Quantitation of Residual *N*-Methylpyrrolidone in Losoxantrone Hydrochloride by Reversed-Phase High-Performance Liquid Chromatography

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Abstract

A reversed-phase high-performance liquid chromatographic method designed to quantitate *N*-methylpyrrolidone (NMP) as a residual solvent in losoxantrone hydrochloride (DuP 941) is described. The experiments performed demonstrate that this procedure can reliably quantitate low concentrations (< 0.01%) of NMP in losoxantrone hydrochloride. The sensitivity, linearity, and reproducibility of this method are also demonstrated.

Introduction

Losoxantrone hydrochloride (DuP 941, CI-941) is a member of the anthrapyrazole class of compounds, intended for the treatment of advanced breast cancer (1–5). The chemical structure is shown in Figure 1. In the quality control of the drug substance, one important test is for residual solvents and organic volatile impurities that may be left over from the synthetic process. Typically, this test is performed by capillary gas chromatography (GC) (6,7) or, less frequently, by headspace GC (8,9). High-performance liquid chromatography (HPLC) is not commonly employed for residual solvent analysis, although there are examples in the literature in which it has been used to determine residual dimethylsulfoxide (10) and pyridine (11) in drug substances.

When *N*-methylpyrrolidone (NMP, structure shown in Figure 1) was added to the synthetic scheme for losoxantrone hydrochloride, a means of determining whether residual NMP was present in the finished drug substance had to be developed. The GC method then used to determine residual solvents in losoxantrone required that the drug be dissolved in dimethyl formamide (DMF) at 50 mg/mL. Of the solvents tested, only water, DMF, NMP, and dimethyl sulfoxide (DMSO) dissolved losoxantrone hydrochloride at concentrations sufficient to provide the required sensitivity (< 0.1%, relative to the drug substance). All three organic solvents interfered with the determination of residual NMP, whereas water was incompatible with the flame ionization detector when used as a sample solvent under these conditions. Because NMP possesses a chromophore that absorbs at low ultraviolet (UV) wavelengths, HPLC with UV detection was investigated.

Methods for the HPLC analysis of NMP have previously been published. Frick (12) developed an isocratic method for determination of NMP in waste water. HPLC with radioactivity detection has been used to detect radiolabeled NMP and 2-pyrrolidone in rat plasma and urine (13,14). We believe this to be the first

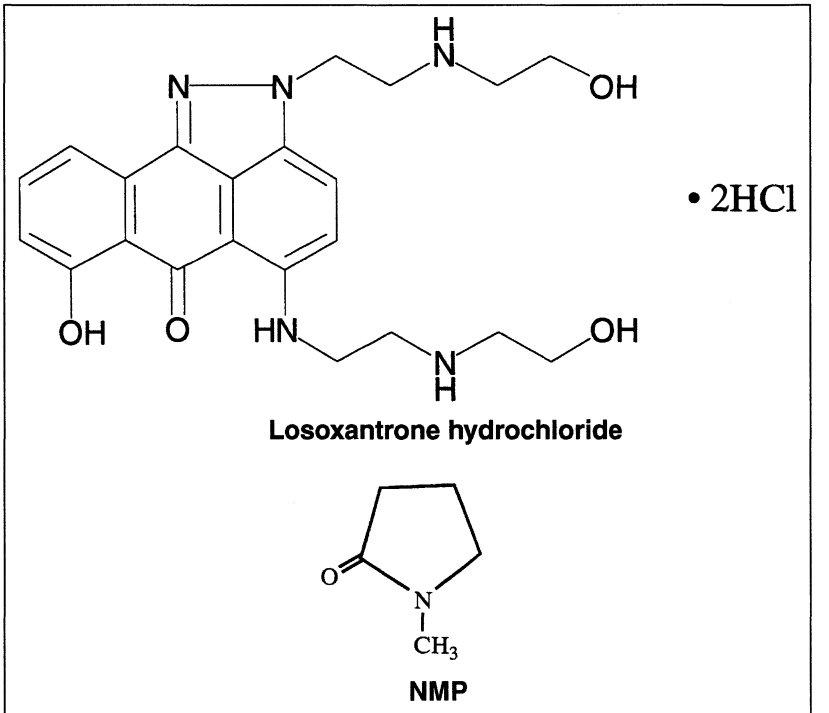

Figure 1. Chemical structures of losoxantrone hydrochloride and NMP.

