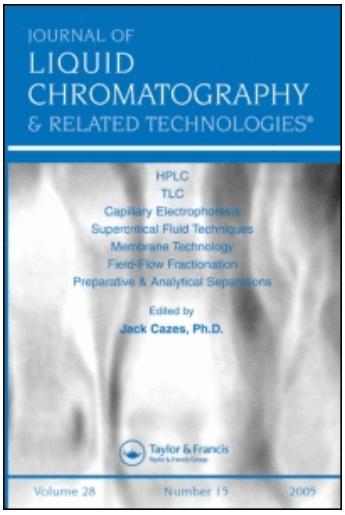

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Quantitation of Propyl Gallate in an Active Pharmaceutical Ingredient (API) Using High-Low Chromatography

Scott M. Thomas[a]; Mark D. Trone[a]; Thomas Hooker[a]; Jason G. Dorwart[a]; Leslie A. Morneweck[a]
[a] Merck and Company, Rahway, New Jersey, USA

Online publication date: 19 June 2003

To cite this Article Thomas, Scott M. , Trone, Mark D. , Hooker, Thomas , Dorwart, Jason G. and Morneweck, Leslie A.(2003) 'Quantitation of Propyl Gallate in an Active Pharmaceutical Ingredient (API) Using High-Low Chromatography', Journal of Liquid Chromatography & Related Technologies, 26: 11, 1743 — 1753
To link to this Article: DOI: 10.1081/JLC-120021282
URL: http://dx.doi.org/10.1081/JLC-120021282

JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES®
Vol. 26, No. 11, pp. 1743–1753, 2003

Quantitation of Propyl Gallate in an Active Pharmaceutical Ingredient (API) Using High-Low Chromatography

Scott M. Thomas,* Mark D. Trone, Thomas Hooker, Jason G. Dorwart, and Leslie A. Morneweck

Merck and Company, Rahway, New Jersey, USA

ABSTRACT

A simple, rapid, and accurate HPLC method was developed using high-low chromatography (H-LC), to determine the level of propyl gallate (PG) in an active pharmaceutical ingredient (API). The method utilizes a Waters Symmetry C-8 column and a variable wavelength UV detector. The method employs isocratic conditions. The mobile phase consists of water and acetonitrile with phosphoric acid. The method is effective in determining the amount of PG in an API. The method was applied to process developmental samples to determine the effectiveness of PG loading and to monitor the levels of PG during the long term and accelerated stability studies. Spiking experiments of PG in the API demonstrated quantitative recovery. Ruggedness was determined as a function of solution stability. Sample solutions stored under ambient

*Correspondence: Scott M. Thomas, Merck and Company, 126 E. Lincoln Ave., Bldg. 818-C208, Rahway, NJ 07065, USA; E-mail: scott_thomas@merck.com.

DOI: 10.1081/JLC-120021282
1082-6076 (Print); 1520-572X (Online)

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

conditions are stable for an overnight period in methanol : acetic acid 99 : 1 v/v. The limit of detection (LOD) was determined to be 0.0006% w/w. The limit of quantitation (LOQ) was determined to be 0.002% w/w. The LOD and LOQ are based on a 10 mg/mL API sample solution.

Key Words: Hi-low chromatography; Propyl gallate; LOD; LOQ.

INTRODUCTION

Propyl gallate (PG) is an antioxidant used to prevent the oxidative deterioration of fatty materials such as edible oils, fats, and cosmetic products.[1] Many pharmaceuticals are prone to oxidation often preceded by formation of free radicals. The addition of a small amount of an antioxidant can inhibit this formation by providing a hydrogen atom or an electron to the free radical. Antioxidants such as butylated hydroxyanisole, tocopherols, and alkyl gallates also block and prevent the free radical chain reaction.[2] To improve the stability of an active pharmaceutical ingredient (API), PG can be added at low concentration to the final stages of API processing. Due to matrix effects and potential interferences from impurities in the API, accurate, and rapid quantitation of low levels of PG can be a challenge.

