ES does not accept this language. As a potential client for DataChem Laboratories, we want this sentence to be changed to "Client's QAPP or other project requirements may supersede this SOP."

In addition, as stated under the corrective action items for SW-846 Method 8330 of Parsons ES On-Site Evaluation Report (October 1999), AFCEE has specific requirements for manual integration. They are listed on Page 4 of the On-Site Evaluation Report as well as below:

AFCEE requires an SOP for manual integration that should address and detail the following issues:

- Description of conditions that lead to manual integration,
- Factors that constitute technically acceptable manual integration events, and
- Proper manual integration procedures with multi-tiered review and approval processes.

In cases where manual integration is performed, AFCEE also requires that the following be submitted with the final data package:

- Reasons(s) for manual integration,
- Signature of the analyst and supervisor, and
- A 'before' and 'after' report with all the raw data.

Please respond with your decision to comply with Parsons' request in writing by 2 PM central time on December 23, 1999. If DataChem agrees to revise this SOP by including all the items listed above, please submit the document by December 28, 1999.

Please call me at (512) 719-6092 if you have any questions.

Sincerely,



Tammy Chang
Senior Scientist

cc: Nancy Stein, AMC
Brian Murphy, CSSA
Jo Jean Mullen, AFCEE/ERD
Ed Brown, AFCEE/ERC
Jonathan Decker, Parsons ES-St. Louis
Susan Roberts, Parsons ES-Austin
Julie Burdey, Parsons ES-Austin
Ken Rice, Parsons ES-Austin
Karuna Mirchandani, Parsons ES-Austin

DATA CHEM LABORATORIES, INC.

STANDARD OPERATING PROCEDURE APPROVAL SHEET

SOP TITLE: Manual Integration for AFCEE projects using method SW846 8330

DOCUMENT CONTROL NUMBER: OL-SW-8330MI

EFFECTIVE DATE: January 10, 2000

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SUBJECT TO REVISION WITHOUT NOTICE

APPROVALS:

MANAGER

Richard H. Stude

Date 1-5-2000

QA MANAGER

R. P. R.

Date 1-5-00

LAB DIRECTOR

James H. ...

Date 1-3-00

STANDARD OPERATING PROCEDURE

Manual Integration

1.0 SCOPE AND APPLICATION

- 1.1 This Standard Operating Procedure (SOP) summarizes the DCL manual integration process used by analysts on AFCEE projects to manually integrate peak areas using chromatographic software. Those individuals involved with SW846 method 8330 use this procedure.
- 1.2 Procedures designated in the AFCEE QAP 3.0 or by the client may supersede requirements specified in this SOP.

2.0 RESPONSIBILITY

- 2.1 It is the responsibility of each operations manager to train analysts concerning procedures outlined in this SOP.
- 2.2 It is the responsibility of the analyst to process data in accordance with Section 4.0 and Section 5.0 of this SOP and to ensure that appropriate data are available in data packages as required.
- 2.3 Extreme caution is required when using manual integration, particularly when the integration affects method compliance. Each manual integration is documented in the data package and a technical justification provided.

3.0 DEFINITIONS

- 3.1 Manual Integration is defined as the process by which an analyst can set the baseline of a peak for reprocess and quantitation, which is different than the automated process set by the run parameters.

- 3.2 Acronyms

None

4.0 GENERAL PROCEDURES

4.1 The following conditions are the only technically acceptable reasons for using manual integration: See Appendix 1 for examples of poorly integrated peaks.

- ◆ **Peak Tailing**

The peak in question has an excessive amount of area (>10%) added to it due to peak tailing. Peak tailing is a delayed return to the chromatographic baseline. This condition only applies if peak tailing is not present in standard chromatograms.

- ◆ **Peaks are split by chromatography software**

A Chromatographic peak is split into two separately integrated peaks due to noise levels at the peak.

- ◆ **Well-defined peaks on shoulders of other peaks.**

Baseline resolution is impossible due to interfering peaks present in the chromatogram. The integration must add area to the peak or miss the peak entirely.

- ◆ **Baseline noise - Signal to noise ratio <3**

Peaks are small compared to the baseline of the chromatogram. The ratio of the peak height (measured from the center of the noise on the baseline to the center of the noise on the peak maximum) to baseline noise (measured from the minimum of the noise on the baseline to the maximum of the noise on the baseline) is less than 3. The condition must exist where multiple peaks are integrated as one or the peak is missed entirely.

- ◆ **Negative Spikes in baseline**

Downward spikes in the chromatographic baseline have caused the integration to add significant area to the peak. This happens only when peak integration begins at the bottom of the negative spike.

