Development and Validation of a Stability-Indicating Method for the Quantitation of Paclitaxel in Pharmaceutical Dosage Forms

Ali Mohammadi[1,2,*], Farnaz Esmaeili[3], Rasoul Dinarvand[3], Fatemeh Atyabi[3], and **Roderick B. Walker[4]**

[1]Department of Drug and Food Control, Faculty of Pharmacy, Tehran University of Medical Sciences, P.O. Box 14155-6451, Tehran 14174, Iran; [2]Pharmaceutical sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran; [3]Medical Nanotechnology Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, P.O. Box 14155-6451, Tehran 14174, Iran; [4]Department of Pharmaceutics, Faculty of Pharmacy, Rhodes University, Grahamstown, South Africa

Abstract

A simple, rapid stability-indicating isocratic assay has been developed and validated for the determination of Paclitaxel (PTX) in commercial injection formulations. The assay is performed using a Nucleosil RP-18 (5 µm, 250 × 4.0 mm i.d) column protected by a Nucleosil C_{18} precolumn (5 µm, 4.0 × 4.0 mm i.d.) with a mobile phase of methanol–water (80:20) and UV detection at 230 nm. The method was found to be specific for PTX in the presence of degradation products with an overall analytical run time of ~ 9 min. Accuracy reported as % bias was found to be 0.1–2.5% bias for all samples tested. Intra-assay precision (repeatability) was found to be 0.22–2.65% RSD, while inter-day precision (intermediate precision) was found to be 1.0–3.0% RSD for the samples studied. The calibration curve was found to be linear with the equation $y = 29.78x + 7.65$, and a linear regression coefficient of 0.9994 over the concentration range 0.05–20 µg/mL. The limits of quantitation and detection were 0.05 and 0.02 µg/mL, respectively. Taxol (30 mg/5 mL), a commercially available dosage form of PTX, was assayed and 100.6–103.6% of the label claim was recovered.

Introduction

Paclitaxel (taxol or PTX) (Figure 1) (1) is a taxane derivative originally derived in limited amounts from the bark of the pacific yew tree *Taxus brevifolia* (Taxaceae). It is now obtained from a taxane precursor derived from the needles of the European yew, *Taxus bacata*, using a semi-synthetic process. It is a BCS class IV drug with a high degree of hydrophobicity and consequently an extremely low aqueous solubility of 4 µg/mL (2,3). PTX has shown significant activity against a wide range of tumors such as those in breast, ovarian, and lung cancer, in addition to head and neck carcinomas (4). Capillary electrophoresis (5), liquid-chromatography–mass spectrometry (6,7), and high-performance liquid chromatography (HPLC) (8–11) methods for the quantitative determination of PTX in biological samples have been reported. These methods are not directly applicable for the determination of PTX in pharmaceutical dosage forms and need further investigation, particularly for method development and validation. In recent years, some HPLC methods (12–14) for the determination of PTX in pharmaceutical dosage forms have also been published. Although these methods can be used for the determination of PTX in the presence of its related substances, none are stability-indicating. Furthermore, the analysis time of the analytical methods used for the quality control of formulated PTX are long (12,13) and require multi-step sample treatment (14) that is laborious and time-consuming. Ciutaru et al. have published a validated method for the determination of the related impurities of PTX in pharmaceutical dosage forms (15). The method, reported in the current United States Pharmacopoeia (USP) (16), requires the use of gradient elution with a mobile phase of water and acetonitrile. There is no information published in the USP with respect to the stability-

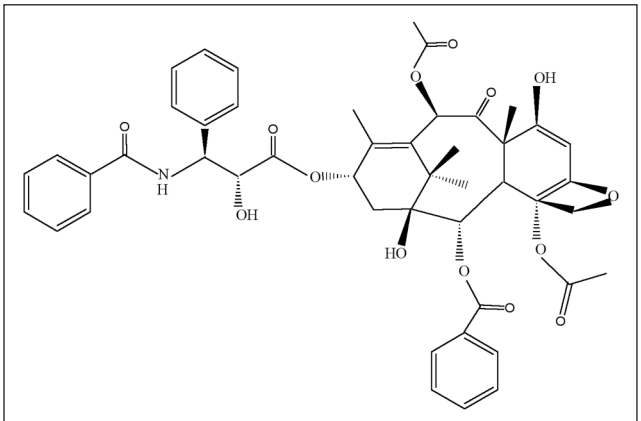

Figure 1. Structural formula of PTX (MW = 853.92).