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example of using HPLC to quantitate NMP in a drug substance, which should prove useful to others in this field.

Experimental

Reagents

The losoxantrone hydrochloride drug substance from DuPont Merck (Wilmington, DE) was used as received. High-purity water (> 18 MΩ-cm resistivity) was obtained from a Millipore Milli-Q purification system (Milford, MA); Omni-Solv HPLC-grade acetonitrile was obtained from EM Science (Gibbstown, NJ); trifluoroacetic acid (TFA) was obtained from Aldrich Chemicals (Milwaukee, WI); and HPLC-grade NMP was obtained from Burdick & Jackson (Muskegon, MI).

Table I. Recovery Data for HPLC Method

NMP spiked into sample (%)	NMP recovered (%)
0	0
0	0
0.18	0.18
0.18	0.18
0.09	0.10
0.09	0.09
0.02	0.02
0.02	0.02

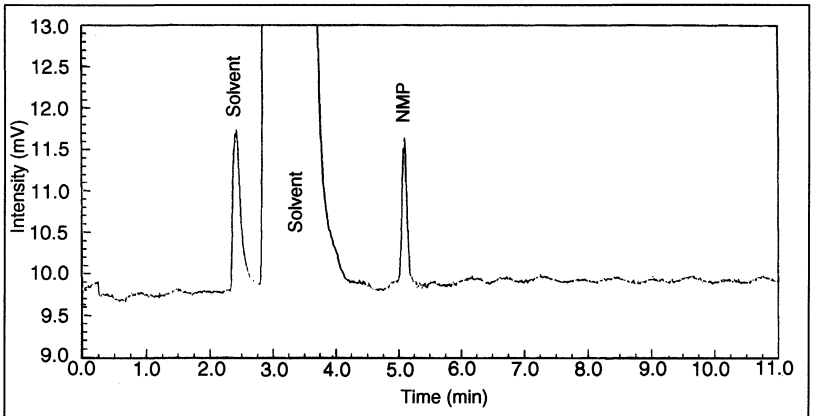

Figure 2. Chromatogram of a 0.54-µg/mL NMP standard injection.

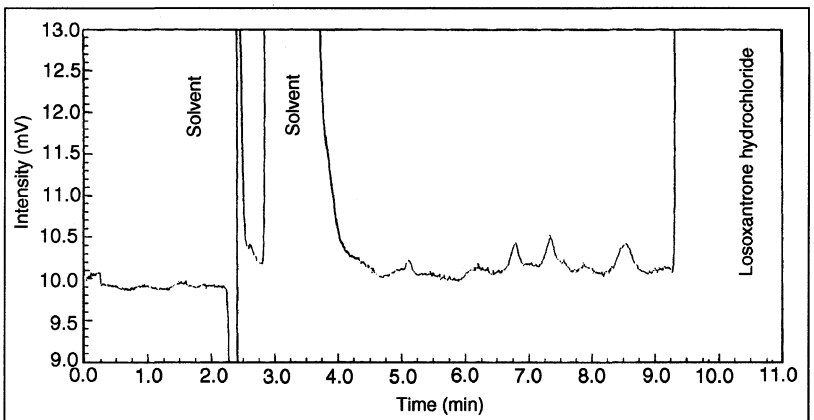

Figure 3. Chromatogram of a losoxantrone hydrochloride sample.