Over the past few years, several papers have been published in the area of measuring antioxidants in food and cosmetic products.[3–14] HPLC is one of the methods often used for the separation and determination of antioxidants.[15–27] These methods frequently require tedious pre-concentration or isolation of the antioxidant.

The goal of this work was to develop and validate a rapid and accurate HPLC method without a concentration or isolation step for the determination of an API of PG, which had been selected to stabilize the API.

EXPERIMENTAL

Apparatus

Experiments were conducted on an HPLC unit consisting of a Thermal Separations (Palo Alto, CA), quarternary pump, model P4000; a Thermal Separations autosampler equipped with a column heater, model AS1000, equipped with a 5 μL injection loop, and a Thermal Separations variable

wavelength UV detector, model UV1000. Integration and data manipulation was performed using Perkin-Elmer Turbochrom (Groton, CT).

Chemicals

Propyl gallate was purchased from Aldrich (Milwaukee, WI), phosphoric acid, acetic acid, methanol, acetonitrile, and water were purchased from Fisher Scientific (Fair Lawn, NJ). All of the solvents were HPLC grade and the reagents were the best available quality and were used without further purification.

Samples of the API were prepared by Dr. Feng Xu and Dr. Michael Sowa of Merck Research Laboratories, Rahway, NJ.

Chromatographic Conditions

High-low chromatography was selected for the determination of the weight percent of PG in an API. High-low chromatography is a technique especially designed for trace analysis, which increases the sensitivity and decreases the limit of quantitation (LOQ) for an analyte. In this technique, the API samples are prepared at high concentrations while PG standards are prepared at low concentrations, similar to the level expected in the API samples. Isocratic elution was employed and the detector wavelength was optimized at 275 nm for PG sensitivity and to eliminate interference from the API, which has a 238 nm UV maximum. In these studies, H-LC provided a higher degree of sensitivity and precision allowing sound conclusions with respect to PG levels in stability studies and other experiments.

A Waters Symmetry C-8 column, 25 cm × 4.6 mm I.D., containing 5 μm particles was used. The column temperature was maintained at 45°C. The eluent flow rate was 1.5 mL/min. The chromatographic conditions were water : acetonitrile : phosphoric acid 70/30/0.1, v/v/v. The detector was operated at 275 nm. Injection volume was 5 μL.

Sample Preparation

The diluent was prepared by adding 10 mL of acetic acid to a 1-L volumetric flask and diluting to mark with methanol. The diluent was mixed and stored under ambient laboratory conditions. Samples of the API were prepared in duplicate at a sample concentration of 10 mg/mL. To insure complete dissolution, samples were sonicated for up to 10 min. Samples were analyzed against a 0.005 mg/mL PG standard solution.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

RESULTS AND DISCUSSION

A typical chromatogram of a PG standard and a typical sample are given in Fig. 1. The API elutes between 32 and 35 min and can be detected in the UV at 220 nm. Using 275 nm, as we describe in this method, the API is not detected. The method was validated according to ICH guidelines and the choice of characteristics for validation is also consistent with USP requirements.

Linearity of the detector response for PG was evaluated over the concentration range of 0.0005 to 0.1 mg/mL. Linearity solutions were

