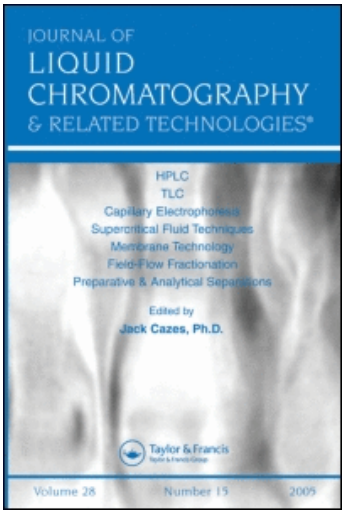

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HPLC Determination of a Vincristine, Doxorubicin, and Ondansetron Mixture in 0.9% Sodium Chloride Injection

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HPLC DETERMINATION OF A VINCRISTINE, DOXORUBICIN, AND ONDANSETRON MIXTURE IN 0.9% SODIUM CHLORIDE INJECTION

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ABSTRACT

A high performance liquid chromatography procedure has been developed for the assay of a vincristine, doxorubicin, and ondansetron mixture in 0.9% sodium chloride injection. The separation and quantitation are achieved on a phenyl column at ambient temperature using a mobile phase of 50:50 v/v 0.02 M phosphate buffer, pH 5.4-acetonitrile at a flow rate of 1.0 mL/min with detection of all three analytes at 233 nm. The separation is achieved within 15 min with sensitivity in the ng/mL range for each analyte. The method showed linearity for vincristine, doxorubicin, and ondansetron in the 0.36 - 3.6, 10.0 - 100, and 11.95 - 119.7 μg/mL ranges, respectively. Accuracy and precision were in the 1 - 3% and 0.2 - 3.3% ranges, respectively, for all three compounds. The limits of detection for vincristine, doxorubicin, and ondansetron were 90, 200 and 200 ng/mL, respectively, based on a signal to noise ratio of 3 and a 20 μL injection.

INTRODUCTION

A mixture of vincristine, doxorubicin, and ondansetron is highly effective in the treatment of certain types of cancer. Interest in our laboratories in the stability and compatibility of the drug mixture over time in 0.9% sodium chloride injection required the development of an HPLC method. A search of the literature indicated that an HPLC method was not available to assay for all three compounds concurrently in a single injection.

Vincristine has been previously analyzed by radioimmunoassay (1), TLC (2) and HPLC (3). The radioimmunoassay method also measured vinblastine and the sensitivity was in the low ng/mL range for both compounds. The TLC separation was achieved on alumina using a dual development technique, first with ethyl acetate, and then a 3:1 mixture of ethyl acetate - ethanol. The HPLC separation is used as the official USP XXII assay for the drug substance. It involves chromatography of the drug on an octylsilane analytical column equipped with an octadecylsilane guard column. The mobile phase is 70:29.5:0.5 methanol-water-diethylamine (pH adjusted to 7.5) and the analyte was detected at 297 nm using a 2 mL/min flow rate.

Assay methods for doxorubicin have included spectrophotometry (4), electrochemistry (5,6), microbiological agar diffusion (4), HPLC (7-10) and TLC (11). The HPLC methods are the most common of the procedures reported and have involved the separation of the drug on

silica, cyanopropyl, octyl, or octadecylsilane columns. The official USP XXII assays for doxorubicin drug substance and injection utilize reverse-phase chromatography on an octadecylsilane column (10).

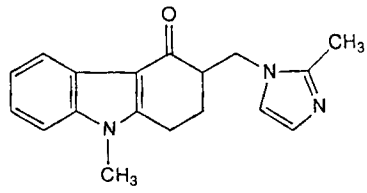
Ondansetron has been assayed by high performance thin-layer chromatography (HPTLC) and HPLC methods (12-14). The HPTLC method was developed especially for plasma samples, but the sample throughput was low and the equipment is not generally available in most laboratories. The HPLC assays used either a silica column with an aqueous-organic mobile phase or a cyanopropyl column operated in the reverse-phase mode.

In this paper, an isocratic HPLC assay is presented that will simultaneously analyze for vincristine, doxorubicin, and ondansetron in 0.9% sodium chloride injection using a single injection. The compounds are separated on a phenyl column using a buffered aqueous -acetonitrile eluent. The separation is achieved within 15 min at ambient temperature with sensitivity in the ng/mL range.

