Quantitation of Residual Dimethylsulfoxide in a Drug Substance (Bisnafide) by Reversed-Phase High-Performance Liquid Chromatography

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Abstract

A reversed-phase high-performance liquid chromatographic method is developed in which low-wavelength ultraviolet detection is used to determine trace levels of residual dimethylsulfoxide (DMSO) in an antineoplastic agent, bisnafide (DMP 840 drug substance). The method is sensitive and free from interferences with a linearity for DMSO over a wide range of concentrations (0.219–3.288 µg/mL). The detection limit is approximately 0.051 µg/mL based on a signal-to-noise ratio of 3 and a 200-µL injection, and the limit of quantitation is approximately 0.219 µg/mL (relative standard deviation, 4.8%).

Introduction

The determination of residual solvents in an active drug substance that is used in a pharmaceutical product is an important analytical control because of both the need to accurately determine drug substance purity and the toxicity of some solvents (1). Traditionally, gas chromatography is the technique used for the analysis of residual solvents in active drug substances.

Bisnafide is a member of the bis-naphthalimide family and is under development as an anticancer agent (2,3). The chemical structure is shown in Figure 1. The synthesis of bisnafide was changed from a batch process to a continuous process, which improved the yield and produced drug substances of equivalent or greater purity (J. Matos and J. Fortunak, DuPont Merck Pharmaceutical Company, personal communication, April, 1994). Acetonitrile was the residual solvent in the batch process and was determined with gas chromatography. Dimethylsulfoxide (DMSO) was used as a sample solution solvent. Synthesis by the continuous process involves the introduction of DMSO as a reaction solvent in the final step of the synthesis. As a result, a method was needed to measure residual DMSO in the drug substance. A high-performance liquid chromatographic (HPLC) method was developed in which low-wavelength detection is used to monitor residual DMSO present in the drug substance.

Previous authors described methods for the detection and quantitation of DMSO by titrimetry (4–6); however, these methods were not specific for DMSO. Other authors described gas chromatographic methods (7–11), but these methods did not measure DMSO directly and involved cumbersome sample preparation steps or were not suitable for trace level determinations. Liquid chromatography (12–15) has been used to determine levels of DMSO in rainwater, sea water, waste water, tissues, and aqueous solutions. To our knowledge, this paper is the first example which implements the use of reversed-phase HPLC with low-wavelength detection to quantitate trace levels of DMSO in a drug substance.

Experimental

Reagents and Chemicals

Bisnafide from DuPont Merck (Wilmington, DE) was used as received. High-purity acetonitrile was obtained from EM Science (Gibbstown, NJ), DMSO was obtained from Burdick and Jackson (Muskegon, MI), high purity water (> 18 MΩ-cm resistivity) was obtained from a Millipore Milli-Q Plus device (Milford, MA), HPLC-grade phosphoric acid (85%) was obtained from Fisher Scientific (Fairlawn, NJ), and sodium chloride was obtained from Fluka Chemie AG (Ronkonkoma, NY).

Chromatographic systems

Chromatographic results were obtained with use of two chromatographic systems. The first consisted of a Waters WISP

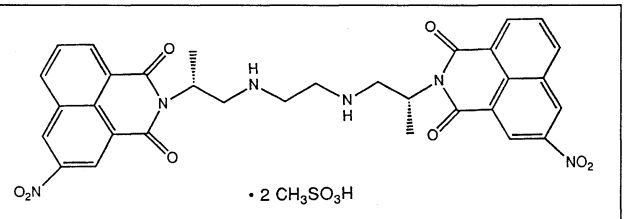

Figure 1. Chemical structure of DMP 840.

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712 sample injector (Milford, MA), a Waters 600E pump, a Waters 490 detector, and a DuPont Instruments column oven. The second consisted of a Waters WISP 717 sample injector, a Waters 600E pump, a Kratos Spectroflow 783 detector, and a DuPont Instruments column oven. Integration of peaks was performed with a Fisons Multichrom data acquisition system (Cheshire, England).

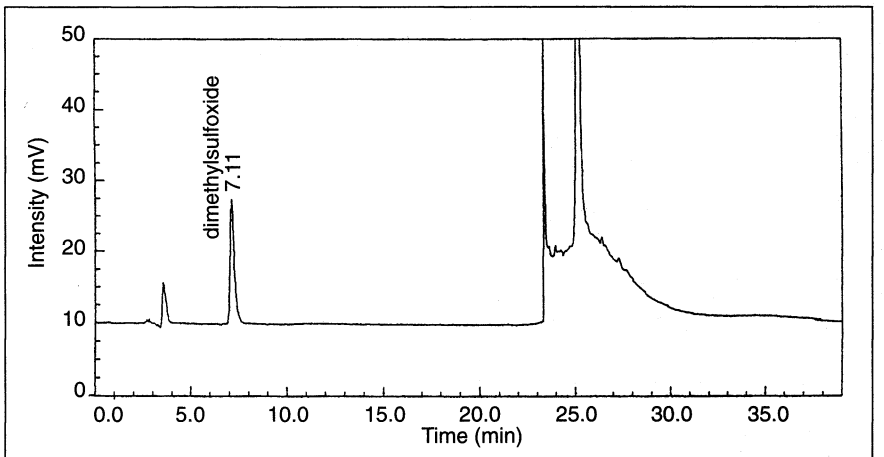

Figure 2. Chromatogram of standard (2.19 µg/mL DMSO)

