Appendix 1: Quantitative migration of contaminants in recycled HDPE by GC-FID and GC-MS

➢ **Method**

Procedure:

1. Prepare 50% ethanol solution (simulant D1) as the food simulant.
2. Use rHDPE specimens with a total surface area of approximately 0.42dm² per 35mL of simulant.
3. Expose rHDPE samples to simulant D1 at 55 ± 2°C for a minimum of 4 hours.
4. Cool samples for at least 30 minutes.
5. Add 100 ± 1µL Internal Standard Solution to test vials (except blank and NOIS).
6. Add 2 ± 0.1mL heptane to each vial.
7. Shake vigorously and allow to settle for 10 minutes.
8. Extract ≥1mL of heptane layer into GC vials.

GC-FID Analysis:

- Column: Zebron ZB-5HT 30m x 530µm x 0.15µm
- Oven: 70°C (5 min) → 20°C/min to 320°C (4.5 min hold)
- Injector: Cool on-column, 2µL injection
- Detector: FID at 340°C

GC-MS Analysis (for unknown identification):

- Column: Rxi-5ms 30m x 250µm x 0.25µm
- Oven: 70°C (5 min) → 20°C/min to 320°C (9.5 min hold)
- Injector: Splitless, 300°C, 5µL injection
- MS parameters: Transfer line 320°C, Source 230°C, Quad 150°C, Mass range 12-700 m/z

Internal Standards:

- Pentadecane (PD)
- Bis(1-butylpentyl) adipate (DNA)
- Glyceryl trioctanoate (GT)

Calibration Substances:

1. Limonene (LIM)
2. 1-Dodecene (DOD)
3. Hexyl salicylate (HS)
4. α-Hexylcinnamaldehyde (HCA)
5. 2-Ethylhexyl benzoate (EHB)
6. Isopropyl myristate (IPM)
7. Isopropyl palmitate (IPP)
8. Dibutyl Sebacate (DBS)
9. Irgafos168 (FOS)
10. Irganox1076 (NOX)
11. Oleic acid (OA)
12. Palmitic acid (PA)

Surface Area to Volume Ratio:

- The test uses 0.42dm² of rHDPE surface area per 35mL (0.035 kg) of simulant.
- This results in a ratio of 0.42 dm² / 0.035 kg = 12 dm²/kg

- Results are reported normalized to the EU conventional food packaging ratio of 6dm²/kg.

Simulant:

- Simulant D1 (50% ethanol) is used as per EU Regulation 10/2011 for dairy products.

Calibration Range:

The calibration range is established using four levels of working calibration solutions:

Cal. Level 1: 250 ± 10µL of Cal. Soln. in 25 ± 0.5mL of 50% (v/v) ethanol

1. Concentration: Approximately 10 ppb (0.01 mg/kg)

Cal. Level 2: 500 ± 10µL of Cal. Soln. in 25 ± 0.5mL of 50% (v/v) ethanol

2. Concentration: Approximately 20 ppb (0.02 mg/kg)

Cal. Level 3: 750 ± 10µL of Cal. Soln. in 25 ± 0.5mL of 50% (v/v) ethanol

3. Concentration: Approximately 30 ppb (0.03 mg/kg)

Cal. Level 4: 1000 ± 10µL of Cal. Soln. in 25 ± 0.5mL of 50% (v/v) ethanol

4. Concentration: Approximately 40 ppb (0.04 mg/kg)

Quality Control (QC) and Limit of Detection (LOD):