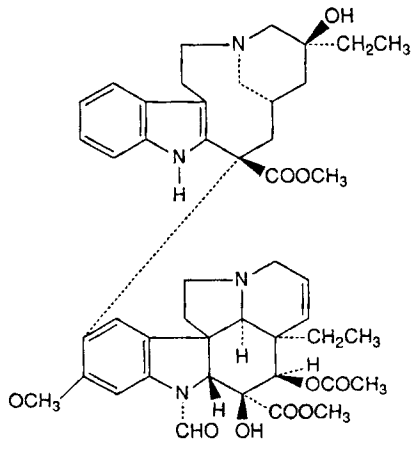
EXPERIMENTAL

Reagents and Chemicals

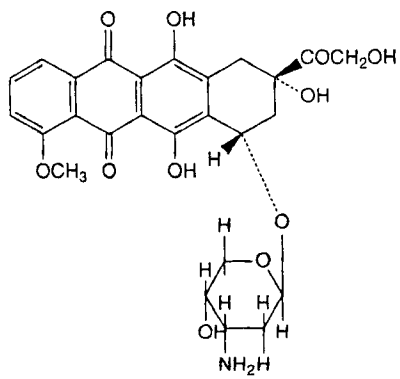
The structure formulae of the compounds studied are shown in Figure 1. Vincristine and doxorubicin were purchased from the United States Pharmacopeial Convention, Inc. (Rockville, MD 20852). Ondansetron (Batch C662/116/1) was a gift from Glaxo, Inc. (Research



ONDANSETRON



VINCRISTINE



DOXORUBICIN

Figure 1 - Chemical structures of compounds studied.

Triangle Park, NC 27709). Acetonitrile (J.T. Baker, Phillipsburg, NJ 08865) was HPLC grade and water was purified by a cartridge system (Continental Water Systems, Roswell, GA 30076). Monobasic sodium phosphate and sodium hydroxide were Baker analyzed reagents.

Instrumentation

The chromatographic separation was performed on an HPLC system consisting of a Waters Model 501 pump (Milford, MA 01757), an Alcott Model 728 auto-sampler (Norcross, GA 30093) equipped with a 20 μL loop, a Beckman Model 163 variable wavelength UV-VIS detector (Fullerton, CA 92634) and a Shimadzu Model CR-3A integrator(Columbia, MD 21046). Separation was accomplished on a 30 cm phenyl column (3.9 mm i.d., 10 μm particle size, Waters μ-Bondapak, Milford, MA 01757). The mobile phase consisted of 50:50 v/v 0.02M aqueous monobasic potassium phosphate, pH 5.4 (adjusted with 1 N sodium hydroxide)-acetonitrile. The mobile phase was filtered through a 0.45 μm Nylon-66 filter (MSI, Westborough, MA 01581) and degassed by sonication prior to use. The flow rate was set at 1 mL/min. The detector was set at 233 nm.

Preparation of Standard Solutions

A combined standard solution containing vincristine, doxorubicin, and ondansetron was prepared by accurately weighing 0.195 mg of vincristine sulfate, 5.45 mg of doxorubicin hydrochloride, and 7.50 mg of ondansetron hydrochloride, transferring to a 50-mL volumetric flask,

manually shaking for 10 min and 0.9% sodium chloride injection added to volume. This combined standard solution along with 4:10 and 1:10 dilutions made from the combined standard solution gave solutions containing 0.36, 1.40 and 3.60 μg/mL of vincristine, 10.0, 40.2, and 100.0 μg/mL of doxorubicin, and 11.95, 47.9 and 119.7 μg/mL of ondansetron expressed as the free base concentrations. Three point calibration curves were constructed for each analyte. Additional dilutions (2:10 and 8:10) of the combined standard solution were prepared in 0.9% sodium chloride injection to serve as spiked samples for each analyte to determine accuracy and precision of the method. Quantitation was based on linear regression analysis of analyte peak height versus analyte concentration in μg/mL.

RESULTS AND DISCUSSION

The goal of this study was to develop an isocratic HPLC assay for the analysis of a vincristine, doxorubicin and ondansetron mixture in 0.9% sodium chloride injection. The mixture is typical of a chemotherapy regimen that would be administered to a cancer patient. Stability studies of the mixture would require an assay procedure that would detect and quantitate each analyte with reasonable accuracy and precision.

There are no reports in the scientific literature describing a separation of these three analytes in a single mixture. Initial studies to

develop a single isocratic HPLC method for the three compounds involved the use of underivatized silica and cyanopropyl columns with various mobile phases containing acetonitrile-aqueous phosphate buffers. The vincristine peak eluted > 14 min and showed tailing on both columns with varied mobile phases. Doxorubicin and ondansetron eluted in that order and generally gave sharp peaks with some overlap on the silica column to less overlap of the peaks on the cyanopropyl column. Changes in mobile phase composition did not significantly improve the resolution of the three compounds on either column. Using an octylsilane column and a 60:40 aqueous pH 5.4 phosphate buffer-acetonitrile, the doxorubicin and ondansetron peaks were not well resolved, but the vincristine peak was well-separated from the other analytes with an 8 min retention time. Also, a deactivated octylsilane column was investigated and, while it generally provided excellent separation of the three compounds, a split peak was observed at higher concentration levels of ondansetron. Next, two commercial brands of octadecylsilane columns were studied. Even though the better separation was achieved on one of the brands using a mobile phase of 50:50 aqueous pH 6.5 phosphate buffer-acetonitrile at 1 mL/min, the peaks were still too close to one another and greater resolution was needed.

