# Simultaneous Determination of Riboflavin, Thiamine and Total Niacin in Extracts of Flour Using Segmented Continuous Flow Analysis

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## Abstract

Simultaneous determination of riboflavin, thiamine and total niacin has been achieved in acidic extracts of flour and corn meal for the purposes of nutrient enrichment quality control. Segmented Continuous Flow Analysis (SFA) utilizes low sample and reagent consumption while offering high sample throughput and data quality. Contingent upon local environmental regulations, much of the waste stream can go into the drain reducing waste-handling costs. Applying AOAC or HPLC sample preparations, extracts or digests are analyzed at 40 per hour with automated sampling and data collection using FASPac II software.


## RIBOFLAVIN
**A530**

1. **Scope and Application**
   This method is used for the determination of riboflavin in food extracts and digests. The standard range of this method is 0.005 to 2.00 µg/ml, however it is also applicable to other ranges.

2. **Summary of Method**
   This method is based upon the automated **AOAC Official Method 981.15** and is similar to **AACC Method 86-70**. All methods use measurement of riboflavin's natural fluorescence under controlled pH conditions. Fluorescence is measured at 510 nm after excitation at 450 nm.

3. **Interferences**
   Sample background fluorescence and interferences are reduced with dialysis and by oxidation with potassium permanganate.

4. **Sample Throughput**
   Analyze 60 samples per hour with a dwell time of 3 minutes from sampling to data collection (40 samples per hour if analyzed concurrent with thiamine).

## NIACIN
**A561**

1. **Scope and Application**
   This method is used for the determination of total niacin in food extracts and digests. The standard range of this method is 1.0 to 35.0 µg/ml as niacin (or niacinamide), however it is also applicable to other ranges.

2. **Summary of Method**
   This method is based upon the automated **AOAC Official Method 975.41** and is similar to **AACC Method 86-50A**. Modifications have resulted in a safer method with equivalent responses from both niacin and niacinamide, eliminating the need for conversion of niacinamide to niacin. Niacin and niacinamide react with cyanogen chloride created *in situ* to form an aldehyde intermediate. Subsequent addition of sulfanilic acid results in the formation of a polymethine dye absorbing light at 470 nm. The absorbance is directly proportional to total niacin concentration.

3. **Interferences**
   Colored food extracts or digests could interfere. Extracts or digests with considerably high or low pH can interfere with color development.

4. **Sample Throughput**
   Analyze 60 samples per hour with a dwell time of 6.5 minutes from sampling to data collection (40 samples per hour if analyzed concurrent with thiamine).

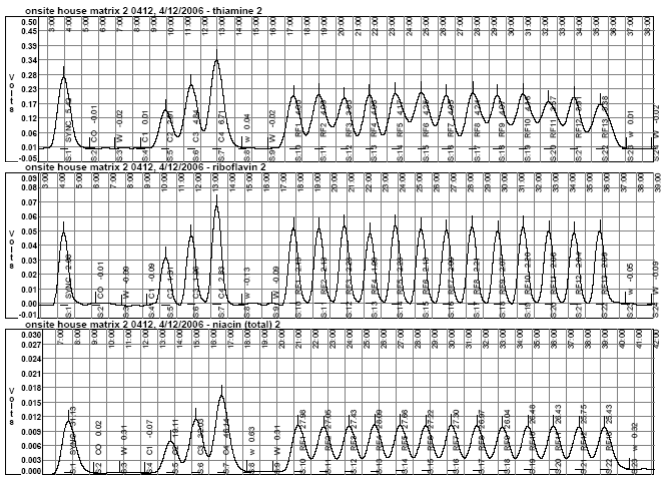
## THIAMINE
**A540**

1. **Scope and Application**
   This method is used for the determination of thiamine in food extracts and digests. The standard range of this method is 0.025 to 2.00 µg/ml as thiamine hydrochloride, however it is also applicable to other ranges.

2. **Summary of Method**
   This method is similar to **AOAC Official Method 953.17** and **AACC Method 86-80**. Thiamine is oxidized to thiochrome by potassium ferricyanide in a strongly alkaline solution. Thiochrome is then extracted into isobutyl alcohol and its fluorescence is measured at 435 nm after excitation at 365 nm. Thiochrome fluorescence is directly proportional to the concentration of thiamine in the sample.

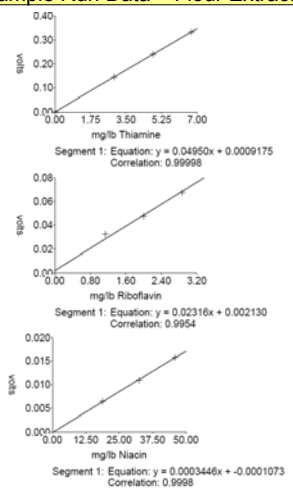
3. **Interferences**
   The presence of compounds that absorb thiamine or interfere with thiochrome fluorescence will affect method accuracy. Compounds that fluoresce at the wavelength of interest may produce false positive results.

4. **Sample Throughput**
   Analyze 40 samples per hour with a dwell time of 3.5 minutes from sampling to data collection.




NOTE: Data shown are in milligrams per pound.

Example Run Data – Flour Extract




## Sample Handling and Preservation

Consult and use appropriate industry guidelines (*e.g.* AOAC, AACC) for the preparation and analysis of samples. Acceptable results are also achieved using simple room-temperature extractions under controlled pH conditions. Samples are filtered to 1.2 µM. Use of amber glassware and limited exposure to light minimizes loss in calibrant and sample preparations.

## Accuracy and Precision

The SFA B vitamin methods correlate well with AOAC Official Methods and initial comparisons against HPLC with UV detection show good agreement. However, in correlation trials involving two different QC labs, the similar HPLC methods did not consistently yield the same results for identical samples. Precision studies indicate that %CV is typically ≤5% based on 10 replicates of the same extract or digest.

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