Chromatographic systems

Chromatographic results were obtained using two different HPLC systems. The first consisted of a Hewlett-Packard model 1050 solvent delivery system, a variable-wavelength UV–visible detector, a variable-volume injector, and a column oven (Palo Alto, CA). The second was an integrated Hewlett-Packard 1090M system incorporating a diode-array detector. Raw data storage and peak integration were performed on a Fisons Multichrom data collection and reduction system (Cheshire, England) operating on a Digital VAX computer (Maynard, MA).

Chromatographic conditions

The analytical method for quantitation of low concentrations of NMP in losoxantrone hydrochloride is a binary gradient reversed-phase HPLC method. Mobile phase A was 0.05% (v/v) TFA in water, and mobile phase B was acetonitrile. The gradient profile began by holding the mobile phase composition for 8 min at 85:15 (A:B), followed by a linear gradient to 15:85 for over 1 min. The composition was held at 15:85 for 4 min, then returned in 1 min to 85:15 and equilibrated for at least 8 min before the next injection. The total mobile phase flow rate was 1.0 mL/min, and both mobile phases were filtered and degassed before use. The column used was a Zorbax RX C8 (150 × 2.1 mm, 5-µm particle size; Mac-Mod Analytical, Chadds Ford, PA), held at a temperature of 40°C. The injection volume was 100 µL for both samples and standards. The detector wavelength was 220 nm.

Standard and sample preparation

Sample solutions of the losoxantrone hydrochloride drug substance were prepared by accurately weighing about 60 mg of losoxantrone hydrochloride into a 100-mL volumetric flask that was then diluted to volume with solvent (0.5% TFA in water). Standards were prepared by pipetting 1.00 mL NMP into a 200-mL volumetric flask. A 1.00-mL aliquot of this stock standard solution was pipetted into a 50-mL volumetric flask to form the intermediate standard solution. Aliquots of 10.0, 5.00, and 1.00 of the intermediate standard solution were then pipetted into separate 1-L volumetric flasks to produce standard solutions of 1.08, 0.54, and 0.11 µg/mL, respectively.

Results and Discussion

Method development

This reversed-phase HPLC method was adapted from previously published chromatographic conditions used to analyze losoxantrone hydrochloride in the finished dosage form and in extracts from

swabs used for cleaning certification of process equipment (15). TFA was used in the mobile phase to improve peak shape and in the sample and standard solvents to prevent losoxantrone hydrochloride and its impurities from adhering to the volumetric glassware. The detector wavelength was lowered to 220 nm from the 254 nm used in the finished dosage form method; although this is not the maximum wavelength (λ_{max}) of NMP, it greatly increased the sensitivity of the method. The gradient to 85% B was employed to ensure that no late-eluting losoxantrone hydrochloride impurities carried over into subsequent injections. Representative chromatograms of a standard, sample, NMP-spiked sample, and solvent blank are shown in Figures 2–5, respectively.

Accuracy and precision

The accuracy of the method was determined by recovery studies in which a losoxantrone hydrochloride reference standard, made by a process that did not employ NMP as a solvent, was spiked with NMP at four concentrations: 0, 0.18, 0.09, and 0.02% (w/w, relative to losoxantrone hydrochloride). Each sample was prepared in duplicate. The results (shown in Table I) showed complete recovery of NMP from the drug matrix. Precision was demonstrated during the recovery studies by repeated injections of standards at the three concentrations described in the *Standard and sample preparation* section. The middle and high standards typically showed relative standard deviations (RSDs) of less than 2%; the low standard RSDs were in the range of 4–6%. This precision is acceptable for a residual solvents test in which solvent concentrations are expected to be less than 0.1%.

Linearity, detection limits, and quantitation limits

The HPLC method was linear over the required concentrations. Over the concentration range of 0.11–1.08 µg/mL, the correlation coefficient was 1.000, the slope was 12213 µV-s-mL/g, and the *y*-intercept was –8 µV-sec, about 0.1% of the response of the 0.54 µg/mL standard.