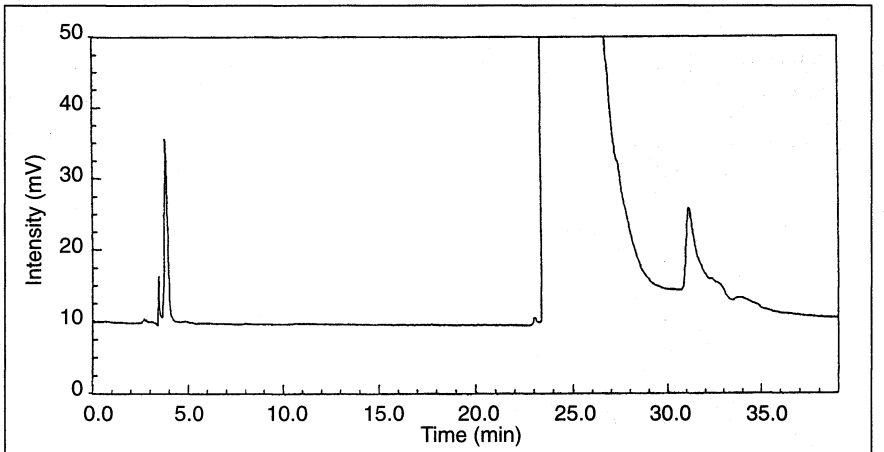

Figure 3. Chromatogram of sample (2 mg/mL DMP 840). Note the absence of a peak at the DMSO retention time.

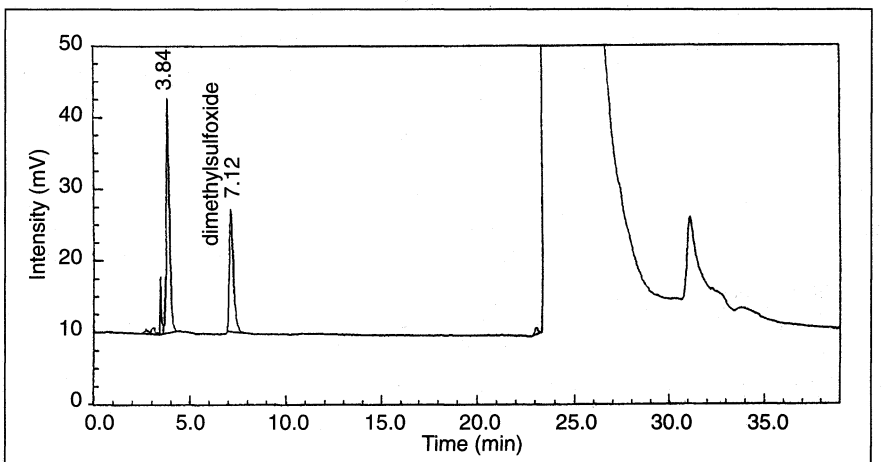

Figure 4. Chromatogram of DMSO spiked sample (2 mg/mL DMP 840 spiked with 2.19 µg/mL DMSO).

Chromatographic conditions

Mobile phase A was an aqueous 0.01M sodium chloride solution that contained 0.10% phosphoric acid by volume. Mobile phase B was composed of 90% acetonitrile and 10% mobile phase A by volume. The following step gradient profile was used: Mobile phase A was pumped for the first 10 min, then mobile phase B was pumped for the next 5 min as a column wash, and finally mobile phase A was pumped for the next 15 min to allow the system to re-equilibrate prior to the next injection. The mobile phases were filtered, degassed, and pumped at a flow rate of 0.80 mL/min. The injection volume was 200 µL for samples and standards. The column used was a Zorbax Rx-C8 from Mac Mod (Chadds Ford, PA). Column temperature was maintained at 45°C ± 2. The detector wavelength was 215 nm.

Sample and standard preparation

Sample solutions of bisnafide were prepared by accurately weighing about 40 mg of the drug substance into an Erlenmeyer flask and then pipetting 20.0 mL of water. After capping the flask, the sample was sonicated to disperse any large particles and stirred at low heat until the sample was completely dissolved. Standard and sample solutions were analyzed within 24 h. Solution stability studies showed that the methylsulfoxide was stable in the sample preparation solvent. Standards were prepared at 3.3, 2.2, and 1.1 µg DMSO per milliliter in water. These values correspond to DMSO levels of 0.16, 0.11, and 0.055% (w/w) in the drug substance. For recovery studies, samples of the drug substance synthesized by using the batch process, free of DMSO, were spiked with known quantities of DMSO.