* Author to whom correspondence should be addressed: email alimohammadi@tums.ac.ir.

indicating nature of the method. Stability is important from a quality control perspective in the pharmaceutical industry, and therefore any analytical method developed should preferably be stability-indicating. To our knowledge, no validated stability-indicating analytical method for the determination of PTX in pharmaceutical dosage forms has been reported in the literature. Therefore, an isocratic stability-indicating HPLC method for the rapid quantitative determination of PTX in presence of degradation products in pharmaceutical dosage forms was developed. This paper reports the forced degradation of PTX under stress conditions including acid and base hydrolysis, oxidation, heat, and UV light. This manuscript also reports the validation of the method for the accurate assay of PTX in a commercially available pharmaceutical dosage form without interference from Cremophor EL (polyoxyethylated castor oil), a component of the formulation.

Experimental

Chemicals and reagents

PTX (99.95% purity) was kindly supplied by Cipla (India) and was used without further purification. Taxol (30 mg/5 mL) was obtained from Bristol-Meyer Squibb's (Canada). Methanol, sodium hydroxide, hydrochloric acid, and hydrogen peroxide were obtained from Merck (Darmstadt, Germany). All reagents used were at least of analytical grade, except methanol, which was HPLC grade. HPLC-grade water was obtained following distillation in glass and passage through a Milli-Q system (Millipore, Milford, MA) and was used to prepare all solutions.

HPLC instrumentation and conditions

Chromatographic analyses were performed using an Agilent HPLC system that consisted of a model G1311A quaternary HPLC pump (Agilent Technologies, Palo Alto, CA), a model G1329A autosampler system (Agilent Technologies) fitted with a 20 µL sample loop, and a model G1315B diode array detector (Agilent Technologies).

Chromatographic data were monitored and analyzed using Agilent ChemStation software (Agilent Technologies). Separation of the compounds of interest was achieved using a Nucleosil RP-18 (5 µm, 250 × 4.0 mm) analytical column protected by a C_{18} Nucleosil precolumn (5 µm, 4.0 × 4.0 mm). The mobile phase was comprised of methanol–water in a ratio of 80:20 v/v and was delivered at a flow rate of 1.0 mL/min, and the eluent was monitored at a wavelength of 230 nm. The column was maintained at ambient temperature (25ºC) and 20 µL samples were introduced onto the HPLC system every 15 min. The mobile phase was filtered through a 0.45 µm Chrom Tech Nylon-66 filter prior to use.

Preparation of stock and standard solutions

A stock solution of PTX (1 mg/mL) was prepared in methanol. The stock solution was protected from light using aluminium foil and stored for 7 days at 4ºC with no evidence of decomposition. Aliquots of the standard stock solution of PTX were transferred into 10-mL A-grade volumetric flasks using A-grade bulb pipettes and the solutions were made up to volume with mobile phase to yield solutions with final concentrations of 0.05, 0.1, 0.5, 1, 6, and 20 µg/mL.

Preparation of commercial products for assay

The content of three ampoules of the relevant commercial product were poured into a volumetric flask and mixed well. One hundred microliters of the resultant solution was added into each of six 100-mL volumetric flasks, made up to volume with mobile phase, and mixed well to yield a 6 µg/mL solution of PTX.