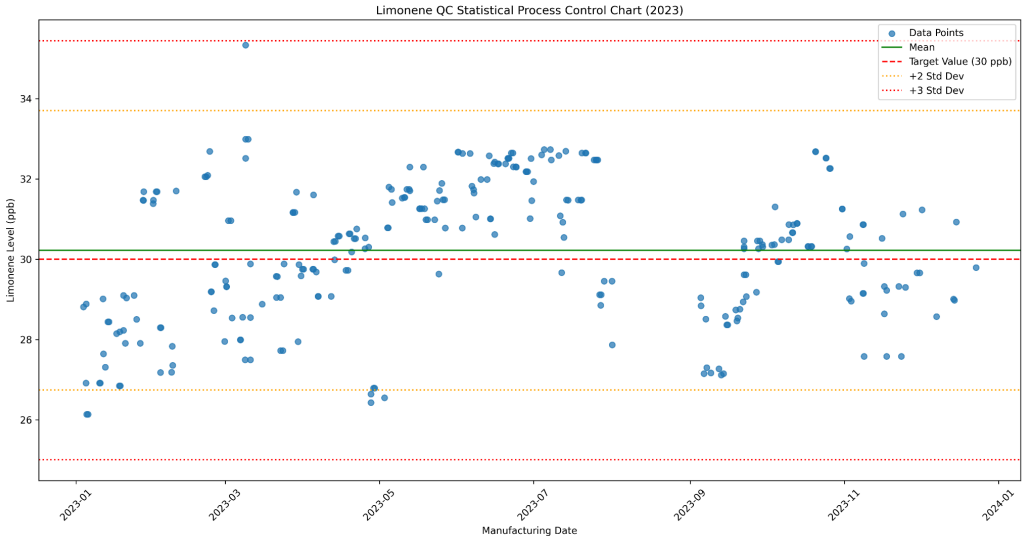
1. GC Calibration Performance Criteria:
    - Correlation coefficient: ≥0.99 for all calibration curves
    - Calibrated limit of detection of dodecane: ≤10ppb
    - 1-Dodecene in QC: Detected
    - Limonene in QC: 30 ppb
    - Dodecene symmetry in Cal. Level 4 (FID only) or Pentadecane symmetry: 0.8-1
2. QC Sample:
    - Prepared with Limonene (30 ppb), 1-Dodecene
    - Used to verify method performance
3. Limit of Detection (LOD):
    - Calculated by regression analysis of the calibration curve
    - Standard error in predicted concentration divided by gradient and multiplied by 3.3
4. Uncalibrated unknown substances:
    - Specific migration limit of 0.01 mg/kg applied (as per EU Reg 10/2011)
    - GC-MS used for identification of unknown peaks

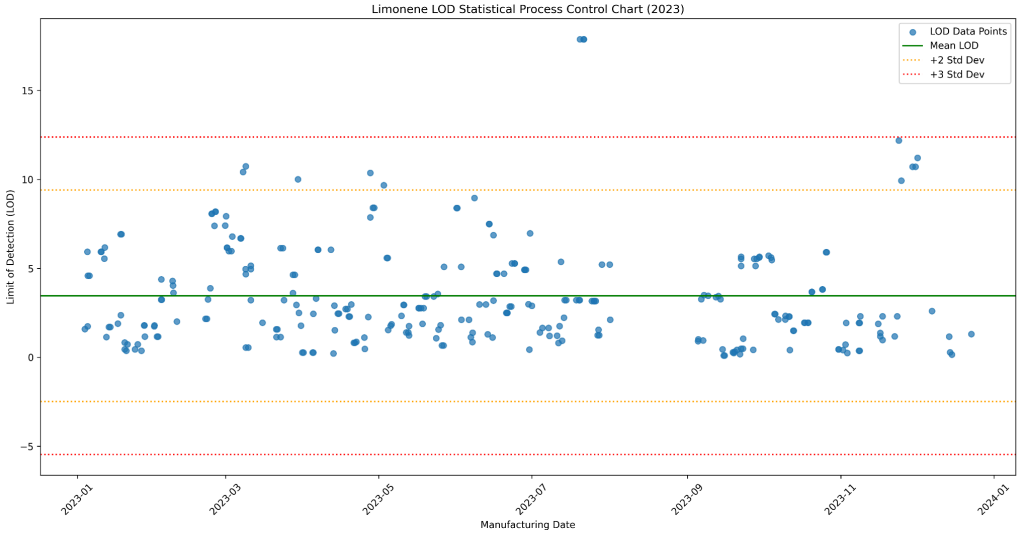
Calculations:

- Migration (mg/kg) calculated based on GC results and sample surface area
- Results normalized from 12 dm²/kg to 6 dm²/kg for reporting
- Maximum blend rate (%) calculated for each substance based on migration limits

➢ Method validation and QC performance

Number of data points: 280, Mean: 30.22 ppb, Standard Deviation: 1.74 ppb, RSD%: 5.75%, Bias from target value (30 ppb): 0.75%, Uncertainty of Measurement: 5.80%





LOD by Standard Error of the Regression

Number of LOD data points: 279, Average LOD: 3.4568 ppb ,LOD: 1.7284 ppb for 50%,

For 40% blend the LOD = 1.38272 ppb, LOQ = 2.8 ppb

The assumption in the analysis is that all substances will give a similar response with the flame ionisation detector (FID) and that the substances are amenable to extraction with heptane and GC analysis. Substances less amenable to heptane extraction and GC analysis will be polar and ionic substances that may be present in the feedstock, but will be easily removed in the washing and cleaning stages and will not therefore be expected to be present in the cleaned HDPE.
The FID responses are similar for the substances used as internal markers and this is expected to hold true also for most organics that will have an affinity for, and are compatible with HDPE, although highly halogenated substances will have a lower response.
The GC-FID analysis was conducted using cold on-column injection. This provides a more sensitive analysis than the traditional splitless technique and extends the range of substances covered up to a molecular weight of around 1000 Daltons.
This method allows for quantitative evaluation of substance migration from rHDPE, with results used to determine safe blend rates with virgin HDPE for food contact applications. The use of simulant D1, the specific surface area to volume ratio, and the normalization of results to 6 dm^2/kg ensure compliance with EU regulations. The QC measures and LOD calculations ensure the reliability and sensitivity of the analysis. The use of GC-MS for unknown identification provides additional confidence in the comprehensive assessment of potential migrants.
The calibration range covers from the lowest concentration of interest (10 ppb, which aligns with the general migration limit for non-listed substances in EU Reg 10/2011) up to 40 ppb, allowing for accurate quantification within this range. This ensures that the method can accurately quantify substances from trace levels up to concentrations that approach or exceed regulatory limits, providing a robust analytical approach for assessing migration from rHDPE materials.

Appendix 2: Quantitative Determination of Limonene Concentration in HDPE by Headspace GC-MS

➢ Method

Purpose:
To determine the concentration of limonene evolving from HDPE by headspace analysis using GC-MS.
Scope:
Primarily for FG rHDPE pellets, but applicable to other forms of HDPE such as flakes or purge.
Principle:
A 1g HDPE sample is heated in a sealed vial to allow gases to escape into the headspace. The gases are analyzed by GC-MS, with limonene quantified using standard addition and an internal standard.
Equipment:

- Agilent 7890A GC
- Agilent 5975 MS
- Gerstel MPS with headspace autosampler

- MSD Chemstation
- Analytical balance (d 0.1mg)
- Various lab equipment (fridge, oven, desiccator, pipettes, vials, etc.)