Results and Discussion

Method development

A reversed-phase HPLC method was developed based on the work of Thumm et al. (12). A gradient to high organic content was implemented to remove organic materials from the column. The pH of the mobile

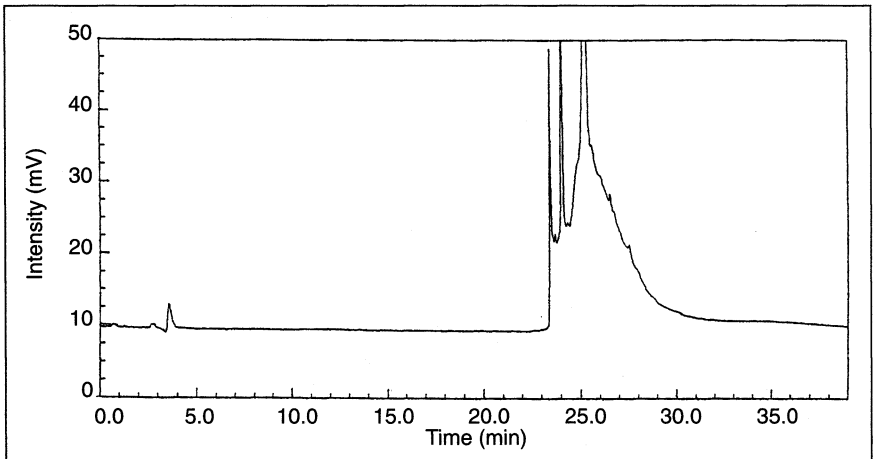

Figure 5. Chromatogram of blank (water).

phases was lowered with phosphoric acid to minimize hydrolysis of bisnafide. Sodium chloride was added to the mobile phases to increase the retention time of the DMSO peak. The wavelength was changed from 195 nm to 215 nm to reduce interferences and minimize noise. Representative chromatograms of a standard, sample, DMSO spiked sample, and blank are shown in Figures 2–5, respectively.

Accuracy and precision

The accuracy and precision of the method were validated by using recovery studies. Bisnafide was spiked with DMSO at three concentration levels: 0.164, 0.110, and 0.055% (%w/w relative to drug). Each sample was prepared in triplicate. The recovery experiments were conducted by two chemists on different days with different instruments. Replicate injections of various standard levels of DMSO yielded good precision, less than 1% relative standard deviation (RSD) on each day that the recovery study was performed. The results show complete recovery of the spiked DMSO from the drug matrix. The results are presented in Table I.

Table I. Percent Recovery of DMSO in DMP 840 Drug Substance

	% Recovery	
% DMSO spiked	Chemist 1	Chemist 2
0.055	99.2	99.4
	99.7	99.1
	101.7	98.8
0.110	99.7	99.4
	99.5	99.2
	99.4	99.2
0.160	99.5	101.0
	98.9	100.0
	99.1	100.9
Mean	99.6	99.7
% RSD*	0.8	0.8

* RSD = relative standard deviation.

Linearity, detection limits, and quantitation limits

The linear range investigated for DMSO was from 0.219 to 3.288 µg/mL. The correlation coefficient was 0.9999, the slope was 1.14×10^5 µVsec mL/µg, and the *y*-intercept was –12.1 µVsec. The standard error of estimate was 3044 µVsec.

The detection limit was defined as the concentration at which the signal-to-noise ratio (S/N) equalled 3. The quantitation limit was defined as the lowest concentration at which the RSD for six replicate injections is less than or equal to 5%, which meets the system suitability criterion. The detection limit with this method was approximately 0.051 µg/mL, and the quantitation limit was 0.219 µg/mL (4.8% RSD). The detection limit and limit of quantitation were 25.5 ppm DMSO and 109.5 ppm DMSO, respectively, in the sample of bisnafide. The limit of quantitation data are presented in Table II.

Specificity

No interferences were noted from sample diluent, sample matrix, or other potential solvents used in the synthesis of bisnafide. The other solvents tested included methanol, acetonitrile, 1,3-dioxolane, tetrahydrofuran, and toluene.

Conclusion

Reversed-phase HPLC with UV detection is a sensitive, accurate, precise, linear, and specific method that provides an alternative means for the determination of DMSO. The advantages of this method are ease of use and sensitivity. The method should be useful for quantitation of residual DMSO in other active drug substances. In addition, reversed-phase HPLC with low-wavelength detection may be used to accurately quantitate other high-boiling solvents, such as *N*-methylpyrrolidone (J.A. Shea, DuPont Merck Pharmaceutical Company, personal communication, June, 1995).

Table II. Determination of the Limit of Quantitation*

No.	Peak area (µV sec)
1	28222.7
2	28596.0
3	31595.3
4	29597.0
5	31456.4
6	29231.0
Mean	29783.1
% RSD†	4.8

* Standard concentration was 0.219 µg/mL.
† RSD = relative standard deviation.

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