# VALIDATION OF EXTRACTABLES AND LEACHABLES METHOD FOR A SULFUR-CURED ELASTOMER


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## Abstract


A protocol for the validation of the quantitation of extractable from a sulfur cured elastomer test article was executed. The test article was extracted using soxhlet extraction, and the material extracted was quantitated by GC/FID using external standards. The validation of the method carried out according to ICH guidelines with the following criteria evaluated:

**Specificity**
**System Suitability**
**Limit of Quantitation**
**Linearity and Range**
**Precision – Repeatability and Intermediate**
**Accuracy**
**Stability**


## Introduction

The objective of this study was to validate a method for the quantitation of materials extracted from a sulfur cured elastomer. Prior to this validation, a method was developed to extract the elastomer and identify the materials which were extracted. The test article was soxhlet extracted in methylene chloride followed by GC/MS to identify extracted material. The conditions for maximum extraction were established by conducting a time course study. The soxhlet extraction was conducted for increasing lengths of time until the amount of extractables reached asymptotic levels. The materials found to extract were: 2,2'-methylene-bis(6-tert-butyl-4-ethyl phenol, n-Docosane, n- tricosane, n-tetracosane, n-pentacosane, n-hexacosane, n-octacosane. Standards of the extracted material were obtained and the extractables were quantified. A validation protocol for the method was developed based upon ICH guidelines. Parameters examined are listed below.

## Methodology

### Experimental:

- All extractions were carried out using methylene chloride spiked with an internal standard (2-fluorobiphenyl). The concentration of the standard was ~ 100 µg/ml.
- All standard and sample materials were dissolved and diluted as needed in methylene chloride spiked with the internal standard.
- For all soxhlet extractions the cellulose thimbles were pre-extracted with methylene chloride for ~ 2 hours.
- The elastomer sample was cut into small squares of approximately 5mm pieces. Approximately 7 grams of sample was then placed in a thimble and extracted with 200 ml methylene chloride for 16 hours.
- After the 16 hours of extraction, the methylene chloride was collected and diluted 1:10 with the extraction solvent described above.
- The diluted material was analyzed by GC

### Instrumental Parameters (GC):

- GC Column: 0.32 mm X 30 m DB-1 1.0 micron film thickness.
- Injector temperature: 280 C
- Injection mode: Splitless
- Injection volume: 1 µl
- Purge Valve: On at 1.00 min; off initially
- Temperature program: 40°C for 1 min then linear ramp to 300°C at 10°C/min. Hold for 10 min.
- Carrier Gas: Helium. Flow rate, 2.7 mL/min constant flow.
- Detector: FID, Air, 400 mL/min $H_2$, 30 mL/min.

## Validation Parameters and Acceptance Criteria

- System Suitability
  - Instrument Precision
  - Chromatographic Resolution and Tailing Factor
- Linearity and Range
- Precision
  - Repeatability
  - Intermediate Precision
- Specificity
- Accuracy
- Limit of Quantitation (LOQ)
- Standard and Sample Stability
- Robustness/Ruggedness

## System Suitability

### Instrument Precision

A test solution of target extractables with internal standard was prepared. Six (6) replicate injections of the test solution were analyzed. Peak area and area ratio measurements of target extractables verses the internal standard were determined. The results for 2,2'MBTBE and n-Pentacosane are shown below.

***Acceptance Criteria: %RSD for each target extractable ≤ 10%***

| Trial | Area 2,2'MBTBE | Area ratio | RRF | Area n-Pentacosane | Area ratio | RRF |
|---|---|---|---|---|---|---|
| 1 | 98.03 | 0.84 | 1.01 | 48.14 | 0.41 | 0.43 |
| 2 | 96.06 | 0.85 | 1.03 | 46.50 | 0.41 | 0.43 |
| 3 | 94.73 | 0.83 | 1.00 | 45.03 | 0.40 | 0.42 |
| 4 | 91.89 | 0.84 | 1.01 | 45.87 | 0.42 | 0.44 |
| 5 | 98.12 | 0.86 | 1.03 | 48.78 | 0.43 | 0.45 |
| 6 | 97.06 | 0.85 | 1.03 | 47.76 | 0.42 | 0.44 |
| **Mean** | **96.0** | **0.85** | **1.02** | **47.0** | **0.41** | **0.43** |
| **% RSD** | **2.48** | **1.25** | **1.25** | **3.08** | **2.54** | **2.54** |

### Chromatographic Resolution and Tailing Factor (USP)

Utilizing the analyses accomplished for Instrument Precision, chromatographic resolution and Tailing Factor (USP) between appropriate peak pairs were be determined.

***Acceptance Criteria: to be determined.***

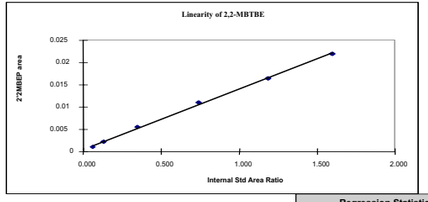
| Trial | Resolution 2,2'MBTBE | Tailing* 2,2'MBTBE | Resolution n-Pentacosane | Tailing* n-Pentacosane |
|---|---|---|---|---|
| 1B | 8.411 | ------ | 2.132 | ------ |
| 2B | 8.336 | ------ | 2.136 | ------ |
| 3B | 8.441 | ------ | 2.135 | ------ |
| 4B | 8.375 | ------ | 2.097 | ------ |
| 5B | 8.324 | ------ | 2.133 | ------ |
| 6B | 8.446 | ------ | 2.113 | ------ |
| **Mean** | **8.39** | ------ | **2.12** | ------ |
| **%RSD** | **0.62** | ------ | **0.75** | ------ |

* Tailing factor was calculated to be 0.9.