Forced degradation studies of PTX

In order to assure the selectivity of the method and to provide an indication of the stability-indicating properties of the proposed method, pure active pharmaceutical ingredient (API) of PTX was stressed under various conditions to conduct forced degradation studies (17). PTX is insoluble in water, soluble in ethanol, and freely soluble and stable in methanol. Therefore, methanol was selected as a co-solvent in all forced degradation studies. All solutions for use in forced degradation studies were prepared by dissolving the API in an equal volume of methanol and solutions of aqueous hydrogen peroxide, distilled water, hydrochloric acid, or sodium hydroxide to achieve a concentration of 120 µg/mL of PTX. Following degradation, the solutions were diluted with mobile phase (80:20) to yield concentrations of ~ 6 µg/mL for PTX.

Oxidation studies

Solutions for use in oxidation studies were prepared in methanol and 10% H_2O_2 (50:50 v/v), and the resultant solutions were analyzed 4 days after preparation.

Acid degradation studies

During initial forced degradation studies, it was observed that acid and base hydrolysis of PTX occurred rapidly and that complete degradation of drug had taken place when the resultant solution was analyzed 24 h after preparation. Therefore, for all subsequent experiments, the exposure time was decreased to 5 h. Solutions for acid degradation studies were prepared in methanol and 1M hydrochloric acid (50:50, v/v), and the resultant solutions were analyzed 5 h after preparation.

Alkali degradation studies

Solutions for alkali degradation studies were prepared in methanol and 0.1M sodium hydroxide (50:50 v/v), and the resultant solutions analyzed 5 h after preparation.

Neutral degradation studies

Solutions for neutral degradation studies were prepared in methanol and water (50:50 v/v), and the resultant solutions protected from light for 24 h prior to analysis.

Temperature stress studies

PTX was exposed to dry heat (150ºC) in a convection oven for 24 h and was then prepared for analysis as previously described.

Photostability studies

PTX powder and solutions of drug were prepared and exposed to light to determine the effects of irradiation on the stability of PTX in solution and in the solid state. Approximately 50 mg of PTX was spread onto a glass dish in a layer that was less than 2 mm in thickness. A solution of PTX (120 µg/mL) was prepared in methanol and HPLC-grade water (50:50, v/v). All samples for photostability testing were placed in a light cabinet (Suntest CPS/CPS+, Atlas Material Testing Technology, Germany) and exposed to light for 30 h, resulting in an overall illumination of ≥ 200 w.h/m^2 at 25ºC with UV radiation in the range 320–400 nm. Control samples were protected from light with aluminium foil and then exposed to light in the light cabinet. Exposure of all samples was concurrent. Following removal of the samples from the light cabinet, samples were prepared for analysis as previously described.

Results and Discussion

HPLC method development and optimization

A Nucleosil RP-18 (5 µm, 250 × 4.0 mm) analytical column protected by a C_{18} Nucleosil precolumn (5 µm, 4.0 × 4.0 mm) maintained at ambient temperature (25ºC) was used for the separation and the method validated for the determination of PTX in Taxol injections. The composition and the flow rate of the mobile phase were changed to optimize the separation conditions using the stressed samples. A mobile phase consisting of methanol and water (80:20, v/v) set at a flow rate of 1 mL/min was ultimately selected for use for further studies after preliminary chromatographic separations. In terms of determining the most appropriate and acceptable separation conditions, the optimization of gradient elution is more complex than for isocratic methods because there are more variables that can influence the selectivity (primarily gradient steepness, initial eluent strength, and secondarily dwell volume) of the method (18). In addition, the transfer of a gradient elution method between columns, instruments, and laboratories is notoriously a more difficult (19) and inherently slower technique than isocratic methods. Furthermore, many chromatographers have experienced "ghost" peaks (20), baseline noise (21), and other disturbances (e.g., eluent mixing) associated with gradient elution that can lead to inaccurate determination of peak area and peak height, thereby preventing the accurate and precise quantitation of the compound of interest. Under the experimental conditions described, all peaks of interest were well defined and free from tailing. The effects of small deliberate changes in the mobile phase composition and flow rate were evaluated as a part method robustness testing.

Validation of the method

The analytical method was validated with respect to several parameters including linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, selectivity, recovery, and robustness/ruggedness (22,23).