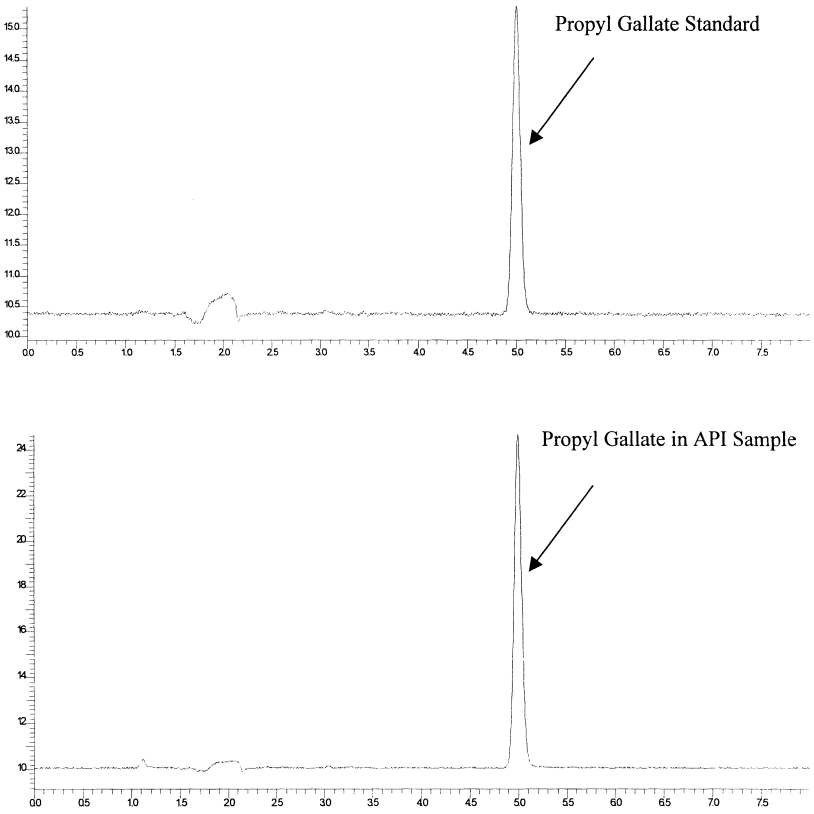

Figure 1. Determination of PG in an API. Conditions: column, Waters Symmetry C-8, 25 cm × 4.6 mm; flow rate 1.5 mL/min; injection volume 5 μL; eluent: water : acetonitrile : phosphoric acid 70/30/0.1 v/v/v; column temperature 45°C; detection: UV at 275 nm. Top: 0.005 mg/mL PG standard solution. Bottom: 10 mg/mL sample solution.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

prepared by serial dilution from a 0.5 mg/mL stock solution. Six injections were made of each solution. A graph of the concentration vs. the area counts for PG can be found in Fig. 2. The method is linear, as demonstrated by a correlation coefficient of 0.9999.

Injection precision was demonstrated by the 1.4% relative standard deviation (RSD) obtained for eight injections of the 0.005 mg/mL standard solution (Table 1). Method precision was determined using API, which had been coated with PG. Samples were prepared in duplicate and each sample solution was injected twice. The RSD ranged from 0.1% to 1.1%.

The limit of detection (LOD) for PG was established by determining the lowest concentration in which PG had a signal to noise ratio of 3 to 1 (Table 2). The standards used for this experiment were prepared by serial dilution from a 0.1 mg/mL stock solution, and six injections were made of each standard. The LOD for PG was 0.00006 mg/mL. This corresponds to a LOD of 0.0006% w/w PG for a 10 mg/mL sample solution.

The LOQ (Table 3) for PG was determined to be 0.0002 mg/mL (0.002% w/w), which was the lowest concentration that satisfied the following requirements: (1) a minimum signal to noise ratio of 10 : 1; (2) a maximum deviation of 20% between the response factor obtained at the LOQ and the

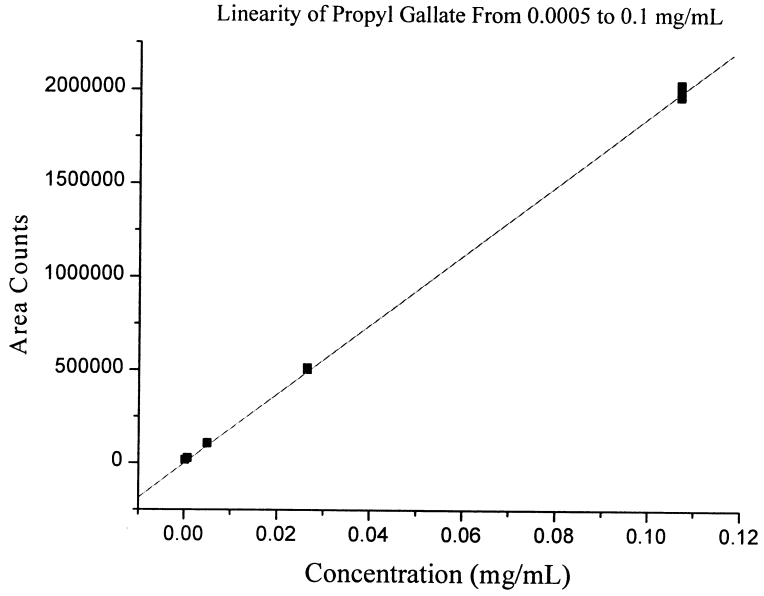

Figure 2. Linearity plot of PG in methanol : acetic acid 99/1 v/v.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Table 1. Injection precision of a 0.005 mg/mL PG standard solution.

