

## Joe Warnick, Hui Zhao

www.eplbas.com

EPL BioAnalytical Services, 9095 West Harristown Blvd., Niantic, IL 62551

### Abstract

Fatty acids have historically been extracted from samples using organic solvent in conjunction with soxhlet glassware and cellulose thimbles. Microwave assisted extraction is used to speed up the extraction process and reduce the amount of solvent. A 40 mL 2:1 mixture of petroleum ether: acetone is used in the microwave assisted extraction as opposed to the 200 mL of petroleum ether used in the standard soxhlet extraction.. The microwave assisted extraction takes about 60 minutes or less depending on matrix, which is considerably less than the 16 hours usually associated with a soxhlet extraction.

### Objective

Develop a microwave assisted extraction to replace soxhlet extraction for analysis of fatty acids..

### Materials

- Analytical balance, capable of weighing to the nearest 0.01 mg (stock standard preparation)
- Top-loading balance, capable of weighing to the nearest 1 mg (sample weighing)
- Mars Microwave or equivalent
- GreenChem Microwave Vessels and Turntable
- Class A volumetric glassware
- Wrench (set at 5 foot pound)
- Gas Chromatograph with Flame Ionization Detector, Hewlett-Packard 6890 or equivalent
- GC Column, DB-WAX 20 m x 0.18 mm x 0.18 $\mu$ m, J&W Scientific or equivalent
- Fume hood with heating mantles

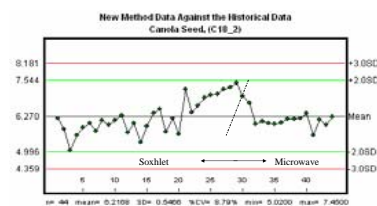
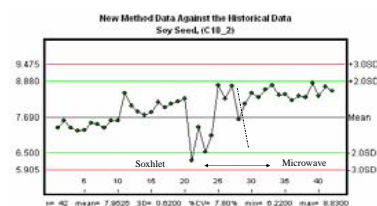
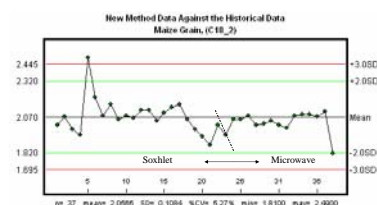
### Procedure

- Sub-samples (1.0 g) of grain and seed weighed into GreenChem microwave vessel.
- 0.5 mL of the tridecanoic acid stock standard solution added to each sample as an internal standard.
- 40ml 2:1 (v:v) petroleum ether:acetone added into the GreenChem microwave vessel.
- Vessels swirled to mix well.
- Vessels assembled and placed into microwave extraction turntable.
- Each vessel capped by hand-tightening, with further tighten using the wrench until hearing an audible 'click'.
- Turn table placed into the microwave and the appropriate program used (Table 1).
- Vessels removed from microwave after cooling to room temperature.
- The liquid is decanted into a 125mL Erlenmeyer flask and evaporated on a heating mantle in a fume hood.
- The samples are methylated with methanolic NaOH and derivatized with Boron Trifluoride.
- Analysis performed by GC-FID.

| Matrix      | Power (w), % | Pressure (Psi) | Temperature ramp          | Hold Time (min) |
|-------------|--------------|----------------|---------------------------|-----------------|
| Maize grain | 1600, 100%   | 100            | Ambient to 80C in 10 min  | 10              |
| Soy Seed    | 1600, 100%   | 100            | Ambient to 75C in 8 min   | 5               |
| Canola Seed | 1600, 100%   | 100            | Ambient to 100C in 10 min | 35              |

Table 1. Microwave program for selected matrices

### Results



| Spike replicates | 1:1 Spike recovery - Soxhlet | 1:1 Spike recovery - Microwave | 1:1 Spike recovery - Control |
|------------------|------------------------------|--------------------------------|------------------------------|
| 1                | 87.6                         | 73.7                           | 88.1                         |
| 2                | 91.4                         | 77.9                           | 72.9                         |
| 3                | 91.0                         | 73.9                           | 78.2                         |
| 4                | 91.4                         | 83.1                           | 77.1                         |
| 5                | 91.1                         | 84.6                           | 79.7                         |
| 6                | 90.7                         | 82.9                           | 80.4                         |
| 7                | 89.2                         | 80.9                           | 77.8                         |
| 8                | 89.3                         | 79.5                           | 78.6                         |
| 9                | 88.5                         | 82.6                           | 80.4                         |
| 10               | 97.2                         | 85.9                           | 82.2                         |
| 11               | 95.9                         | 86.0                           | 77.1                         |
| 12               | 94.9                         | 84.6                           | 79.4                         |
| 13               | 95.2                         | 88.6                           | 78.9                         |
| 14               | 88.2                         | 88.8                           | 82.2                         |

Table 2. Spike recovery for internal standard tridecanoic acid

### Discussion & Conclusion

- The new procedure saves both time and solvent and improves safety.
- Elimination of expensive soxhlet extraction glassware.
- Recoveries of tridecanoic acid presented are acceptable. (Table 2)
- Satisfactory results were obtained for accuracy and precision.
- Increased throughput by dedication of fume hoods for the methylation and derivatization of samples.
- Does not require a hood to perform extractions, self contained exhaust system in microwave.

#### Method Summary

Accurate; precise; reproducible; high-throughput; safe.

|                                | Soxhlet extraction | Microwave assisted |
|--------------------------------|--------------------|--------------------|
| Sample extraction time (hours) | 16                 | 1                  |
| Extraction solvent (mL)        | 200                | 40                 |
| Samples processed per week     | 160                | 200+               |

### References

- AOAC International Method 939.05. In Official Methods of Analysis of the AOAC International, 17th Edition. Association of Official Analytical Chemists International, Gaithersburg, Maryland.
- Official Method of Analysis of AOCS International, Ce 2-66.
- Official Method of Analysis of AOCS International, Ce 1e-91