## Linearity and Range of 2,2'-MBTBE

Determined by analyzing selected target extractables at 6 different concentration levels (in duplicate).

***Acceptance Criteria: to be determined.***

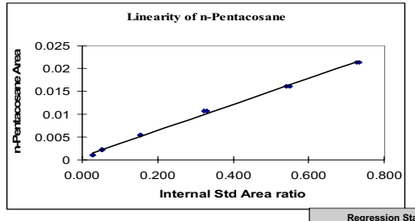



| Regression Statistics | |
|---|---|
| R Square | 0.999 |
| Standard Error | 0.0003 |
| Observations | 12 |
| Y Intercept | 0.0006 |
| Slope | 0.0135 |

## Linearity and Range of n-Pentacosane

Determined by analyzing selected target extractables at 6 different concentration levels (in duplicate).

***Acceptance Criteria: to be determined.***




| Regression Statistics | |
|---|---|
| R Square | 0.998 |
| Standard Error | 0.0004 |
| Observations | 12 |
| Y Intercept | 0.0007 |
| Slope | 0.0286 |

## Precision

Repeatability

Six separate extractions were performed and all analytes were quantitated.

***Acceptance criteria: %RSD for each target extractable = 10%***

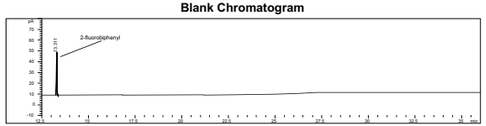
| Trial ID | n-Doco (mg/g) | 2,2'-MBTBE (mg/g) | n-Trico (mg/g) | n-Tetra (mg/g) | n-Penta (mg/g) | n-Hexa (mg/g) | n-Octa (mg/g) |
|---|---|---|---|---|---|---|---|
| 1 | 35 | 501 | 60 | 72 | 56 | 45 | 11 |
| 2 | 35 | 503 | 57 | 72 | 53 | 43 | 11 |
| 3 | 37 | 543 | 58 | 71 | 52 | 40 | 9 |
| 4 | 32 | 492 | 52 | 64 | 47 | 36 | 8 |
| 5 | 29 | 464 | 45 | 53 | 37 | 38 | 12 |
| 6 | 30 | 464 | 46 | 55 | 39 | 34 | 13 |
| **AVG** | **33** | **494** | **53** | **64** | **47** | **39** | **11** |
| **% RSD** | **4.49** | **2.98** | **6.15** | **6.72** | **8.21** | **5.38** | **7.46** |

## Specificity

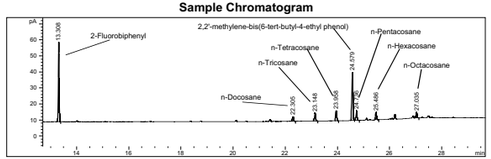
Below appear the standard, and sample chromatograms with all the GC/MS assignments for target extractable peaks.

***Acceptance Criteria: Confirm peak identifications and no significant co-eluting peaks.***

**Blank Chromatogram**




**Sample Chromatogram**




## Accuracy

Standard additions of approximately one, two, and three times the target analyte concentration in test solution were dosed, in triplicate, into 50 ml of the Soxhlet extract test solution. Weights expressed below represent total amount of material spiked into 50 ml in triplicate.

***Acceptance Criteria: Mean recovery for each target extractable at each spiking level should be between 80% and 120% of known spiking level.***

**Recovery Data for n-Pentacosane**

| Trial ID | mgs spiked | mgs recovered | % Recovery |
|---|---|---|---|
| Low Spike | 352 | 396.3333 | 112.6 |
| Mid Spike | 704 | 687 | 97.6 |
| High Spike | 1056.0 | 992.3 | 94.0 |

## Accuracy *(cont'd)*

**Recovery Data for 2,2'-methylene-bis(6-*tert*-butyl-4-ethyl phenol)**

| Trial ID | mgs spiked | mgs recovered | % Recovery |
|---|---|---|---|
| Low Spike | 554.0 | 525.7 | 94.9 |
| Mid Spike | 1108.0 | 1083.0 | 97.7 |
| High Spike | 1662.0 | 1591.7 | 95.8 |

## Limit of Quantitation (LOQ)

The target analytes n-Pentacosane and 2,2'-methylene-bis(6-tert-butyl-4-ethyl phenol) were analyzed at very low levels in test solution to determine at which point the signal to noise ratio approaches 10. The data below represents 0.5 µg/ml of both analytes in test solution.

***Acceptance Criteria: Report results based on extrapolated LOQ's***

| Trial ID | S/N n-Pentacosane | S/N 2,2'-methylene-bis(6-*tert*-butyl-4-ethyl phenol) |
|---|---|---|
| 1 | 8.8 | 13.6 |
| 2 | 8.0 | 13.7 |
| 3 | 8.2 | 13.3 |
| 4 | 8.6 | 13.8 |
| 5 | 9.9 | 16.2 |
| 6 | 9.3 | 11.3 |
| **AVG S/N** | **8.8** | **13.7** |

## Standard and Sample Stability

Mixed standards of target extractables were ran daily for five days and the area ratios calculated. The data for standards appears below.

***Acceptance Criteria: Area ratios for target extractables on each subsequent day should be ±10% of those determined on day 1***

| Day number | Internal Std Area Ratio n-Pentacosane | Internal Std Area Ratio 2,2'-MBTBE |
|---|---|---|
| 1 | 0.43 | 0.86 |
| 2 | 0.42 | 0.84 |
| 3 | 0.46 | 0.84 |
| 4 | 0.46 | 0.84 |
| 5 | 0.49 | 0.85 |