Linearity

Linearity was established by least squares linear regression analysis of the calibration curve (24,25). The calibration curves (n = 3) that were constructed for PTX were linear over the concentrations of 0.05, 0.1, 0.5, 1, 6, and 20 µg/mL. Peak areas of PTX were plotted versus concentration and linear regression analysis performed on the resultant curves. Three correlation coefficients of R1 = 0.9994, R2 = 0.9993, and R3 = 0.9991 with % relative standard deviation (RSD) values ranging from 0.2–3% across the concentration range studied were obtained following linear regression analysis. Typically, the regression equation for the calibration curve was found to be $y = 29.78X + 7.65$.

LOQ and LOD

The LOQ was determined as the lowest amount of analyte that was reproducibly quantified above the baseline noise following triplicate injection. The resultant % RSD for these studies was ≤ 0.35%. The LOQ that produced the requisite precision and accuracy was found to be 50 ng/mL. The LOD was determined based on signal-to-noise ratio using an analytical response of three times the background noise (26). The LOD for PTX was found to be 20 ng/mL using this method. In a previously published method (13) for the determination of PTX in pharmaceutical dosage forms, the LOQ and LOD of the method were found to be 240 and 72 ng/mL, respectively. Recently, two other HPLC methods (12,14) developed for the determination of PTX in pharmaceutical dosage forms have also been published, and neither the LOQ nor the LOD were reported.

Precision

The intra- and inter-day variability or precision data are summarized in Table I and were assessed by the use of standard solutions of three different concentrations of drug. Repeatability or intra-day precision was investigated by injecting nine replicate samples of PTX solutions of three different concentrations. Inter-day precision was assessed by injecting the same three samples over three consecutive days. The data reported in Table I reveal that the method precision has a RSD below 2.13% for intra-assay precision and 2.20% for inter-assay precision. In a previously published method (13) for the determination of PTX in pharmaceutical dosage forms, the RSD for repeatability and intermediate precision were determined to be below 1% and 0.76%, respectively.

Table I. Intra- and Inter-Assay Precision Data (n = 9)*

Actual concentration PTX (µg/mL)	Measured concentration (µg/mL), RSD (%)	
	Intra-day	Inter-day
0.05	0.051, 1.43	0.05, 2.15
2	2.04, 2.13	2.01, 1.25
20	20.45, 0.35	19.80, 2.20

* Data expressed as mean for "measured concentration" values.

Accuracy

Accuracy data for the assay following the determination of PTX are summarized in Table II. Accuracy was determined by interpolation of replicate (n = 6) peak areas of three accuracy standards (0.05, 1, and 20 µg/mL) from a calibration curve that had been freshly prepared as previously described. In each case, the percent relative error and accuracy were calculated and found to be less than 2.5%. In a previously published method (13), accuracy determined in the interval 80–120% of the working concentration of the PTX and evaluated by the parameter "recovery" was within the proposed limits (100 ± 2%), with results ranging from 98.2% to 100.5% and a RSD of 0.83%.

Specificity

The results of stress testing studies indicated a high degree of specificity of this method for PTX. Taxol injection is a clear colorless to slightly yellow viscous solution. It is supplied as a nonaqueous solution intended for dilution with a suitable parenteral fluid prior to intravenous infusion. Taxol is available in 30 mg (5 mL), 100 mg (16.7 mL), and 300 mg (50 mL) multidose vials. Each mL of sterile nonpyrogenic solution contains 6 mg paclitaxel, 527 mg of purified Cremophor EL, and 49.7% (v/v) dehydrated alcohol. Peak purity values for PTX in chromatograms of stressed samples and Taxol injections were in the range of 0.999–1, indicating that the peaks were homogenous, thereby establishing the selectivity of this assay method. Typical chromatograms obtained following the assay of untreated PTX and stressed PTX solutions are shown in Figure 2.