Concentration, mg/mL	Area counts
0.00536	97,439
0.00536	100,581
0.00536	98,518
0.00536	97,758
0.00536	100,919
0.00536	97,334
0.00536	97,522
0.00536	98,839

Note: $\bar{X}_{AC} = 98{,}614$; RSD = 1.4%.

response factor of a standard solution, which is five times more concentrated; and (3) a maximum RSD of 15% for the area counts obtained from the solution at the LOQ.

Robustness was assessed by studying the solution stability of a sample solution under ambient laboratory conditions. A sample of the API containing PG was prepared at a concentration of 10 mg/mL and was injected once an hour for 15 hours (Table 4). The area counts for PG did not change over the 15 hour period ($\bar{X} = 393{,}883$, RSD = 0.2%).

Accuracy (recovery of PG) was demonstrated by spiking in known amounts of PG into a sample of the API, which did not contain PG. Two spiking experiments were performed at the following weight percent levels of PG with respect to the API: 0.05%, 0.10%, and 0.20%. A control, which contained no PG was also prepared and two injections of each sample solution were made. The recovery was 100.0–101.0%, further demonstrating the effectiveness of the method (Table 5).

Five batches of API processed with PG were analyzed. The results are presented in Table 6. These results demonstrate the precision of the method to accurately and reliably quantitate low levels of PG in an API. The method was also used to monitor PG levels in the API in long term and accelerated stability studies, which were performed on the API. Propyl gallate results from these

Table 2. Signal to noise ratio (S/N) for 0.00006 mg/mL PG solution.

S/N	2.628	3.901	3.986	3.622	4.150	1.459

Note: $\bar{X}_{S/N} = 3.291$.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Table 3. Limit of quantitation for PG.

Sample conc., mg/mL	Area counts	Response factor (RF)	S/N
0.0002038	1931.89	9,479,342	11.151
0.0002038	1726.57	8,471,884	14.200
0.0002038	1620.79	7,952,845	10.739
0.0002038	1737.85	8,527,232	10.927
0.0002038	1822.27	8,941,462	10.910
0.0002038	1822.76	8,943,866	11.234
0.001019	9959.41	9,773,710	
0.001019	10118.48	9,929,814	
0.001019	9684.98	9,504,396	
0.001019	10073.27	9,885,447	
0.001019	10067.16	9,879,450	
0.001019	9633.99	9,454,357	

Note: 0.0002038: $\bar{X}_{RF} = 8{,}719{,}439$, $\bar{X}_{S/N} = 11.53$; 0.001019: $\bar{X}_{RF} = 9{,}737{,}862$.

Table 4. Solution stability of PG in an API over a 15 h period.

Time (h)	Area counts
0	393,639
1	393,384
2	396,336
3	394,763
4	393,786
5	393,598
6	392,546
7	394,002
8	393,693
9	393,941
10	394,110
11	393,456
12	394,406
13	393,741
14	393,530
15	393,190

Note: $\bar{X}_{AC} = 393{,}883$; RSD = 0.2%.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Table 5. Recovery of PG from an API.

Sample identification	PG added, wt.%	PG found, wt.%	Recovery, %
Control, no PG	None	None detected	—
0.05% PG spike	0.0544	0.0549	100.9
0.05% PG spike	0.0543	0.0548	100.9
0.10% PG spike	0.1084	0.1095	101.0
0.10% PG spike	0.1085	0.1085	100.0
0.20% PG spike	0.2169	0.2178	100.4
0.20% PG spike	0.2172	0.2172	100.0

Table 6. Propyl gallate results for five lots of API.

