Curcuminoids Content of Turmeric Spice and Oleoresins

Purpose: To determine the percent total curcuminoids present in turmeric preparations by spectrophotometric procedure.

Principle: Curcuminoid pigments are extracted from turmeric powder in hot acetone or alternate extraction method or diluted from turmeric extracts in acetone and quantified by spectrophotometric absorbance at 420 nm.

A. Apparatus

1. Spectrophotometer, with visible range light source, capable of accurately measuring absorbance within the 415 - 425 nm range.
2. Stirring hot plate.
3. Stir bars, 1" X 3/8".
4. Cuvettes, 1 cm square, silica.
5. Erlenmeyer flask, 125 mL, TS 24/40, amber. Wrapping equipment with Aluminum foil to protect from light is acceptable.
6. Condenser, West Type, 400 mm, TS 24/40
7. Volumetric flasks, 100 and 200 mL, amber (or covered with Aluminum foil to protect from light)
8. Pipets Class A, 1mL, volumetric,
9. Funnel, fluted, Pyrex^{R}, 60-degree angle, 65 mm I.D.,
10. Filter paper, fluted, grade 588 (or equivalent)

B. Reagents

1. Acetone, Reagent Grade

C. Preparation of Sample

1. Raw Spice - Prepare sample as directed in ASTA Method 1.0, except for grinding to pass U.S. 40 mesh.

2. Oleoresins – Sample must come from a well stirred container (pail shaker) as the Oleoresin may settle into two distinct layers. Use as is.

D. Procedure

1. Raw Spice (see section G4 for alternate extraction methods):
   a. Weigh to the nearest 0.001 g an appropriate weight (see section G1) of sample into a 125 mL Erlenmeyer flask; add ca. 75 mL of acetone and stir bar; gently reflux on stirring hot plate with West Condenser for one hour.

   b. Cool to room temperature and filter (Optional. See G, note 3) quantitatively into 200 mL volumetric flask. Transfer extracted residue to filter with acetone. Wash thoroughly and dilute to volume with acetone.

c. Pipet 1 mL of (b) solution into a 100 mL volumetric flask; dilute to volume with acetone and mix.
d. Zero spectrophotometer with acetone in the cuvette at 420 nm. Determine absorbance of solution from (c) at 420 nm.

2. Oleoresins:
   a. Weigh to the nearest 0.001 g appropriate weight (See section G1) of a well-mixed sample and transfer into 100 mL volumetric flask. Dissolve in acetone; dilute to volume with acetone and mix until fully dissolved.
   b. Pipet 1 mL of (a) into a 100 mL volumetric flask; dilute to volume with acetone and mix well to homogenize.
   c. Zero spectrophotometer with acetone in the cuvette at 420 nm. Determine absorbance of solution from (b) at 420 nm.

E. Calculation

$$\% \text{ Curcuminoids} = \frac{D_s}{100} \times \frac{A_s}{W_s \times 1650} \times 100\%$$

D_s = dilution volume, sample, mL, where if using the dilution schedule as presented in this method is 20,000 for Raw Spice and is 10,000 for Oleoresins.

W_s = weight of sample, in grams

A_s = absorbance of the sample solution to read (double dilution)

$1650 = E^{1\%}_{1\ cm}$ for mixed curcuminoids at 420 nm (see note 2 and reference a)

F. Statistics

A short collaborative study was initiated between several ASTA members to compare data on the same sample of ground Turmeric using different extraction methods. Each test was performed in triplicate. The results published below show that all extraction methods seem to provide an equally acceptable level of confidence for the intended purpose of this test based on the Relative Standard Deviation (% RSD). Additional data, possibly collected through the ASTA check sample program, would certainly help refine such preliminary work. Results expressed in % Total Curcuminoids.