Our attention turned to the use of a phenyl column for the separation of the three analytes. Using various proportions of 0.02M pH

5.4 phosphate buffer - acetonitrile as mobile phases, the best separation and excellent resolution of the three analytes were obtained using a 50:50 v/v phosphate buffer-acetonitrile mobile phase with a total run time of 15 min. It was discovered later in these studies that the phenyl column also allowed the separation of methylparaben from the analytes, an important consideration since both ondansetron and vincristine commercial injections contained significant amounts of methylparaben as a preservative. Thus, the phenyl column with a mobile phase consisting of 50:50 v/v 0.02 M phosphate buffer pH 5.4 - acetonitrile was selected for the assay. A typical chromatogram showing the separation of the three analytes is shown in Figure 2.

In the acetonitrile-phosphate buffer mobile phase, the absorption maxima for vincristine, doxorubicin, and ondansetron were 254, 233, and 216 nm, respectively. It was decided to use 233 nm as the detection wavelength for the mixture since this was the wavelength that provided the best accuracy and precision data for all three components.

The HPLC method showed concentration versus absorbance linearity for vincristine, doxorubicin, and ondansetron in the 0.36 - 3.6, 10.0 - 100.0 and 11.95 - 119.7 μg/mL ranges, respectively at 233 nm. Table 1 gives other analytical figures of merit for each analyte. A photodiode array detector (Model 990, Waters Associates, Milford, MA 01757) was used to verify that none of the degradation products of any of the three analytes would interfere with the quantitation of each drug

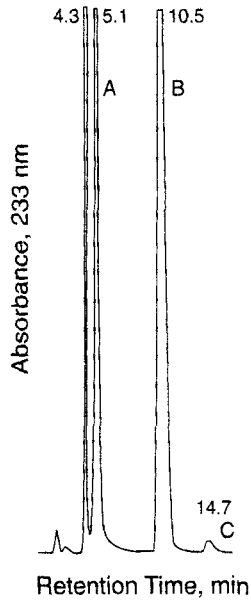


Figure 2- Typical HPLC chromatogram of doxorubicin (A), ondansetron (B) and vincristine (C) on phenyl column with acetonitrile - aqueous phosphate buffer pH 5.4 mobile phase. The peak at 4.3 min retention time is methylparaben, a component in ondansetron and vincristine injections. See Experimental Section for assay conditions.

at 233 nm. These experiments were performed on solutions of the three drugs in 0.9% sodium chloride injection after they has been degraded for 6 hr at 80°C in both 1.0N acid and 1.0N base.

Percent error and precision of the method were evaluated using spiked samples containing each analyte. The results shown in Table 2 indicate that the procedure gives acceptable accuracy and precision for all three analytes.

TABLE 1

Analytical Figures of Merit For Doxorubicin, Ondansetron, and Vincristine.

Analyte	r^{2a}	System Suitability[b]	LOD[c] ng/mL	k′	Theoretical Plates[d]	Tailing Factor[e]	Rs
Doxorubicin	0.9993	1.52	200.0	1.19	419	2.0	
							5.08
Ondansetron	0.9998	0.49	200.0	3.40	1591	1.5	
							2.94
Vincristine	0.9999	0.74	90.0	5.20	1127	1.5	

[a] Range examined from 0.36 - 3.60 μg/mL vincristine(n = 9) 10.0 - 100 μg/mL doxorubicin (n = 9), and 11.95 - 119.7 μg/mL ondansetron (n = 9). Mobile phase consisted of 50:50 v/v 0.02 M phosphate buffer, pH 5.4-acetonitrile at 1.0 mL/min with detection at 233 nm.

[b] RSD % of 6 replicate injections at 2.9 μg/mL vincristine, 80.0 μg/mL doxorubicin, and 95.7 μg/mL ondansetron at 233 nm.

[c] Limit of detection, S/N = 3.

[d] Calculated as $n = 16 (t/w)^2$

[e] Calculated at 10% peak height.

TABLE 2

Accuracy and Precision Using Spiked Drug Samples

	Concn Added (μg/mL)	Concn Found[a] (μg/mL)	Percent Error	RSD (%)
Vincristine	0.72	0.70 ± 0.004	2.40	0.58
	2.90	2.87 ± 0.009	1.14	0.30
Doxorubicin	20.1	19.92 ± 0.38	0.90	1.91
	80.4	79.50 ± 1.47	1.12	1.85
Ondansetron	23.9	24.63 ± 0.82	3.05	3.33
	95.8	96.70 ± 0.15	0.94	0.16

[a] Based on n = 3.

Intra-day variabilities of the assay for vincristine, doxorubicin, and ondansetron expressed as % RSD were 1.58, 1.39 and 0.87% (n = 4), respectively. Inter-day variabilities of the assay for these drugs were 0.74, 1.52 and 0.49% (n = 30 over 6 days), respectively.

In summary, a phenyl column with an aqueous 0.02 M pH 5.4 buffer-acetonitrile mobile phase has been shown to be amenable for the separation and quantitation of a vincristine-doxorubicin-ondansetron mixture in 0.9% sodium chloride injection. This study suggests that the HPLC method can be used to investigate the chemical stability of all three drugs in sodium chloride injection.

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