Sample identification	PG (dry basis), %	RSD, %
Lot #40	0.103	0.32
Lot #44	0.134	0.37
Lot #45	0.147	1.05
Lot #46	0.155	0.09
Lot #47	0.152	0.21

stability studies are presented in Table 7. These results show, that over a six month period under accelerated stability conditions, 40°C and 75% relative humidity, the amount of PG is reduced approximately 50%, while under long term stability conditions, 30°C and 60% relative humidity, PG levels are essentially unchanged.

CONCLUSION

A method has been developed, which employs Hi–Lo HPLC for the determination of PG in the presence of a bulk pharmaceutical, which is

Table 7. Propyl gallate weight percent results from a stability study.

Storage conditions	Initial	1 Month	2 Month	3 Month	6 Month
30°C/60% RH	0.11	0.11	0.11	0.11	0.10
40°C/75% RH	0.11	0.10	0.09	0.07	0.06

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

achieved in less than 10 min. This method has been validated to be linear, precise, sensitive, and rugged. The chromatographic conditions have been optimized to insure no interferences are observed from the absorbance of the API. The method is used routinely to determine the amount of PG present in an API.

REFERENCES

1. Robard, K.; Dilli, S. Analytical chemistry of synthetic antioxidants. Analyst **1987**, *112* (6), 933–938.
2. Bhargava, H.H.; Nicolai, D.W.; Oza, B.J. Topical suspensions. In *Pharmaceutical Dosage Forms: Disperse Systems*, 2nd Ed.; Lieberman, H.A., Rieger M.M., Banker, G.S., Eds.; Marcel Dekker Inc.: New York, 1996; Vol. 2, 183–242.
3. Ma, Y.; Zhang, J.-Z.; Cong, J.-B.; Wu, K.; Wang, S.; Sun, C.-P.; Duan, S.-J. Microstudies on kinetics of propyl gallate autooxidation by use of oxygen spin probe. Chinese Sci. Bull. **1994**, *39* (5), 397–401.
4. Summanen, J.; Vuirela, H.; Hiltunen, R.; Siren, H.; Riekkola, M.L. Determination of phenolic antioxidants by capillary electrophoresis with ultraviolet detection. J. Chrom. Sci. **1995**, *33* (12), 704–711.
5. Panadero, S.; Gomez-Hens, A.; Perez-Bendito, D. Kinetic determination of propyl gallate in edible and cosmetic oils with sensitized terbium (III) luminescence detection. Analyst **1995**, *120* (1), 125–128.
6. Agui, L.; Lopez-Huertas, M.A.; Yanez-Sedeno, P.; Pingarron, J.M. Voltammetric behaviour of poly(3-methylthiophene)-coated cylindrical carbon fibre microelectrodes: electrochemical oxidation of the antioxidant propyl gallate. J. Electroanal. Chem. **1996**, *414* (2), 141–148.
7. Espinosa-Mansilla, A.; Salinas, F.; del Olmo M.; Orbe-Paya, I. Determination of synthetic food antioxidants in multicomponent mixtures using UV-visible spectrophotometry and partial least-squares calibration. Appl. Spectrosc. **1996**, *50* (3), 449–453.
8. Aguilar-Caballos, M.P.; Gomez-Hens, A.; Perez-Bendito, D. Simultaneous kinetic determination of butylated hydroxyanisole and propyl gallate by coupling stopped-flow mixing technique and diode array detection. Anal. Chim. Acta **1997**, *354* (2–3), 173–179.
9. Luque, M.; Rios, A.; Valcarel, M. A poly(vinyl chloride) graphite composite for flow-injection amperometric determination of antioxidants. Anal. Chim. Acta **1999**, *395* (1–2), 217–223.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