Reagents:

- D-Limonene ≥ 99%
- Toluene ≥ 99% (Internal Standard)
- Methanol
- Low limonene HDPE pellets (< 1 ppm)
- Positive control HDPE pellets

Procedure:

1. Sample Preparation:
    - Weigh 1 ± 0.02 g of sample into a 20 mL headspace vial
    - Spike with 10 ± 0.5 µL of 0 ppm calibration solution (internal standard)
    - Seal with magnetic cap and PTFE/silicone septum
2. GC-MS Analysis:
    - Place sample in autosampler tray
    - Set up sequence in MSD Chemstation
    - Run analysis (approximately 2 hours per sample)
3. Calibration:
    - Prepare calibration solutions (0, 0.5, 1, 2, 3 ppm limonene)
    - Prepare calibration samples using low limonene HDPE
    - Run calibration samples weekly or after Atune
4. QC Samples:
    - Run daily QC samples (QC Sample and 0.5 PPM)

GC-MS Parameters:

- Column: Rxi-5ms 30 m x 250 µm x 0.25 µm
- Inlet: Split, 250°C
- Oven: 40°C (2 min) → 20°C/min to 300°C (5 min hold)
- MS: Transfer line 320°C, Source 230°C, Quad 150°C, Mass range 30-550 m/z
- Headspace: Incubator 120°C for 90 min, 2500 µL injection

Calculations:

- Limonene concentration determined by ratio of limonene to toluene response
- Calibration curve used to convert ratio to ppm
- Matrix effect factored in using x-intercept of calibration curve

Quality Control:

- Daily QC samples
- Weekly calibration
- Atune of GC-MS system before calibration

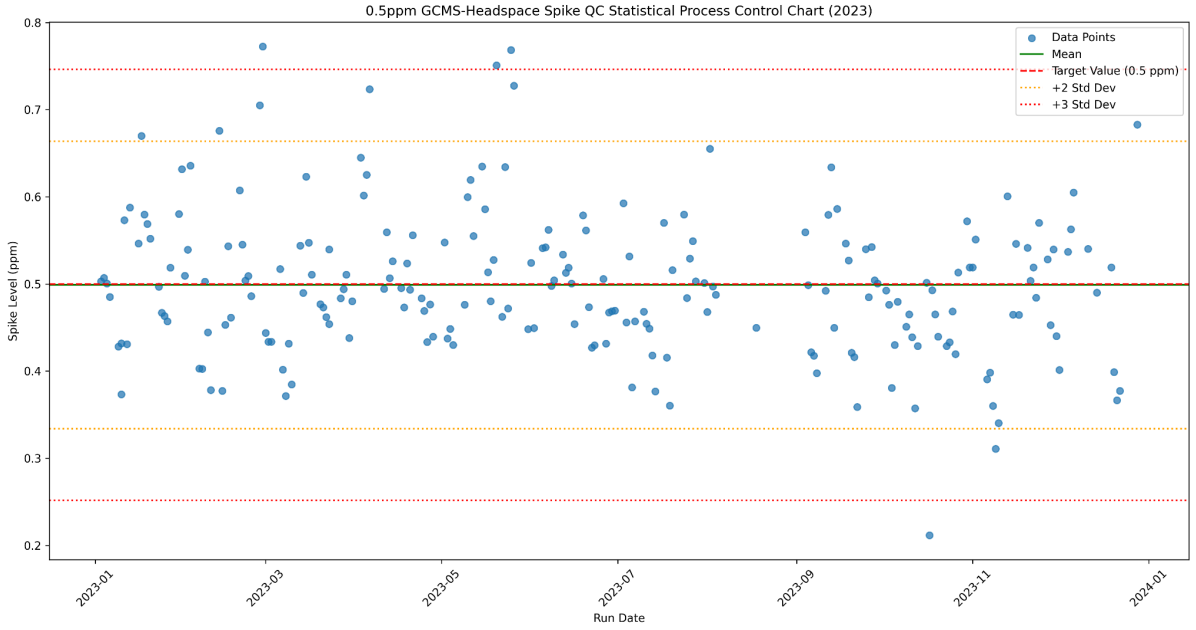
This method allows for the quantitative determination of limonene in HDPE samples, with a focus on recycled materials. The use of headspace analysis, internal standards, and matrix-matched calibration ensures accurate results for this volatile compound. The detailed QC measures and frequent system checks maintain the reliability of the analysis.

