

47.2.02

AOAC Official Method 983.15
Phenolic Antioxidants
in Oils, Fats, and Butter Oil

Liquid Chromatographic Method
First Action 1983
Final Action
Revised First Action 1992
Final Action 1994

IUPAC–AOAC Method

(Applicable to propyl gallate [PG], 2,4,5-trihydroxybutyrophenone [THBP], *tert*-butylhydroquinone [TBHQ], nordihydroguaiaretic acid [NDGA], 2- and 3-*tert*-butyl-4-hydroxyanisole [BHA], 2,6-di-*tert*-butyl-4-hydroxymethylphenol [Ionox-100], and 3,5-di-*tert*-butyl-4-hydroxytoluene [BHT] at 20–200 g/g in oils and fats, 10–100 g/g in butter oil, and to octyl and dodecyl gallate [OG and DG] at 10–100 g/g in butter oil.)

See Tables 983.15A–C for the results of the interlaboratory study supporting acceptance of the method.

A. Principle

Antioxidants are extracted into acetonitrile. Extract is concentrated and diluted with 2-propanol. Antioxidants are separated by liquid chromatography and measured by ultraviolet detection at 280 nm.

B. Apparatus

(a) *Gradient liquid chromatograph*.—With 10 mV recorder or integrator to electronically measure peak heights, 10 L loop injection valve, and detector to measure absorbance at 280 nm. Typical operating conditions: detector sensitivity, 0.05 AUFS; temperature, ambient; flow rate, 2.0 mL/min.

(b) *LC column*.—Packed with C18-bonded spherical (preferred) silica, or equivalent. Use guard column if desired. Must be capable of baseline resolution of all 9 antioxidants as shown in Figure 983.15. Verify peak identities, if necessary, by injection of individual standards.

(c) *Glassware*.—Rinse all glassware with CHCl₃, acetone, and methanol, successively, and blow dry with N₂.

C. Reagents

(a) *Solvents*.—Acetonitrile, 2-propanol, and hexane. Distilled-in-glass grade.

(b) *Mobile phase*.—(1) 5% Acetic acid in H₂O.—LC grade. (2) Acetonitrile–methanol (1 + 1, v/v).—LC grade.

Table 983.15A. Interlaboratory study results for phenolic antioxidants in oils, liquid chromatographic method

Antioxidant ^a	Added, mg/g ^b	Recovered, mg/g	Results based on mean recovery, %	Repeatability		Reproducibility		
				s _r	RSD _r , %	s _R	RSD _R , %	HorRat
PG	193.7	184	95.2	16.0	8.66	16.0	8.66	1.20
PG	96.7	93.8	96.9	4.50	4.80	4.50	4.80	0.60
PG	19.4	17.6	90.9	2.01	11.5	2.52	14.3	0.25
THBP ^c	203.2	199	98.1	9.99	5.01	9.99	5.01	0.70
THBP	101.6	99.1	97.5	5.25	5.30	5.58	5.63	0.71
THBP	20.3	19.6	96.7	0.97	4.94	1.53	7.77	0.77
TBHQ	196.1	201	103	8.77	4.36	22.8	11.4	1.58
TBHQ	98.1	100	102	3.63	3.63	21.5	21.5	2.69
TBHQ	19.6	19.1	97.5	1.50	7.85	3.17	16.6	1.63
NDGA	93.8	91.7	97.8	5.06	5.51	6.39	6.97	0.87
NDGA	18.8	18.4	98.3	0.34	1.83	0.60	3.24	0.32
BHA	198.5	197	99.1	6.54	3.32	6.61	3.36	0.47
BHA	99.2	99.7	101	5.43	5.45	6.15	6.17	0.77
BHA	19.9	19.5	98.0	0.43	2.19	0.76	3.92	0.39
IONOX-100	208.2	198	95.3	17.7	8.92	19.4	9.76	1.37
IONOX-100	104.1	98.7	94.8	10.2	10.4	10.5	10.6	1.34
IONOX-100 ^d	20.8	20.2	97.0	0.71	3.54	1.14	5.63	0.56
BHT ^d	202.9	170	83.8	3.50	2.06	4.54	2.67	0.37
BHT ^d	101.5	84.3	83.1	2.36	2.80	2.36	2.80	0.35
BHT	20.3	17.2	85.1	0.90	5.22	1.06	6.18	0.61

^a PG = Propyl gallate, THBP = 2,4,5-trihydroxybutyrophenone, TBHQ = *tert*-butylhydroquinone, NDGA = nordihydroguaiaretic acid, BHA = 2- and 3-*tert*-butyl-4-hydroxyanisole, Ionox-100 = 2,6-di-*tert*-butyl-4-hydroxymethylphenol, BHT = 3,5-di-*tert*-butyl-4-hydroxytoluene.

^b Antioxidant values measured to 4 significant figures, g/g, but rounded to nearest 0.1 g/g.

^c Two of 7 laboratories rejected as outliers by the pair Grubbs test.

