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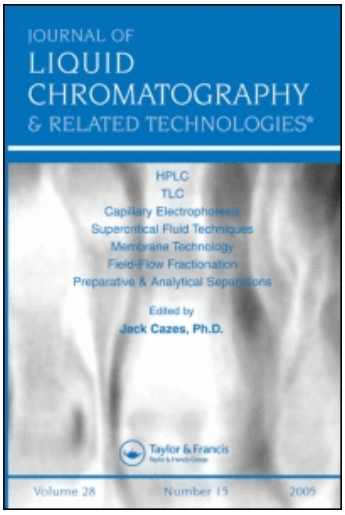

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HPLC DETERMINATIONS OF DOXORUBICIN WITH SELECTED MEDICATIONS IN 0.9% SODIUM CHLORIDE INJECTION USP

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ABSTRACT

High performance liquid chromatographic methods have been developed for the assays of mixtures containing doxorubicin-morphine, doxorubicin-granisetron, and doxorubicin-cyclophosphamide in 0.9% sodium chloride injection USP. The separation and quantitation of each mixture were achieved on octylsilane columns at ambient temperature using a mobile phase of aqueous sodium dihydrogen phosphate - acetonitrile at a flow rate of 1.0 mL/min with UV detection. The separations were achieved within 13 min. The methods showed linearity for the analytes in their respective calibration ranges. Accuracy and precision were in the 0.1 - 3.3% and 0.2 - 3.1% ranges, respectively, for the various analytes.

INTRODUCTION

Mixtures of doxorubicin hydrochloride with morphine sulfate (Mixture A), granisetron hydrochloride (Mixture B), and cyclophosphamide (Mixture C) are highly effective in the treatment of certain types of cancer.

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They are usually prepared in polyvinyl chloride bags in hospitals and administered to cancer patients by the intravenous route. Interest in this laboratory in the compatibility and stability of each drug mixture over time in 0.9% sodium chloride injection USP required the development of HPLC methods. A search of the literature indicated that analytical methods were not available to concurrently assay for each analyte in Mixture A, Mixture B or Mixture C.

Doxorubicin hydrochloride is probably the most important anticancer drug available because of its relatively broad spectrum of activity. It has been analyzed by a variety of methods including spectrophotometry,[1,2] electrochemistry,[3] HPLC,[4-6] and TLC[7]. The HPLC methods are the most common of the procedures reported and have involved the separation of the drug on silica, phenyl, cyanopropyl, octylsilane, or octadecylsilane columns. The official USP 23 assays for doxorubicin hydrochloride drug substance and injection dosage form utilize reversed-phase chromatography on a trimethylsilane column.[8]

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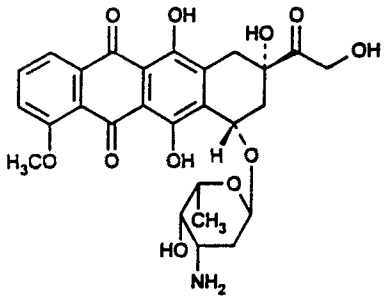
Morphine sulfate is the most often used analgesic for severe, acute, and chronic pain. Many analytical techniques have been employed for the determination of morphine. The spectrophotometry of morphine has been studied using either a color-producing reagent such as potassium iodate or second-derivative spectroscopy.[9,10] The TLC separation was achieved on silica gel G by overspotting the sample spots with dansyl chloride.[11] Flow-injection analysis with chemiluminescence has been reported with sensitivity in the low ng/mL range for morphine.[12] The HPLC methods, including the official USP 23 assay for morphine, involve chromatography of the drug on an octadecylsilane column[13-15] and phenyl column[16] with different mobile phases.

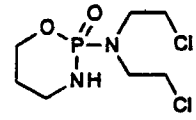
Granisetron is used as an antiemetic for cancer chemotherapy patients. The assay methods reported for granisetron are all involved with HPLC procedures for analyzing biological samples. Most of them use reversed-phase chromatography with fluorescence[17-19] or MS detection.[20] The use of silica column has also been reported in the HPLC determination of the drug.[21]

Cyclophosphamide is also an anticancer agent and has been assayed by different methods such as GC-MS,[22] spectrophotometry,[23] electrochemistry,[24] TLC[25] and HPLC.[26] The official USP 23 assay for cyclophosphamide is based on a reversed-phase HPLC method using an octadecylsilane column.[27]

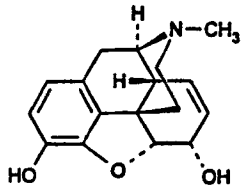
In this paper, isocratic HPLC assays are presented that simultaneously analyze for Mixture A, B, and C in 0.9% sodium chloride injection USP using a single injection. Each mixture is separated on an octylsilane column using an aqueous phosphate - acetonitrile eluent. The separations are achieved within 13 min at ambient temperature.