Table II. Accuracy Data (n = 5)*

PTX concentration (µg/mL)	Interpolated concentration (mean ± SD)	RSD (%)	RE (%)
0.05	0.049 ± 0.001	2.05	2.0
1	1.018 ± 0.025	2.45	1.83
20	20.50 ± 0.120	0.58	2.48

* Data obtained from five replicates at each concentration.

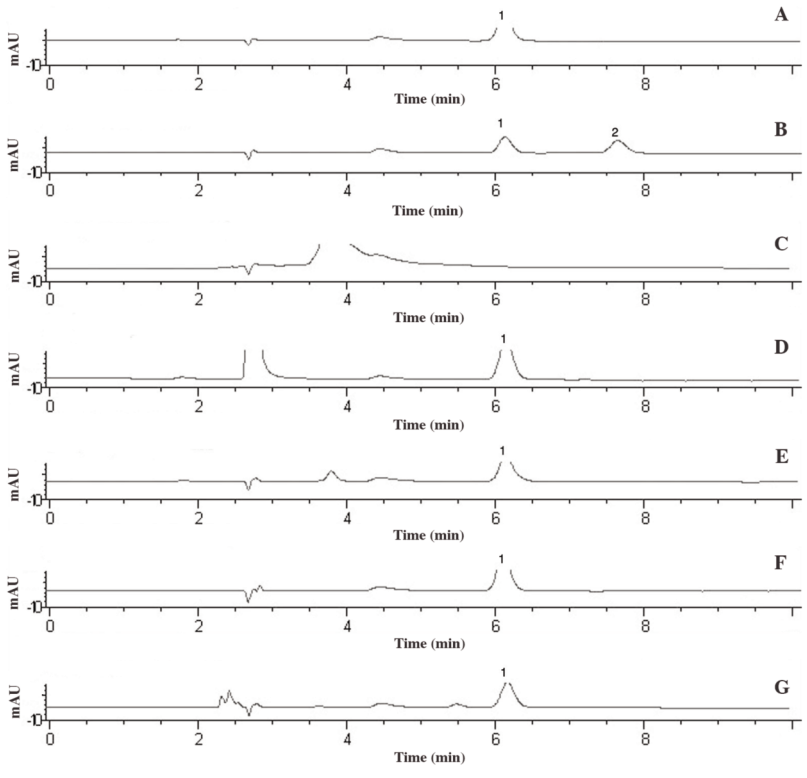

Figure 2. Typical HPLC chromatograms of: untreated API (A); dry-heated API, showing PTX (peak 1) and 7-epi-taxol (peak 2) (B); base hydrolysis-degraded API (C); oxidative degraded API (D); photodegraded API (E); neutral-hydrolysis degraded API (F); and acid hydrolysis-degraded API (G).

Ruggedness and robustness tests

As recommended in the International Conference on Harmonization (ICH) Guidelines, a robustness assessment was performed during the development of the analytical method (27). The ruggedness (28) of the method is assessed by comparison of the intra- and inter-day assay results for PTX that were performed by two analysts in two different laboratories. The % RSD values for intra- and inter-day assays of PTX in the Taxol injections performed in the two different laboratories by two analysts did not exceed 3.5%, indicating the ruggedness of the method. In addition, the robustness of the method was investigated under a variety of conditions including changes to flow rate, laboratory temperature, and eluent composition (29). The degree of reproducibility of the results obtained as a result of small but deliberate variations in these method para-meters in addition to changing the analyst proves that the method is robust. The percent recoveries of PTX in Table III are good under most conditions and did not show a significant change when the critical parameters were modified.