The limit of detection (LOD) was defined as the concentration of NMP at which the signal-to-noise ratio equalled 3. The limit of quantitation (LOQ) was defined as the lowest concentration of NMP at which the RSD of the peak areas for six injections was comparable with or less than the system suitability criterion of 10%. Both limits were determined using both the variable-wavelength and diode-array detectors. The LOD and LOQ were 0.02 and 0.03 µg/mL, respectively (0.003 and 0.005%, relative to losoxantrone hydrochloride), using the variable-wavelength detector. The LOD and LOQ were 0.07 and 0.14 µg/mL, respectively (0.012 and 0.023%, relative to losoxantrone hydrochloride), using the diode-array detector. The LOQ data for both detectors are shown in Table II.

Specificity

No interferences with the NMP peak were noted from the sample solvent, sample matrix, or other solvents likely to be used in the synthesis of losoxantrone hydrochloride. Tested solvents included isopropanol, methanol, methylene chloride, pyridine, and tetrahydrofuran.

Conclusion

This reversed-phase HPLC method provides an accurate and precise means of determining residual NMP in losoxantrone hydrochloride. The method is linear and specific and provides

Table II. LOQ Determination

Replicate number	Peak area (µV sec) on an HP1050 at 0.03 µg/mL	Peak area (µV sec) on an HP1090 at 0.14 µg/mL
1	440	3399
2	461	2499
3	452	2586
4	385	2798
5	400	3037
6	401	2778
Mean	423	2850
RSD (%)	7.5	11.5

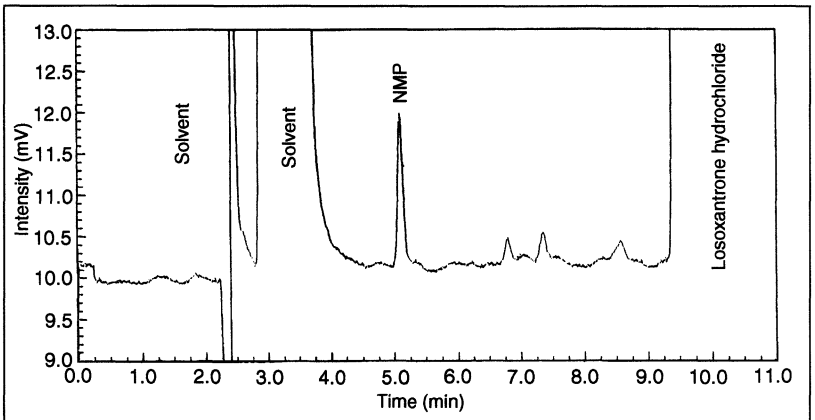

Figure 4. Chromatogram of a losoxantrone hydrochloride sample spiked with 0.09% NMP.

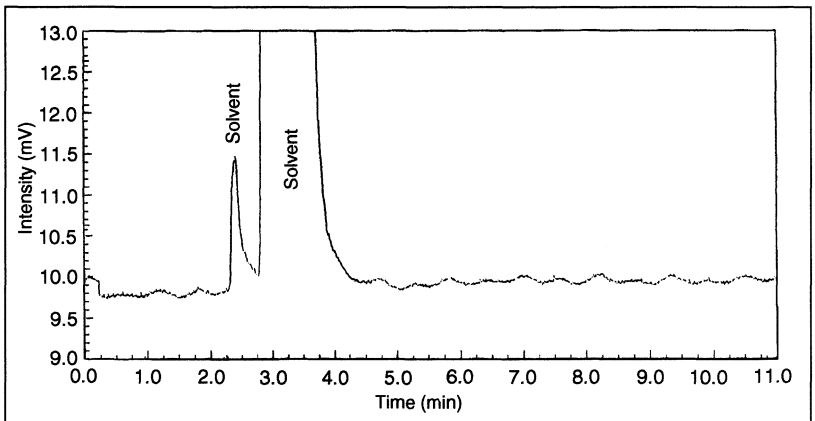

Figure 5. Chromatogram of a solvent blank.

excellent sensitivity, shorter run times, and easier setup than alternative GC procedures. This method should also be useful for the quantitation of NMP in other drug substances.

Acknowledgment

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