(Extraction)	ASTA 18.0 (D1)	Sonic 1h (G4a)	Orbital 1h (G4b)	Steam table (G4c)	16h static (G4d)
lab					
1		6.49		6.53	6.46
2		6.53	6.48		6.38
3	6.55	6.53		6.46	6.40
4	6.57	6.51	6.45	6.45	6.42
5	6.65		6.52	6.33	6.49
6	6.52	6.49	6.38	6.58	6.44
Avg	6.57	6.51	6.46	6.47	6.43
1st STD	0.0556	0.0200	0.0591	0.0946	0.0402
% RSD	0.998	0.307	0.914	1.46	0.625

Each lab result is the average of 3 different test results. Individual details are available upon request.

G. Notes

1. Appropriate sample weight for ground or Oleoresin Turmeric is determined as:

$$\frac{0.03 \text{ gram}}{\text{expected \%}} \text{ X } 100\%$$

Example: for a 6% Curcuminoids: (0.03/0.06) x 100%= 0.500 g

2. The coefficient of extinction (E) is dependent on the solvent being used and the measuring wavelength as determined by experience. If using a different wavelength or solvent, it is imperative to verify the E of a 2-3 ppm solution of pure Curcuminoids (>98%) and extrapolate to a 1% solution. The method described here and the statistics were validated using Acetone and the corresponding dilution at 420nm. See reference H.a.

3. Filtration of the extraction solution is optional when extracting regular ground turmeric, since using a rather low sample size (0.5-0.6 g) greatly lowers the impact of any turbidity on the absorbance reading especially after the second dilution. For preparations requiring a much larger sample size and leading to high turbidity of the extraction solution, filtration may become necessary.

4. Alternate extraction methods for Raw Spice (ground Turmeric) only.

Reminder: Trade disputes should be settled based on a mutually agreed upon method.

a. Ultrasonic bath for 1 h. Weigh sample as per note G1 into a 100 mL amber (or equivalent) volumetric flask at ambient, dilute to volume, let cool down to ambient and verify volume, adjust if necessary. Prepare the second dilution (1 ml into 100 mL acetone), shake a few times and read at 420 nm. Ds is 10,000.

b. Orbital shaker for 1h at about 300 rpm. Weigh sample as per note G1 into a 100 mL amber (or equivalent) volumetric flask at ambient, add about 50 mL of acetone, to swirl sample in partial volume of acetone, let cool down to ambient if necessary, dilute to final volume with acetone, shake flask a couple times, then prepare the second dilution (1 mL into 100 mL acetone), shake flask a few times, then read at 420 nm. Ds is 10,000.

c. Steam bath for 30 min under hood. Weigh sample as per note G1 into a 100 mL amber (or equivalent) volumetric flask, add about 50 mL of acetone, to swirl sample in partial volume of acetone. Gently heat un-stoppered flask on steam bath for 30 minutes, adding acetone only if necessary, to prevent full evaporation, let cool down to ambient then adjust to volume with acetone. Stopper flask and shake a few times. Prepare the second dilution (1 ml into 100 mL acetone), shake a few times and read at 420 nm. Ds is 10,000.

d. Static method, 16 h (concept similar to ASTA 20.1). Weigh sample as per note G1 into a 100 mL volumetric flask, dilute to volume with acetone. Shake a few times and let sit in a dark cabinet for 16 h minimum. Shake a few times before diluting a second time (1 mL into 100 mL acetone), shake flask a few times and read at 420 nm. Ds is 10,000.

H. References:

a. The extinction coefficient was verified by measuring the absorbance at 420 nm of a 1.97 ppm solution of Curcuminoids standard (MP Bio #190133 @ 98.5%) in Acetone. Reading was 0.3251 Abs, equivalent to 1650 for a 1% solution (10,000 * 0.3251/1.97).
b. AOAC method 2016.16 (HPLC) allowing quantification of each Curcuminoid, mostly used in the Nutraceutical industry.

I. Revision History

1/30/2015: Added instructions to procedure for collection of absorbance values (D.1.d. and D.2.c.). Changed curcumin to curcuminoids in multiple places. Added a Principle section.

April 2019: Renaming method as Total Curcuminoids, removed obsolete apparatus references and made filtration optional for straight turmeric extracts (5-6%) in notes G3, added an example for sample size in note G1, introduced four alternate extraction methods in note G4, provided some statistical data (F) to support their validity and added references including the origin and verification of the coefficient of extinction used in the calculation.