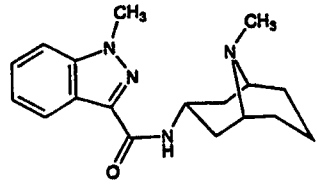
doxorubicin



cyclophosphamide



morphine



granisetron

Figure 1. Chemical structure of analytes studied.

EXPERIMENTAL

Reagents and Chemicals

The structure formulae of the compounds studied are shown in Figure 1. Reference standards of doxorubicin hydrochloride, morphine sulfate, and cyclophosphamide were purchased from the United States Pharmacopeial Convention, Inc. (Rockville, MD 20852). The working standard of granisetron hydrochloride was obtained from Smith Kline Beecham Pharmaceuticals (Philadelphia, PA 19101). Acetonitrile (J.T. Baker, Phillipsburg, NJ 08865) was HPLC grade and water was purified by a cartridge system (Continental Water Systems, Roswell, GA 30076). Sodium dihydrogen phosphate was J.T. Baker analyzed reagent (Phillipsburg, NJ 08865).

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Instrumentation

The chromatographic separations were performed on an HPLC system consisting of a Beckman Model 110B Solvent Delivery Module (Beckman, San Ramon, CA 94583), an Alcott Model 738 HPLC Autosampler (Alcott Chromatography, Norcross, GA 30093), an ABI Model 759A Absorbance Detector (Applied Biosystems, Foster City, CA 94404), and an HP Model 3394A Integrator (Hewlett-Packard Company, Avondale, PA 19311).

The doxorubicin-morphine separation was accomplished on an octylsilane column (IB-SIL C8, 250mm × 4.6 mm i.d., 5 μm particle size, Phenomenex, Torrance, CA 90501) at ambient temperature (23 ± 1°C). The mobile phase consisted of 60:40 v/v 0.02 M aqueous sodium dihydrogen phosphate - acetonitrile.

Both doxorubicin-granisetron and doxorubicin-cyclophosphamide separations were achieved on a Symmetry C8 column (150 × 3.9 mm i.d., 5 μm, Waters, Milford, MA 01757) at ambient temperature. The mobile phase for the doxorubicin-granisetron separation consisted of 80:20 v/v 0.02 M aqueous sodium dihydrogen phosphate - acetonitrile. The mobile phase for the doxorubicin-cyclophosphamide separation consisted of 77:23 v/v 0.03 M aqueous sodium dihydrogen phosphate - acetonitrile.

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The mobile phases were filtered through a 0.45 μm Nylon-66 filter (MSI, Westborough, MA 01581) and degassed by sonication prior to use. The flow rates were 1.0 mL/min and the injection volumes were 20 μL for each mixture. The UV detector was set at 285 nm for Mixture A, 307 nm for Mixture B and 195 nm for Mixture C.

Preparation of Standard Solutions

A combined standard solution containing doxorubicin hydrochloride and the other drug in each mixture was prepared by accurately weighing each reference or working standard powder into an appropriate size volumetric flask and adding 0.9% sodium chloride injection USP to volume.

Dilutions of each combined standard solution in the sodium chloride injection were prepared to achieve the concentration ranges shown in Table 1. Other dilutions within the concentration ranges examined were prepared in the sodium chloride injection to serve as spiked samples for each analyte to determine accuracy and precision. Quantitation was based on linear regression analysis of peak area versus analyte concentration in μg/mL.