This method allows for the comprehensive analysis of decontamination performance for volatile compounds. It is quantitative and quicker than the migration testing so more frequent measurements can be made. The decontamination process can be monitored much closer to real-time. Also comparing input (flakes) and output (pellets) gives an ongoing assessment

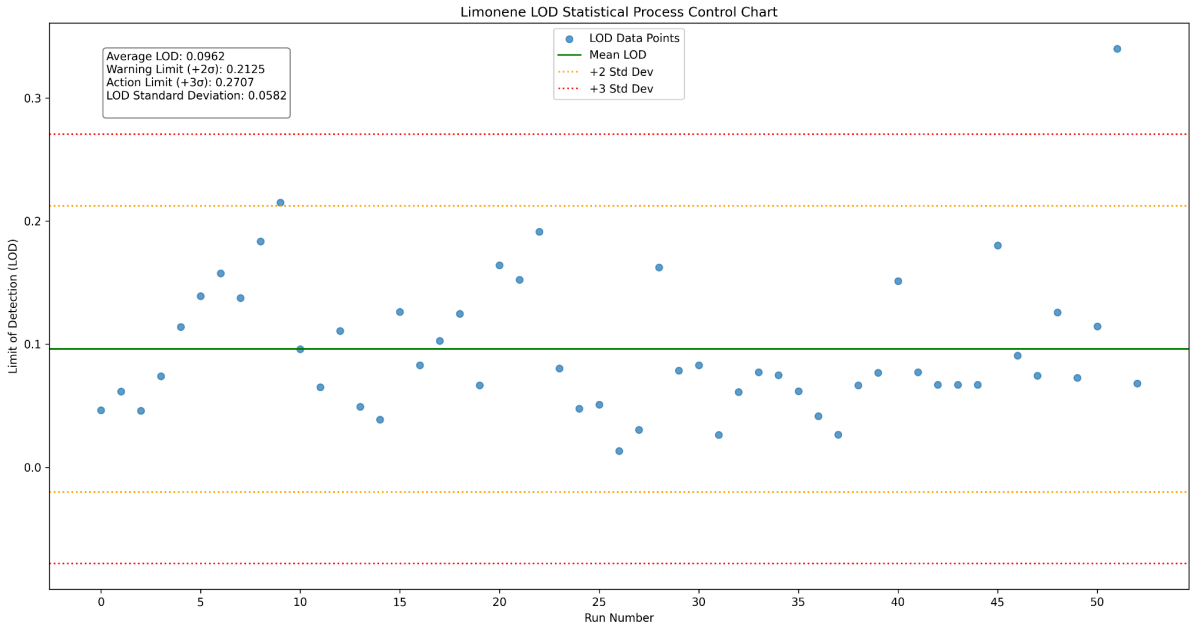
of the decontamination efficiency of the Erema process. The use of extraction, GC-MS analysis, and internal standard quantification provides a robust approach to identifying and quantifying potential contaminants in the recycled material.

➢ Method validation and QC performance

Number of data points: 223, Mean: 0.4989 ppm, Standard Deviation: 0.0825 ppm, RSD%: 16.53%, Bias from target value (0.5 ppm): -0.23%, Uncertainty of Measurement: 16.53%



LOD by Standard Error of the Regression, Number of LOD data points: 53, Average LOD: 0.0962 ppm, LOQ = 0.30 ppm



Appendix 3: Analysis of Non-Intentionally Added Substances (NIAS) in Recycled HDPE

➢ **Method**

Purpose:
To analyze Non-Intentionally Added Substances (NIAS) in recycled HDPE material before and after the extrusion process, evaluating the decontamination efficiency of the Erema process. This method is based on principles outlined in ASTM D7210-21.
Scope:
Applicable to rHDPE washed flake (input) and rHDPE pellets (output) materials.
Principle:

1. Extraction of material by heating with THF under reflux.
2. Analysis of extracts using GC-MS with injection standard quantification.