^d One of 7 laboratories rejected as outlier by the single Grubbs test.

Table 983.15B. Interlaboratory study results for phenolic antioxidants in lard, liquid chromatographic method

Antioxidant ^a	Added ^b , mg/g	Recovered, mg/g	Results based on mean, recovery, %	Repeatability		Reproducibility		HorRat
				s _r	RSD _r , %	s _R	RSD _R , %	
PG	96.9	90.1	93.0	3.18	3.53	3.18	3.53	0.44
PG	38.7	34.6	89.4	1.55	4.48	1.55	4.48	0.49
THBP	101.6	97.9	96.4	6.75	6.89	14.3	14.6	1.84
THBP ^c	40.7	36.4	89.5	1.35	3.71	1.47	4.06	0.45
TBHQ	98.1	95.6	97.5	7.71	8.07	22.1	23.2	2.91
TBHQ	39.3	35.0	89.0	6.04	17.3	11.6	33.2	3.62
NDGA	93.8	87.8	93.6	2.65	3.02	4.74	5.40	0.67
NDGA	37.5	35.3	94.2	1.06	3.01	1.79	5.06	0.55
BHA	99.2	97.4	98.2	2.49	2.56	3.72	3.82	0.48
BHA	39.7	38.3	96.6	1.90	4.97	1.90	4.97	0.54
IONOX-100	104.1	99.3	95.4	4.83	4.87	5.45	5.49	0.69
IONOX-100	41.7	40.7	97.7	3.49	8.56	4.90	12.0	1.32
BHT	101.5	87.9	86.6	4.63	5.27	4.90	5.58	0.70
BHT	40.6	34.6	85.2	1.11	3.22	1.17	3.39	0.37

^a See Table 983.15A for identification of antioxidants.

^b Antioxidant values measured to 4 significant figures, g/g, but rounded to nearest 0.1 g/g.

^c One of 7 laboratories rejected as outlier by the single Grubbs test.

Table 983.15C. Interlaboratory study results for phenolic antioxidants in butter oil, liquid chromatographic method

Antioxidant ^a	Mean added, ^b mg/g	Mean recovered, mg/g	Recovery, %	s _r	RSD _r , %	s _R	RSD _R , %	HorRat
PG	46.0	46.9	102	3.86	8.23	4.54	9.67	1.08
PG	9.20	9.53	104	0.450	4.72	0.875	9.17	0.81
THBP	87.0	82.3	94.6	5.34	6.48	11.2	13.6	1.67
THBP	43.6	42.8	98.1	3.77	8.81	6.68	15.6	1.73
THBP	8.69	8.7	101	1.39	15.9	2.00	22.9	1.99
TBHQ	105.9	111	105	10.9	9.78	24.3	21.8	2.76
TBHQ	52.8	51.8	98.2	2.01	3.87	11.0	21.2	2.42
TBHQ	10.5	11.3	107	1.55	13.8	3.82	34.0	3.05
NDGA ^c	96.5	93.0	96.4	6.27	6.74	6.27	6.74	0.84
NDGA	48.3	47.0	97.3	3.22	6.86	4.66	9.91	1.12
NDGA	9.63	8.9	92.5	2.11	23.7	2.39	26.8	2.37
BHA	101.3	96.3	95.2	8.49	8.81	8.49	8.81	1.11
BHA	50.6	48.8	96.5	2.29	4.70	2.50	5.12	0.58
BHA	10.1	10.2	101	0.515	5.06	0.597	5.87	0.52
IONOX-100	105.7	103	97.4	4.79	4.66	7.52	7.31	0.93
IONOX-100	52.9	50.2	94.9	3.77	7.51	4.13	8.24	0.94
IONOX-100	10.6	9.36	88.6	1.22	13.0	1.25	13.4	1.20
OG	89.2	86.3	96.8	3.80	4.40	4.37	5.06	0.62
OG	43.7	42.0	96.2	2.89	6.87	2.89	6.87	0.76
OG	8.76	8.19	93.5	1.69	20.6	1.69	20.6	1.80
BHT	96.5	76.7	79.4	9.16	12.0	9.51	12.4	1.55
BHT ^c	48.4	38.8	80.2	2.58	6.64	3.16	8.12	0.91
BHT ^d	9.65	7.47	77.4	0.443	5.94	1.03	13.8	1.22
DG	101.1	96.7	95.7	4.02	4.16	7.94	8.21	1.03
DG	50.6	48.8	96.5	2.98	6.12	3.05	6.24	0.71
DG	10.1	9.76	96.4	0.468	4.80	0.742	7.61	0.68

^a See Table 983.15A for identification of antioxidants.

^b Antioxidant values measured to 4 significant figures, g/g, but rounded to nearest 0.1 mg/g.

^c Two of 9 laboratories rejected as outliers by the pair Grubbs test.

^d Two of 9 laboratories rejected as outliers; by the Cochran test, then by single Grubbs test.