- ◆ **Rising or falling baselines**

The chromatographic system is experiencing baseline events, either rising or falling baselines, due to temperature fluctuations, carryover, or column bleed. The peak in question must have significant area (>10%) added before manual integration is used.

- ◆ The chromatographic software identifies the wrong peak.

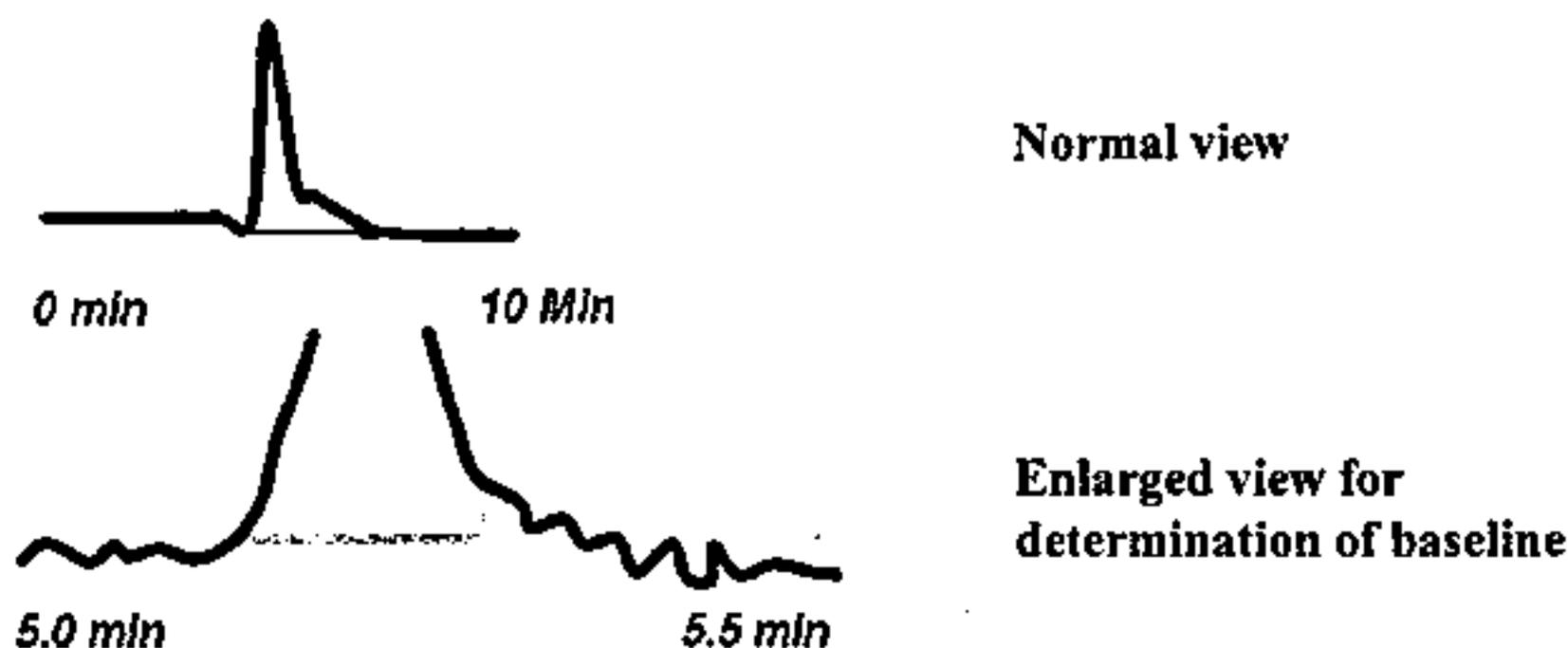
The peak is present but for some reason was not integrated by the chromatographic software. This occurs when peak integration parameters are incorrectly set.

- 4.2 Manual integration is used to provide accurate quantitation of peaks areas where the original integration provided by the data system is in error. Additionally, in some cases the data system may call the wrong peak and must be corrected by manual integration.

Manual integration is used only when conditions in section 4.1 are present. All other cases not cited in section 4.1 must be approved by the QA manager or operations manager prior to use.

Manual integration is never used with the intent of passing calibration criteria or other method/QC compliance.

- 4.3 To carry out a manual integration it is necessary to determine the chromatographic baseline and the start and stop points of the peak along the baseline. When manual integration is used it is best to zoom in on the baseline and display in a window of less than 0.5 minutes (See Figure below). A technically sound rule for determination of a baseline is the average noise (measured from the minimum of the noise on the baseline to the maximum of the noise on the baseline). Set start and stop points for the peak at the average noise of the baseline. Manual integration should only be used when peaks are clearly resolved with a valley between peaks. All manual integration must be clearly documented as discussed in section 5.0. See Appendix 2 for properly integrated chromatograms.