Equipment:

- Agilent 7890A GC
- Agilent 5975 MS
- Multi-extraction heating mantle with magnetic stirrer
- Gerstel MPS with headspace autosampler
- MSD Chemstation
- Various lab equipment (analytical balance, fridge, oven, glassware, etc.)

Reagents:

- Tetrahydrofuran (THF) Spectroscopic grade

- Internal Standard: Bis(1-butylpentyl) adipate (DNA)

Internal Standard Preparation:

1. Stock Solution:
    - Weigh 0.175 g of DNA into a 100 mL volumetric flask
    - Fill to the mark with THF
    - Final concentration: 1750 ppm (1.75 mg/mL)
2. Intermediate Solution:
    - Pipette 1 mL of stock solution into a 100 mL volumetric flask
    - Fill to the mark with THF
    - Final concentration: 17.5 ppm (17.5 µg/mL)

Procedure:

1. Sample Collection:
    - Collect Erema feed samples (wash flake) from all three Eremas once per shift
    - Collect corresponding output pellet samples 3 hours later
2. Extraction:
    - Weigh 2.5 ± 0.1g of sample into a 250 mL round bottom flask
    - Add 25 ± 0.5 mL of THF
    - Reflux for minimum 1 hour at THF's boiling point (approximately 66°C)
    - Cool the extract to room temperature
    - Collect extract in 40 mL amber vial
3. GC-MS Analysis:

    - Take 700 µL of cooled extract
    - Add 300 µL internal standard intermediate solution (17.5 ppm)
    - Final concentration of internal standard in the injection mixture: 5.25 ppm
    - Total volume of injection mixture: 1 mL
    - Run on GC2 using "NIAS2023 Ver1" method
    - Perform extractions and analyses for both flakes and pellets

GC-MS Parameters:

- Column: Rxi-5ms 30m x 250µm x 0.25µm
- Inlet: Split, 250°C
- Oven: 40°C (2 min) → 20°C/min to 300°C (5 min hold)
- MS: Transfer line 320°C, Source 230°C, Quad 150°C, Mass range 30-550 m/z

Quality Control:

- Include blank samples treated in the same manner, excluding material
- Use well-known rHDPE as a control sample
- Prepare and analyze QC samples (details to be specified)

Data Analysis and Evaluation:

- Store all relevant data on appropriate results spreadsheet
- Retain all GC data indefinitely for reference
- Use DNA internal standard for quantification and to account for variations in extraction efficiency and instrument response
- Calculation:

The concentration of NIAS in the sample is calculated using the following formula:

C_NIAS = (A_NIAS / A_DNA) * (C_DNA_final / F_dilution) * (V_THF / W_sample) * RF

- Where:
- C_NIAS = Concentration of NIAS in the sample (µg/g)
- A_NIAS = Peak area of NIAS
- A_DNA = Peak area of DNA (internal standard)
- C_DNA_final = Final concentration of DNA in the injection mixture (5.25 µg/mL)
- F_dilution = Dilution factor of the extract (0.7 mL extract / 1 mL total = 0.7)
- V_THF = Volume of THF used for extraction (25 mL)
- W_sample = Weight of the sample (2.5 g)

RF = Response factor (assumed to be 1 if not determined experimentally)

- Example calculation:

If A_NIAS = 50000, A_DNA = 100000, and assuming RF = 1:

- C_NIAS = (50000 / 100000) * (5.25 µg/mL / 0.7) * (25 mL / 2.5 g) * 1
- = 0.5 * 7.5 * 10 µg/g

  = 37.5 µg/g
- Conversion Factor:
- To convert between concentration in analyzed solution (µg/mL) and concentration in polymer (µg/g):

Conversion factor = 10 (V_THF / W_sample = 25 mL / 2.5 g)

- To convert from solution to polymer: Multiply by 10
- To convert from polymer to solution: Divide by 10

Reporting:

- Report NIAS concentrations in µg/g (mg/kg) relative to the polymer sample.