10. Ni, Y.; Chao, L. Artificial neural networks and multivariate calibration for spectrophotometric differential kinetic determinations of food antioxidants. Anal. Chim. Acta **1999**, *396* (2–3), 221–230.
11. Ni, Y.; Wang, L.; Kokot, S. Voltammetric determination of butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate, and *tert*-butylhydroquinone by use of chemometric approaches. Anal. Chim. Acta **2000**, *412* (1–2), 185–196.
12. Aguilar-Caballos, M.P.; Gomez-Hens, A.; Perez-Bendito, D. Simultaneous stopped-flow determination of butylated hydroxyanisole and propyl gallate using a T-format luminescence spectrometer. J. Agric. Food Chem. **2000**, *48* (2), 312–317.
13. Ceballos, C.; Fernandez, H. Synthetic antioxidants determination in lard and vegetable oils by the use of voltammetric methods on disk ultramicroelectrodes. Food Res. Intl. **2000**, *33* (5), 357–365.
14. Boyce, M.C. Simultaneous determination of antioxidants, preservatives, and sweeteners as additives in food by mixed micellar electrokinetic chromatography. J. Chromatogr. A **1999**, *847* (1–2), 369–375.
15. Page, B.D. High performance liquid chromatographic determination of nine phenolic antioxidants in oils, lards, and shortenings. J. Assoc. Off. Anal. Chem. **1979**, *62* (6), 1239–1246.
16. King, W.P.; Joseph, K.T.; Kissinger, P.T. Liquid chromatography with amperometric detection for determining phenolic preservatives. J. Assoc. Off. Anal. Chem. **1980**, *63* (1), 137–142.
17. Page, B.D. High performance liquid chromatographic determination of seven antioxidants in oil and lard: collaborative study. J. Assoc. Off. Anal. Chem. **1983**, *66* (3), 727–745.
18. Page, B.D.; Charbonneau, C.F. Liquid chromatographic determination of seven antioxidants in dry food. J. Assoc. Off. Anal. Chem. **1989**, *72* (2), 259–265.
19. Grosset, C.; Cantin, D.; Villet, A.; Alary, J. HPLC analysis of antioxidants. Talanta **1990**, *37* (3), 301–306.
20. Boussenadji, R.; Porthault, M.; Berthod, A. Microbore liquid chromatography with electrochemical detection for the control of phenolic antioxidants in drugs and foods. J. of Pharm. Biomed. Anal. **1993**, *11* (1), 71–78.
21. Hall, C.A., III; Zhu, A.; Zeece, M.G. Comparison between capillary electrophoresis and high-performance liquid-chromatography separation of food grade antioxidants. J. Agric. Food Chem. **1994**, *42* (4), 919–921.
22. Bianchi, L.; Colivicchi, M.A.; Della Corte, L.; Valoti, M.; Sgaragli, G.P.; Bechi, P. Measurement of synthetic phenolic antioxidants in human tissues by high-performance liquid chromatography with coulometric electrochemical detection. J. Chromatogr. B **1997**, *694* (2), 359–365.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

23. Rafecas, M.; Guardiola, F.; Illera, M.; Codony, R.; Boatella, J. Liquid chromatographic determination of phenolic antioxidants in bakery products. J. Chromatogr. A **1998**, *822* (2), 305–309.
24. Noguera-Orti, J.F.; Villanueva-Camanas, R.M.; Ramis-Ramos, G. Direct injection of edible oils as microemulsions in a micellar mobile phase applied to the liquid chromatographic determination of synthetic antioxidants. Anal. Chim. Acta **1999**, *387* (2), 127–134.
25. Ruiz, M.A.; Garcia-Moreno, E.; Barbas, C.; Pingarron, J.M. Determination of phenolic antioxidants by HPLC with amperometric detection at a nickel phthalocyanine polymer modified electrode. Electroanalysis **1999**, *11* (7), 470–474.
26. Noguera-Orti, J.F.; Villanueva-Camanas, R.M.; Ramis-Ramos, G. Determination of synthetic antioxidants in dairy products and dietetic supplements by micellar liquid chromatography with direct sample injection. Chromatographia **2000**, *51* (1/2), 53–60.
27. Aparicio, A.; San Andres, M.P.; Vera, S. Separation and determination of phenolic antioxidants by HPLC with surfactant/*n*-propanol mobile phases. J. High Resol. Chromatogr. **2000**, *23* (4), 324–328.

Received August 10, 2002
Accepted December 17, 2002
Manuscript 5931

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016