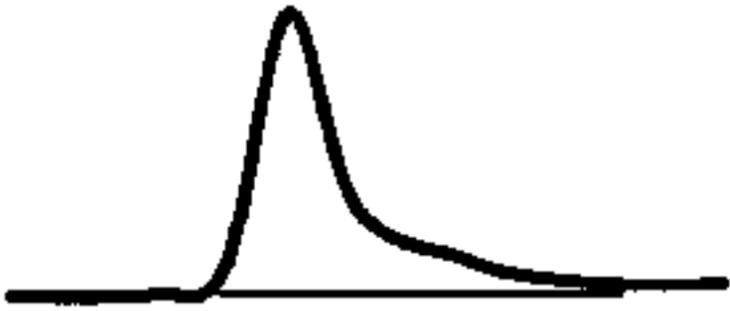
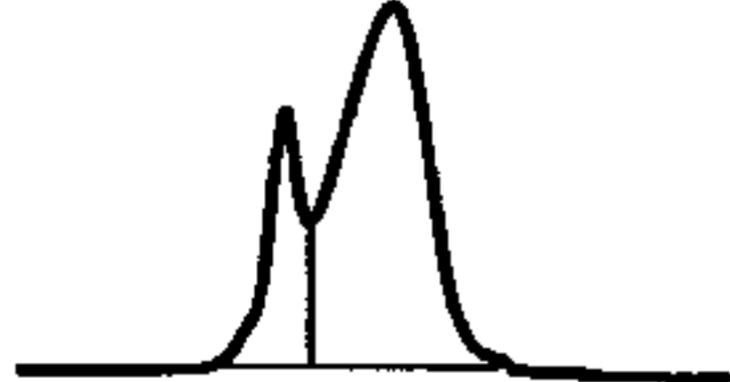
5.0 OPERATIONAL SECTION PROCEDURES

Both the original and manually integrated chromatograms must be present in the data package for peer review. The manually integrated peak must be clearly defined by using a reason described in section 4.1 as justification for the manual integration. A signature of the analyst, peer reviewer, and supervisor is required. A before and after quantitation is included with the chromatogram. All original and manually integrated chromatograms are included in the data package.

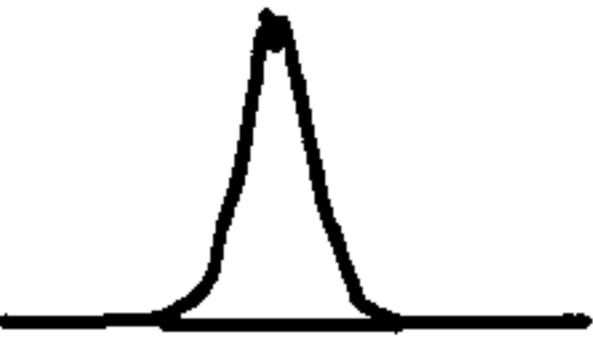
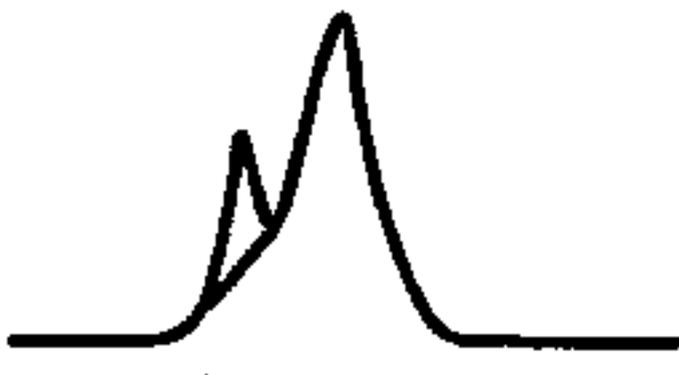
6.0 REFERENCES

- 6.1 DCL SOP XX-DC-020 "Deliverable and Data Package Preparation and Review."
- 6.2 DCL SOP XX-DC-023 "Peer Review"

Appendix 1 - Improper Peak Integration

Peak Tailing:	
Split Peaks	
Peaks on shoulders	
Baseline Noise	
Negative Spikes	
Rising and falling baselines	

Appendix 2 - Acceptable Peak Integration

Peak Tailing:	
Split Peaks	
Peaks on shoulders	
Baseline Noise	
Negative Spikes	
Rising and falling baselines	