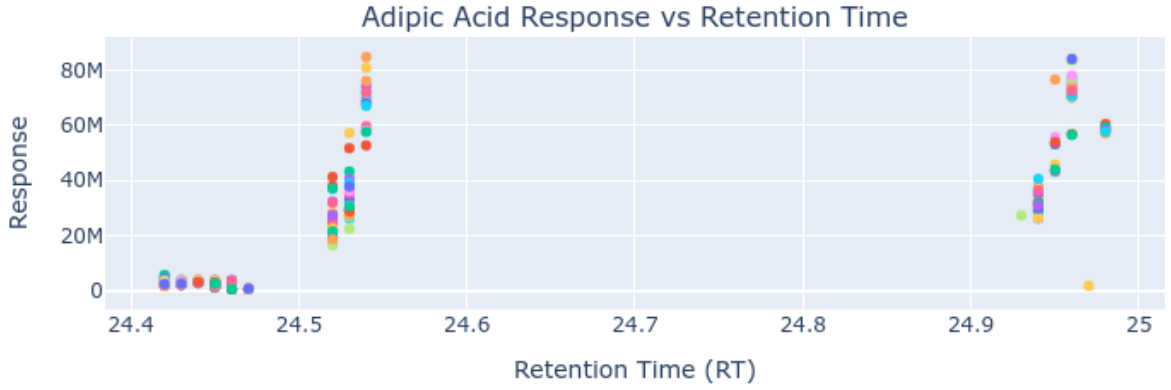
- The internal standard concentration is 52.5 µg/g in the polymer sample, corresponding to 5.25 ppm in the injection solution.

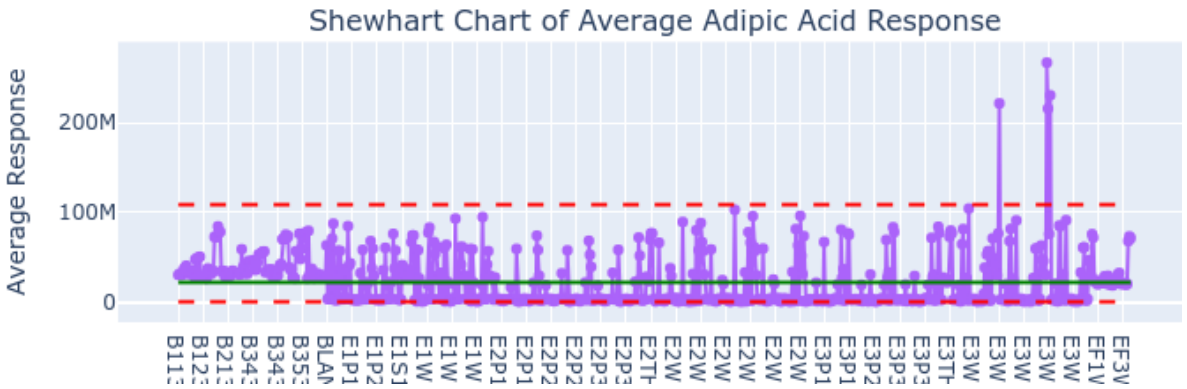
➢ **Method validation and QC performance**

This is a semi-quantitative method that has been implemented according to the ASTM D7210-21 and the original validation data.

Integration settings with a low peak threshold were used to integrate as many low peaks as possible .

Ongoing monitoring of the performance is done by statistically monitoring of the response of the DNA internal standard for variation and checking the cleanliness of the blanks making sure any peaks over 1.5ppm are noted and subtracted from the samples





Overall mean response: 21781957.68
Upper Control Limit (UCL): 108628873.58
Lower Control Limit (LCL): 0.00
843 samples

Current External Proficiency scores with can be found on the website
https://dagenhamplastics.veolia.co.uk/