Results of forced degradation studies

All stressed samples tested in the solid state and in solution remained colorless following exposure, and PTX was found to be completely stable under oxidative (Figure 2D) and neutral (Figure 2F) stress conditions, whereas exposure of PTX to alkaline (Figure 2C) conditions in methanol and 0.1M sodium hydroxide (50:50 v/v) resulted in 100% decomposition within 5 h. On the other hand, PTX decomposed with a resultant 12%, 18%, and 50% degradation under photolytic (Figure 2E), acidic (Figure 2G), and thermal (Figure 2B) stress conditions, respectively. The major impurity formed under thermal stress in the solid state was observed to be 7-epi-taxol, the thermodynamically more stable isomer (Figure 2B). It was not the intention of the study to identify degradation products of API and excipients but merely to

show that they would not interfere if and when present. Peak purity results determined using diode array detection confirm that the PTX peak is homogeneous and pure in all stress samples. The percentage recoveries of PTX are presented in Table IV. The stability of the stock solution was determined by quantitation of drug in solution in comparison to the response obtained for freshly prepared standard solutions. No significant changes (< 1%) were observed for the chromatographic responses for the stock solutions relative to freshly prepared standards.

Table III. Influence of Changes in Experimental Conditions on the Performance of the HPLC System

Parameter	Modification	PTX (% recovery)
Flow rate (mL/min)	0.9	101.6
	1.0	101.3
	1.1	99.9
Methanol composition (%)	77	99.9
	80	100.6
	83	101.5
Lab temperature (°C)	20	101.8
	25	99.8
	30	102.3

Table IV. Degradation of Pure API of PTX Under Stress Conditions

Storage condition	Time	Recovered (%)
Methanol and 10% H_2O_2 (50:50, v/v), 25°C	4 days	Not decomposed
Methanol and 1M HCl	24 h	Completely decomposed
(50:50, v/v), 25°C	5 h	80
Methanol and 0.1M NaOH (50:50, v/v), 25°C	5 h	Completely decomposed
Methanol and water (50:50, v/v), 25°C	24 h	Not decomposed
Dry heat of 150°C	24 h	50
UV radiation at 320–400 nm		
In methanol and water (50:50, v/v), 25°C	30 h	88
In the solid state	30 h	Not decomposed

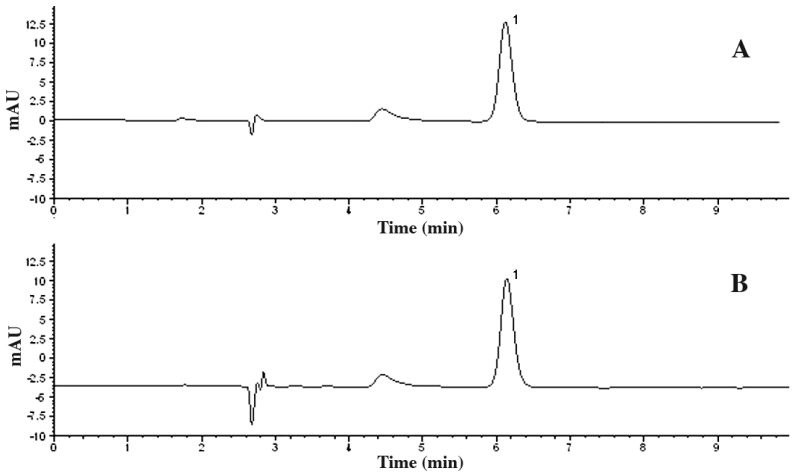

Figure 3. Resultant HPLC chromatograms following the analysis of a standard solution of paclitaxel (6 µg/mL) (A), and Taxol injections (B) showing paclitaxel (peak 1).

Assay

The validated analytical method was applied to the determination of PTX in commercially available Taxol injections. Figure 3 depicts typical HPLC chromatograms obtained following the analysis of Taxol injections (A) and from a standard solution (B). The peak at 4.5 min is a related substance of PTX. The sources of the PTX API and PTX, which were used for the preparation of the injectable formulation were the same. The concentration of standard solution and assay solution is the same; therefore, the peak is visible in both chromatograms. The results of the assay (n = 6) yielded 101.17% (% RSD = 1.20%) of label claim for PTX. The observed concentration of PTX was found to be 6.070 ± 0.073 µg/mL (mean ± SD). The mean retention time of PTX was 6.17 min with associated % RSD values of 0.10%. The results of the assay indicate that the method is selective for the analysis of PTX without interference from the excipients used to formulate and produce these dosage forms. Peak purity values for PTX in chromatograms of injection samples were in the range of 0.999–1, indicating that the peaks were homogenous, thereby establishing the selectivity of this assay method.