Table 1

Analytical Figures of Merit for Doxorubicin Hydrochloride Mixtures

Mixture	Conc. Range (μg/mL)	r^2 [a]	System Suitability[b]	k'	N[c]	Tailing Factor[d]	RS
A. Doxorubicin hydrochloride	7.5-120	0.9996	1.91	2.3	1840	1.8	
							4.6
Morphine Sulphate	30-480	0.9999	0.54	1.1	1860	1.1	
B. Doxorubicin hydrochloride	38-604	0.9999	1.22	9.1	2145	1.3	
							10.6
Granisetron	0.47-7.6	0.9999	0.89	3.2	4190	1.0	
C. Doxorubicin hydrochloride	7.5-120	0.9995	1.44	4.1	4100	1.3	
							3.6
Cyclopho-sphamide	35-560	0.9999	0.26	5.3	5160	1.0	

[a] n=6

[b] Mean RSD% of 6 replicate injections of 37.8 μg/mL doxorubicin HCl and 151 μg/mL morphine sulfate for mixture A, 189 μg/mL doxorubicin HCl and 2.37 granisetron for Mixture B, and 38.7 μg/mL doxorubicin HCl and 151 μg/mL cyclophosphamide for Mixture C.

[c] Calculated as $N = 16(t/w)^2$.

[d] Calculated at 5% peak height.

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RESULTS AND DISCUSSION

There were no reports in the scientific literature describing concurrent assays of mixtures of doxorubicin hydrochloride and selected medications. Initial studies to develop HPLC methods for each mixture using isocratic conditions involved the use of underivatized silica, phenyl, octylsilane, and octadecylsilane columns with various mobile phases containing methanol and/or acetonitrile - aqueous phosphate at 1 mL/min. Our investigation indicated that chromatographic separations of doxorubicin with selected medications in the mixtures were best performed on octylsilane columns with aqueous phosphate - acetonitrile mobile phases. Typical chromatograms showing the separation of each of the three drug mixtures are shown in Figure 2.

The ionic strength of the mobile phase was the important parameter affecting retention of the analytes. Increasing the ionic strength decreased the retention time of doxorubicin more than that of the other analytes. Also, as the concentration of acetonitrile in the mobile phase increased, the retention of each

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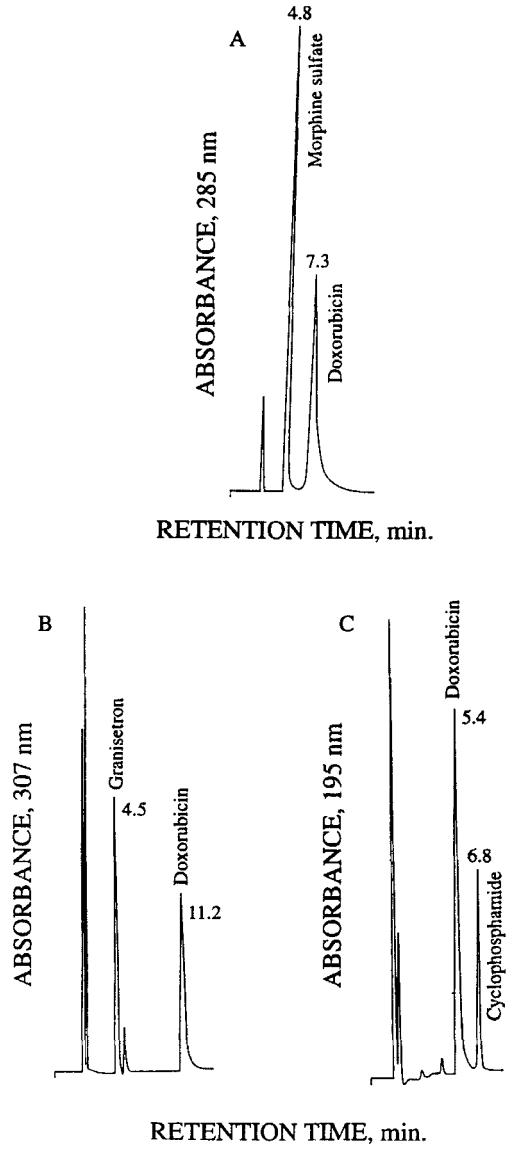

Figure 2. Typical HPLC chromatograms of the doxorubicin-morphine sulfate mixture (A), the doxorubicin-granisetron mixture (B) and the doxorubicin-cyclophosphamide mixture (C). See Experimental Section for HPLC assay conditions.