Conclusions

A simple, rapid, accurate, and precise stability-indicating HPLC analytical method has been developed and validated for the routine analysis of PTX in raw material and injectable dosage forms. The results of stress testing, undertaken according to the ICH guidelines, reveal that the method is selective and stability-indicating. The proposed method has the ability to separate this drug from its degradation products and can be applied to the analysis of samples obtained during accelerated stability experiments.

Acknowledgments

The authors would like to acknowledge financial assistance from Pharmaceutical Sciences Research Center, as well as the Novel Drug Delivery Systems Laboratories, Tehran University of Medical Sciences, Tehran, Iran, for providing facilities used throughout this research.

References

1. M.J. O'Neil, A. Smith, and P.E. Heckelman. *The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals*, 14th ed. Merck & Co. Inc., White House Station, NJ, 2006, p. 1204.
2. S.C. Sweetman. *Martindale the Complete Drug Reference*, 35th ed. Pharmaceutical Press, London, UK, 2006, p. 685.
3. G.M. Zentner, R. Rathi, C. Shih, J.C. McRea, M. Seo, H. Oh, B.G. Rhee, J. Mestecky, Z. Moldoveanu, M. Morgan, and S. Weitman. Biodegradable block copolymers for delivery of proteins and water-insoluble drugs. *J. Controlled Release* **72:** 203–215 (2001).
4. S.G. Arbuck and B. Blaylock. TAXOL: clinical results and current issues in development. In *TAXOL: Science and Applications*. M. Suffness, ed. CRC Press, New York, NY, 1995, p. 379.
5. G. Hempel, D. Lehmkuhl, S. Krumpelmann, G. Blaschke, and J. Boos. Determination of paclitaxel in biological fluids by micellar electrokinetic chromatography. *J. Chromatogr. A* **745:** 173–179 (1996).
6. G. Theodoridis, G. Laskaris, E.L.M. van Rozendaal, and R. Verpoorte. Analysis of taxines in Taxus plant material and cell cultures by HPLC photodiode array and HPLC-electrospray mass spectrometry. *J. Liquid Chromatogr. Rel. Tech.* **24:** 2267–2282 (2001).
7. S.F. Baldrey, R.R. Brodie, G.R. Morris, E.H. Jenkins, and S.T. Brookes. Comparison of LC-UV and LC-MS-MS for the determination of taxol. *Chromatographia* **55:** S187–S192 (2002).
8. A. Sparreboom, P. de Bruijn, K. Nooter, W.J. Loos, G. Stoter, and J. Verweij. Determination of paclitaxel in human plasma using single solvent extraction prior to isocratic reversed-phase high-performance liquid chromatography with ultraviolet detection. *J. Chromatogr. B* **705:** 159–164 (1998).
9. S.H. Lee, S.D. Yoo, and K.H. Lee. Rapid and sensitive determination of paclitaxel in mouse plasma by high-performance liquid chromatography. *J. Chromatogr. B* **724:** 357–363 (1999).
10. T. Nguyen, J. Eshraghi, G. Gonyea, R. Ream, and R. Smith. Studies on factors influencing stability and recovery of paclitaxel from suspension media and cultures of *Taxus cuspidata* cv Densiformis by high-performance liquid chromatography. *J. Chromatogr. A* **911:** 55–61 (2001).
11. S.C. Kim, J. Yu, J.W. Lee, E.S. Park, and S.C. Chi. Sensitive HPLC method for quantitation of paclitaxel (Genexol®) in biological samples with application to preclinical pharmacokinetics and biodistribution. *J. Pharm. Biomed. Anal.* **39:** 170–176 (2005).