Table 2

Inter-Day Accuracy and Precision of HPLC Methods

Mixture	Analyte	Conc. Added (μg/mL)	Conc. Found[a] (μg/mL)	Percent Error	RSD %
A	Doxorubicin hydrochloride	15.42	15.77 ± 0.42	2.3	2.7
		61.65	59.77 ± 0.86	3.0	1.4
	Morphine sulfate	60.66	60.49 ± 0.95	0.3	1.6
		242.6	243.8 ± 1.87	0.5	0.8
B	Doxorubicin hydrochloride	75.52	74.20 ± 1.34	1.7	1.8
		302.1	301.3 ± 3.3	0.3	1.1
	Granisetron	0.9472	0.9323 ± 0.0093	1.6	1.0
		3.789	3.779 ± 0.079	0.1	2.1
C	Doxorubicin hydrochloride	15.47	15.13 ± 0.17	2.2	1.1
		61.88	62.01 ± 0.37	0.3	0.6
	Cyclophos-phamide	60.40	60.64 ± 0.30	0.4	0.5
		241.5	245.6 ± 4.18	1.7	0.3

[a] Mean ± standard deviation based on n = 15.

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analyte decreased. Thus, different aqueous phosphate and acetonitrile concentrations were used in the mobile phase to obtain the best separation of doxorubicin from the other analyte in their respective mixture in the shortest run time.

The concentrations of morphine sulfate or cyclophosphamide in Mixture A or C were several times greater than that of doxorubicin hydrochloride, yet the UV absorbance for morphine and cyclophosphamide at 254 nm were much lower than that of doxorubicin. Using the UV maxima of 285 nm for morphine and doxorubicin in Mixture A and 195 nm for cyclophosphamide and doxorubicin in Mixture C provided increased responses for morphine and cyclophosphamide with a reasonable absorbance response for doxorubicin at the respective wavelength. On the other hand, the concentration of granisetron in Mixture B was about ten times lower than that of doxorubicin hydrochloride. Therefore, the UV maximum of 307 nm for granisetron was used to determine both doxorubicin and granisetron in Mixture B.

Table 3

Intra-Day Accuracy and Precision of HPLC Methods

Mixture	Analyte	Conc. Added (μg/mL)	Conc. Found[a] (μg/mL)	Percent Error	RSD %
A	Doxorubicin hydrochloride	15.12	15.15 ± 0.47	0.2	3.1
		60.46	59.19 ± 0.45	2.1	0.8
	Morphine sulfate	60.24	60.81 ± 0.54	1.0	0.9
		241.0	243.6 ± 0.56	1.1	0.2
B	Doxorubicin hydrochloride	75.52	73.04 ± 0.95	3.3	1.3
		302.1	296.9 ± 1.5	1.7	0.5
	Granisetron	0.9472	0.9442 ± 0.0085	0.3	0.9
		3.789	3.781 ± 0.038	0.2	1.0
C	Doxorubicin hydrochloride	15.50	15.12 ± 0.21	2.5	1.4
		61.99	62.65 ± 0.56	1.1	0.9
	Cyclophos-phamide	60.32	60.13 ± 0.24	0.3	0.4
		241.3	242.8 ± 0.49	0.6	0.2

[a] Mean ± standard deviation based on n = 5.

The HPLC methods showed concentration versus absorbance linearity for the analytes studied. Table 1 gives the analytical figures of merit for the analytes in each mixture. A photodiode array detector (Model 990, Waters Associates, Milford, MA 01757) was used to verify that none of the degradation products of the analytes in each mixture (analyzed under their respective analytical conditions) interfered with the quantitation of each drug at the selected detection wavelengths. These experiments were performed on solutions of each drug in 0.9% sodium chloride injection USP after they had been degraded (10 - 20%) with 1.0 N hydrochloric acid or 1.0 N sodium hydroxide at 80°C or 30% hydrogen peroxide at both ambient temperature and 80°C.

Percent error and precision of each method in inter-day (Table 2) and intra-day (Table 3) assays were evaluated using spiked samples containing each analyte. The results in the tables shown that accuracy and precision for all the procedures were in the 0.1 - 3.3% and 0.2 - 3.1% ranges, respectively, for the analytes in the three mixtures studied.

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In summary, octylsilane columns with aqueous phosphate - acetonitrile mobile phases have been shown to be amenable for the separation and quantitation of doxorubicin and selected medications in the two component mixtures prepared in 0.9% sodium chloride injection USP. These HPLC methods provide good accuracy and precision and have been used to investigate the chemical stability of the analytes in each mixture.

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