12. H.Y. Aboul-Enein and V. Serignese. Liquid chromatographic determination of taxol and related derivatives using a new polyfluorinated reversed-phase column. *Anal. Chim. Acta* **319:** 187–190 (1996).
13. I. Badea, D. Ciutaru, L. Lazar, D. Nicolescu, and A. Tudose. Rapid HPLC method for the determination of paclitaxel in pharmaceutical forms without separation. *J. Pharm. Biomed. Anal.* **34:** 501–507 (2004).
14. J.D. Perdue, P.J. Seaton, J.A. Tyrell, and D.R. DeVido. The removal of Cremophor® EL from paclitaxel for quantitative analysis by HPLC–UV. *J. Pharm. Biomed. Anal.* **41:** 117–123 (2006).
15. D. Ciutaru, I. Badea, L. Lazar, D. Nicolescu, and A. Tudose. A HPLC validated assay of paclitaxel's related impurities in pharmaceutical forms containing Cremophor® EL. *J. Pharm. Biomed. Anal.* **34:** 493–499 (2004).
16. United States Pharmacopeia 29, National Formulary 24, U.S. Pharmacopeial Convention Inc., Rockville, 2006, pp.1624–1627.
17. ICH, Stability Testing of New Drug Substances and Products (Q1AR2). International Conference on Harmonization, IFPMA, Geneva, 2003.
18. U.D. Neue and J.R. Mazzeo. A theoretical study of the optimization of gradients at elevated temperature. *J. Sep. Sci.* **24:** 921–929 (2001).
19. W. Dolan. The hazards of adjusting gradients. *LC-GC* **20:** 940–946 (2002).
20. S. Williams. Ghost peaks in reversed-phase gradient HPLC: a review and update. *J. Chromatogr. A* **1052:** 1–11 (2004).
21. H.F.M. Boelens, R.J. Dijkstra, P.H.C. Eilers, F. Fitzpatrick, and J.A. Westerhuis. New background correction method for liquid chromatography with diode array detection, infrared spectroscopic detection and Raman spectroscopic detection. *J. Chromatogr. A* **1057:** 21–30 (2004).
22. A. Mohammadi, I. Haririan, N. Rezanour, L. Ghiasi, and R.B.Walker. A stability-indicating high performance liquid chromatographic assay for the determination of orlistat in capsules. *J. Chromatogr. A* **1116:** 153–157 (2006).
23. A. Mohammadi, N. Rezanour, M. Ansari Dogaheh, F. Ghorbani Bidkorbeh, M. Hashem, and R.B. Walker. A stability-indicating high performance liquid chromatographic (HPLC) assay for the simultaneous determination of atorvastatin and amlodipine in commercial tablets. *J. Chromatogr. B* **846:** 215–221(2007).
24. J.C. Miller and J.N. Miller. *Statistics for Analytical Chemistry*, vol. 22. Ellis Horwood, Chichester, UK, 1984. p. 82.
25. A. Mohammadi, A. Mehramizi, F. Aghaee Moghaddam, L. Erfani Jabarian, M. Pourfarzib, and H.N. Kashani. Development and validation of a stability-indicating high performance liquid chromatographic (HPLC) assay for biperiden in bulk form and pharmaceutical dosage forms. *J. Chromatogr. B* **854:** 152–157 (2007).
26. ICH Draft Guidelines on Validation of Analytical procedures: Definitions and Terminology, Federal Register, Vol. 60, IFPMA, Switzerland, 1995.
27. M. Zeaiter, J.M. Roger, V.B. Maurel, and D.N. Rutledge. Robustness of models developed by multivariate calibration. Part I: the assessment of robustness. *Trends Anal. Chem.* **23:** 157–170 (2004).
28. M. Mulholland. Ruggedness testing in analytical chemistry. *Trends Anal. Chem.* **7:** 383–389 (1988).
29. Y.V. Heyden, A. Nijhuis, J.S. Verbeke, B.G.M. Vandeginste, and D.L. Massaret. Guidance for robustness/ruggedness tests in method validation. *J. Pharm. Biomed. Anal.* **24:** 723–753 (2001).

Manuscript received September 18, 2007;
revision received April 24, 2008.